Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 21

7 April, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: 1316/18 (4136811-00)

Signalment:
12 year old, female, worm-blooded, *Equus caballus*, horse

History:
A 12-year-old Warmblood mare was presented to the Surgery Clinic with a 5 month history of epistaxis and facial swelling. At admission, a swollen area size 5 x 3 cm proximal of crista facialis was obvious together with epiphora and blepharospasm on the right eye. A strong stertor could be heard during rest. Sinus centesis revealed the presence of a large volume of red-yellow, viscous fluid.

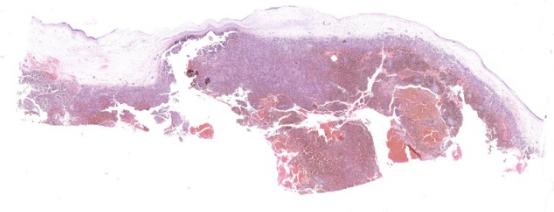
Radiography noted osteolytic processes with complete shading of maxillary and frontal sinuses and mild deviation of the nasal septum. Endoscopic findings included constriction of ventral nasal meatus of the affected side and the presence of reddish yellow mass within sinuses that started to bleed. A suspected diagnosis of sinus cysts was established.

Standing surgery was attempted but due to mare temper, the surgery had to be performed in general anesthesia. A frontonasal flap was performed and a cavity with a red-yellow, smooth surface was observed. The cavity was stretching through all ipsilateral sinuses and it was not possible to differentiate between each sinus compartment. During the surgery the owner was contacted and due to poor prognosis, the animal was euthanized and submitted for necropsy.

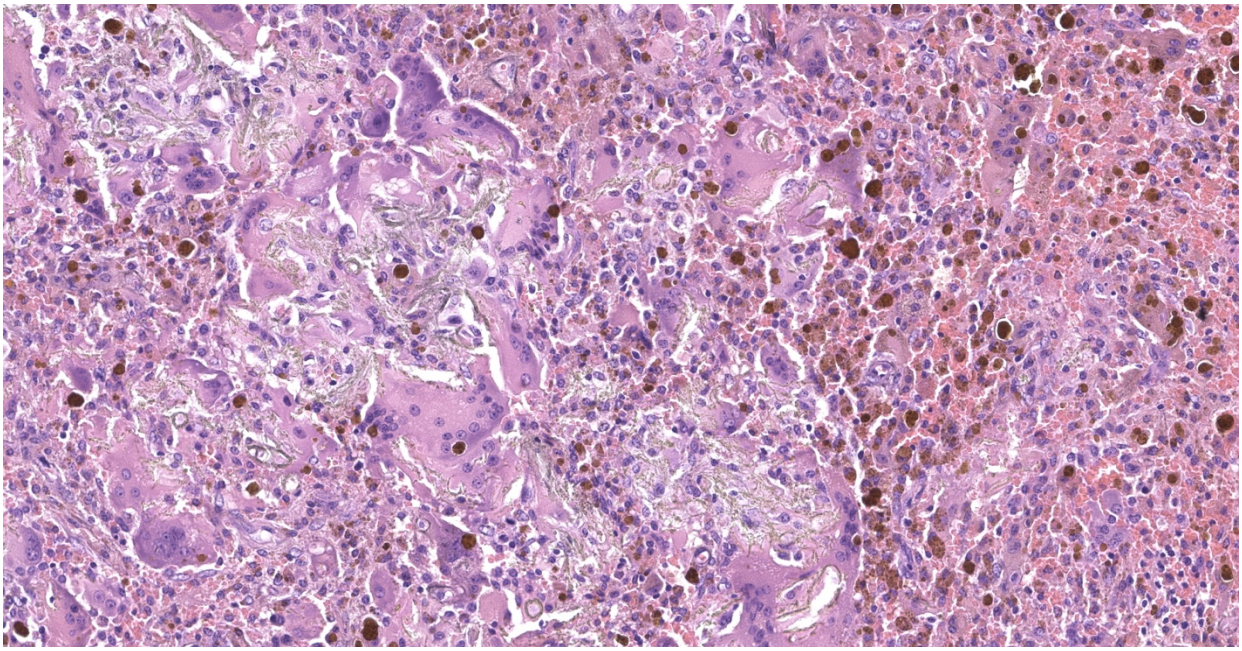
Gross Pathology:
Necropsy just revealed the size of the cavity that stretched through the nasal cavity and all ipsilateral sinuses at the right side. Nasal conchae were completely destroyed. The cavity was filled with dark red to yellow, friable mass covered with fresh coagulated blood on the surface (the mass was partially removed by the surgery so we could not estimate the real size).

Laboratory results:
None.

Microscopic description:
Respiratory epithelium (paranasal sinus); at one side the section is covered by columnar or

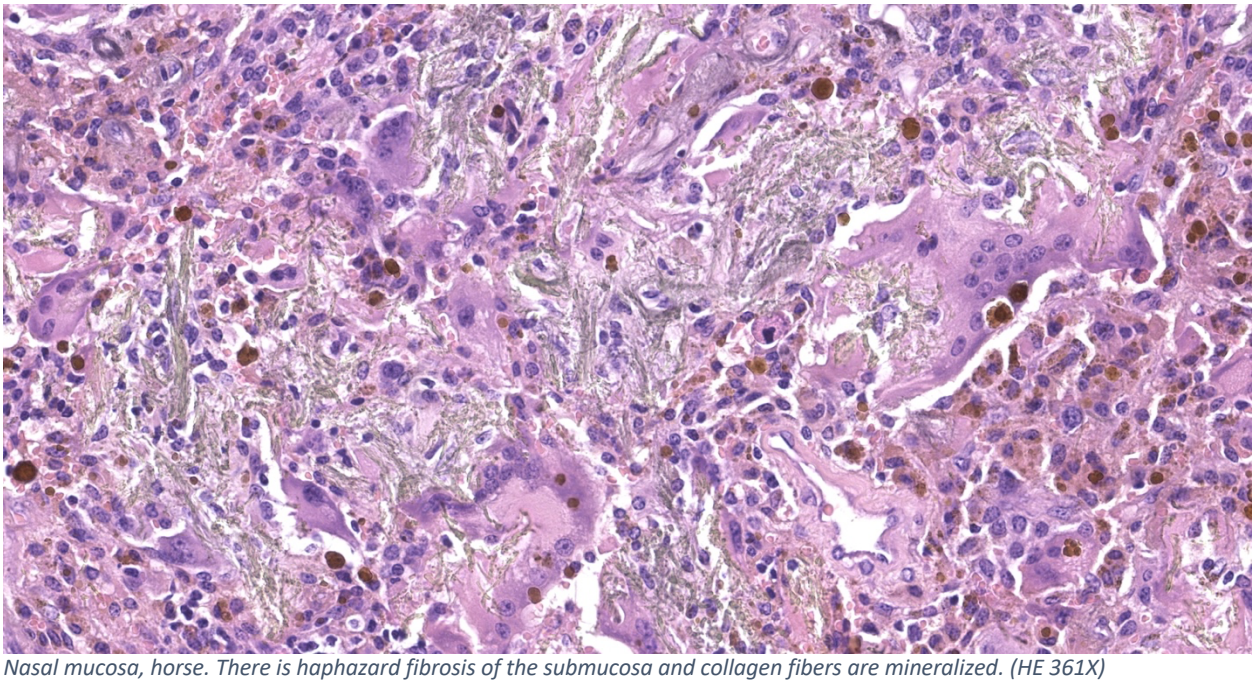


Nasal mucosa, horse. The submucosa is effaced by a dense cellular infiltrate and abundant hemorrhage and hemosiderin. (HE, 6X)

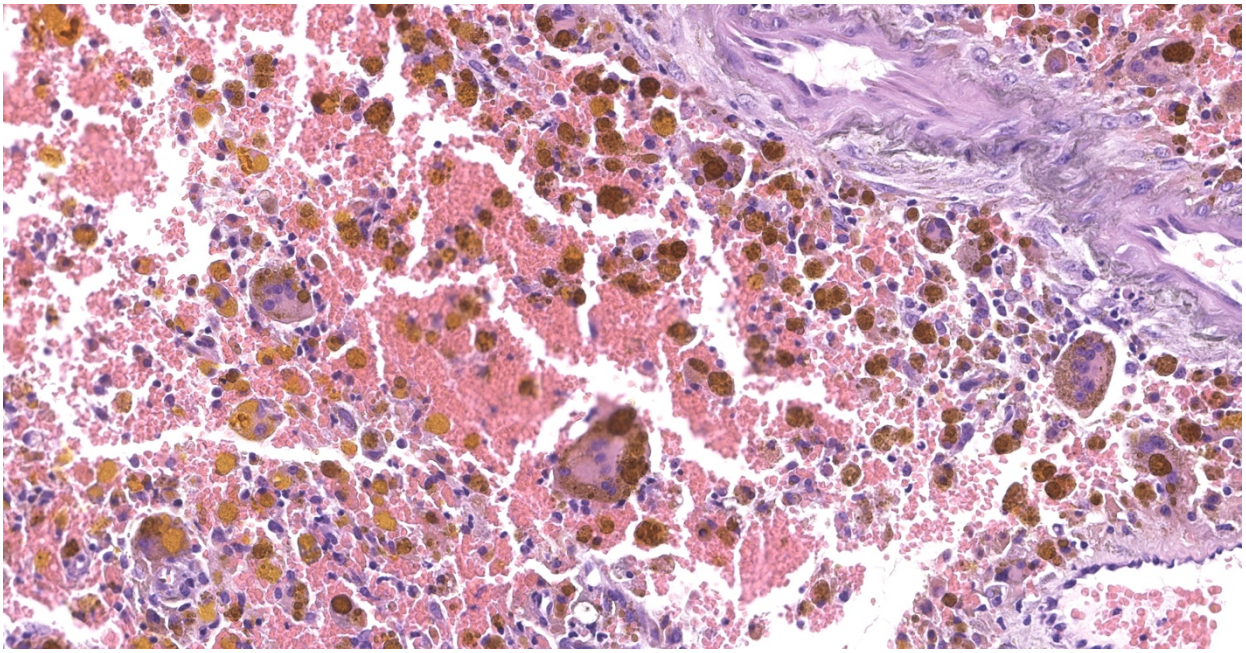


Nasal mucosa, horse. The submucosa is infiltrated by innumerable macrophages and multinucleated giant cell macrophages that contain hemosiderin granules. Hemosiderin deposition is more intense in areas of acute hemorrhage (right). (HE, 227X)

cuboidal ciliated epithelium that is occasionally flattened or missing. Subepithelial connective tissue is edematous with dilated lymphatics and infiltrated with scant to moderate lymphoplasmacytic and histiocytic infiltrate. An edematous fibrous layer progress to the mass composed of numerous macrophages and multinucleated giant cells, moderate numbers of lymphocytes and plasma cells, admixed with hemorrhage, fibrin, and multifocal increased numbers of small to medium caliber vessels (neovascularization). Many giant cells and macrophages contain abundant golden-yellow granular pigment (hemosiderin/hematoidin) and there are large, extracellular globular aggregates of yellow hematoidin pigment (ceroid sequins).



Nasal mucosa, horse. There is haphazard fibrosis of the submucosa and collagen fibers are mineralized. (HE 361X)



Nasal mucosa, horse. Multiple pigments are present in this section. Macrophages at lower left contain hematoidin (degraded hemoglobin without iron), hemosiderin (center) and there is mineralization of the wall of a large vessel at upper right. (HE, 300X)

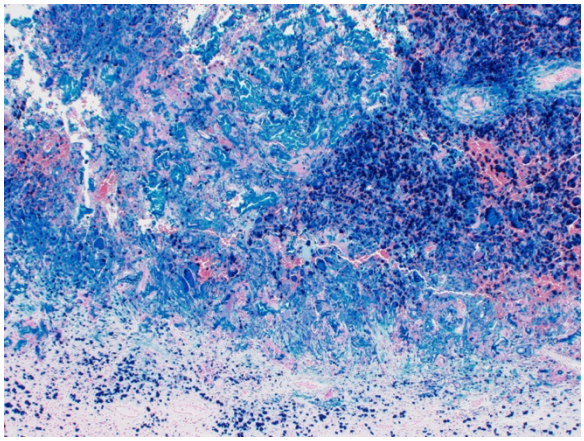
There are multifocal small aggregates of deeply basophilic, finely granular pigment (mineralization) that mostly accumulate in the basement membranes of capillaries.

Contributor's morphologic diagnosis:
Paranasal sinus mucosa: granulomatous inflammation with hemorrhage, hemosiderosis, hematoidin and mineralization.

Condition: Progressive ethmoid hematoma

Contributor's comment:
The paranasal sinus system of horses is complex, comprising six pairs of sinuses: the frontal sinus, the conchal sinuses (dorsal and ventral), the maxillary sinus, and the sphenopalatine sinus. Disease processes that can develop in the sinuses include ethmoid hematomas, cysts, neoplasia, and bacterial and fungal infections. Horses that develop paranasal sinus disease vary widely in age. Because of the anatomic location of the paranasal sinuses and associated chronic conditions that affect many patients, many disease processes involving the paranasal sinuses require surgical correction for a favorable prognosis. Fungal and neoplastic processes have a less favorable prognosis than bacterial and other processes.[12]

A progressive ethmoid hematoma (PEH) is a nonneoplastic, progressive, and locally destructive growth in the paranasal sinuses that resembles a tumor. Even though its etiology is unknown, it is hypothesized that PEH originates in the submucosa of the ethmoid labyrinths resulting from repeated episodes of hemorrhage which lead to formation of an angiomatous-like mass, covered by the respiratory epithelium. The mass grows slowly but progressively, protruding



Nasal mucosa, horse. A totally unnecessary iron stain demonstrates the amount of iron in the section, largely in the form of intracellular hemosiderin. Hematoidin, as it lacks iron, will not stain. (Perl's iron, 100X)

ventrocaudally into the ipsilateral ethmoidal meatus and then into the nasopharynx, contralateral nasal cavity and adjacent paranasal sinuses. Clinical signs appear when the mass reaches a size big enough to induce severe local destructive effects, which are characterized by intermittent monolateral epistaxis, particularly during exercise.[2,4,6] Histologically, the lesion is covered by pseudostratified columnar epithelium containing mucus cells, but focal ulceration, secondary infection, or focal squamous metaplasia of the mucosa may occur. A submucosal fibrous tissue pseudocapsule surrounds the central mass of hemorrhage, and there can be areas of loose vascular channels and sinuses lined by plump spindle-shaped cells with hyperchromatic nuclei. Foci of recent hemorrhages are accompanied by large macrophages and multinucleated foreign body-type giant cells that usually contain large amounts of hemosiderin. The organization of the hemorrhage proceeds by the development of capillaries and fibrous tissue. Both the blood vessels, some of which may be thrombosed, and the collagen fibers, have calcareous deposits on them, along with globin-derived pigments and hemosiderin.[6]

In the presented case, a sinus cyst with repeated chronic hemorrhage cannot be excluded. Sinus cysts are extensive lesions also of unknown etiology that are single or loculated, fluid-filled cavities with an epithelial lining. They develop in the maxillary sinuses and ventral conchae but can extend into the frontal sinus and nasal cavity. Abnormal development of embryonic germ tissue and cystic development caused by repeated submucosal hemorrhage have been proposed as etiologic factors.[8,13,14] It is characterized by firm facial swellings and nasal airflow obstruction.[4] Histologic findings in sinus cysts include granulation tissue, neovascularization, ulceration, hemorrhage, fibroplasia, inflammatory cell infiltration, mineralization, and bony trabeculation in the cyst wall.[12,13] Lane et al. (1987) suggested an association of sinus cyst with progressive ethmoidal hematoma based on the histological evidence of repeated hemorrhages within cysts wall and PEH. However, in one other study there was sufficient evidence to confirm such an association.[12] In this study, recent and older hemorrhages were also observed in some cases of chronic sinusitis. Additionally, in this study, the double epithelial lining found in most sinus cysts was not found in any of the (solid) PEH. Moreover, unlike many sinus cysts, PEH sections examined in this study never contained bony spicules. Thinning and remodeling of the overlying bones was rare with PEH in contrast to sinus cysts, where this feature (and subsequent gross facial swelling) was common, indicating further significant differences in the nature of these lesions.[12] Taking into consideration all of the above, in the presented case, macroscopic findings of facial swelling cannot exclude sinus cyst as underlying disease, but the presence of large hemorrhagic mass and histologic findings are more consistent with PEH.

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JPC diagnosis:
Nasal mucosa: Rhinitis (sinusitis acceptable), granulomatous, chronic, focally extensive, severe, with acute and subacute hemorrhage, hemosiderosis, and hematoidin deposition.

JPC comment:
Progressive ethmoid hematoma (PEH) usually occurs in older horses, not dissimilar to this case, and is more common in Thoroughbred, Arabian, and warmblood horses. Growth is progressive and can continue to grow until it extends to the external nares.[1]

While survey radiography can be useful in identifying a sinus mass, endoscopy of the ethmoid conchae is required to confirm a diagnosis of PEH, with CT being the preferred diagnostic imaging modality. While they can arise unilaterally, approximately 30-50% of affected horses are affected bilaterally. There are several interventions to treat PEH, but regardless of treatment choice, PEH recurs in 17-50% of

cases within months to years following treatment.[11]

Other species also develop non-neoplastic proliferative sinus lesions, including the cat and dog. Feline mesenchymal nasal hamartoma is histologically distinct from the nasopharyngeal polyp and is most often composed of woven bone mixed with proliferating fibrous to myxomatous stroma and erythrocyte filled cavities, with a respiratory epithelial lining.[5] Both respiratory epithelial adenomatoid hamartoma (REAH)[9] and chondo-osseous respiratory epithelial adentomatoid hamartoma (COREAH) have been reported in the dog and are histologically similar to their human counterparts.[3,10] REAH is composed of a proliferation of glandular spaces lined by respiratory epithelium, overlying a proliferative spindle cell background, with COREAH having an additional chondroid or osseous component.[7] These described lesions are currently considered hamartomas, are not invasive, but may cause compression necrosis of adjacent tissues.

The moderator reiterated that this condition has an unknown etiology but is potentially associated with chronic inflammation. During the conference, definitional differences between erosion and ulceration were debated. Erosion is typically described as a loss of mucosa without compromise of the underlying basement membrane. Ulceration has loss of mucosal epithelium and a break in the basement membrane. In human literature, their definition of ulceration requires break through the muscularis mucosa layer, which is not seen in this case.

Also, of note for this entity, the deposits of hematoidin are commonly referred to as "ceroid sequins", though no part of the material is derived from ceroid. Ceroid is a material similar to lipofuscin, associated with peroxidation of fat deposits, and is also yellow. In this case, it is very likely hematoidin and not ceroid, in the context of chronic and acute hemorrhage, and extensive hemosiderin.

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sinunasal cysts. *Equine Vet J.* 2006;38(3):198-202.

CASE 2: 143/16 (4085104-00)

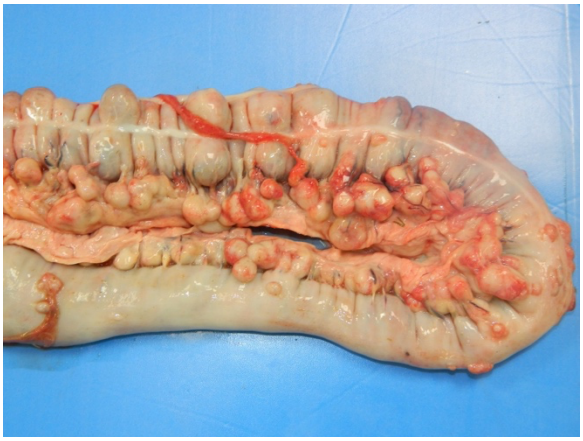
Signalment:
2-month-old, male foal, Brazilian Sport Horse, *Equus caballus*, equine.

History:
The horse was presented at the Veterinary Hospital of the Universidade Federal de Minas Gerais with left hind limb lameness and swelling of the left stifle joint. Few days after admission at the Veterinary Hospital, it developed recurrent fever, lethargy, increased respiratory rate, swelling of the left shoulder joint, and fluid diarrhea. Antibiotics and anti-inflammatory drugs were administered but no clinical improvement was observed.

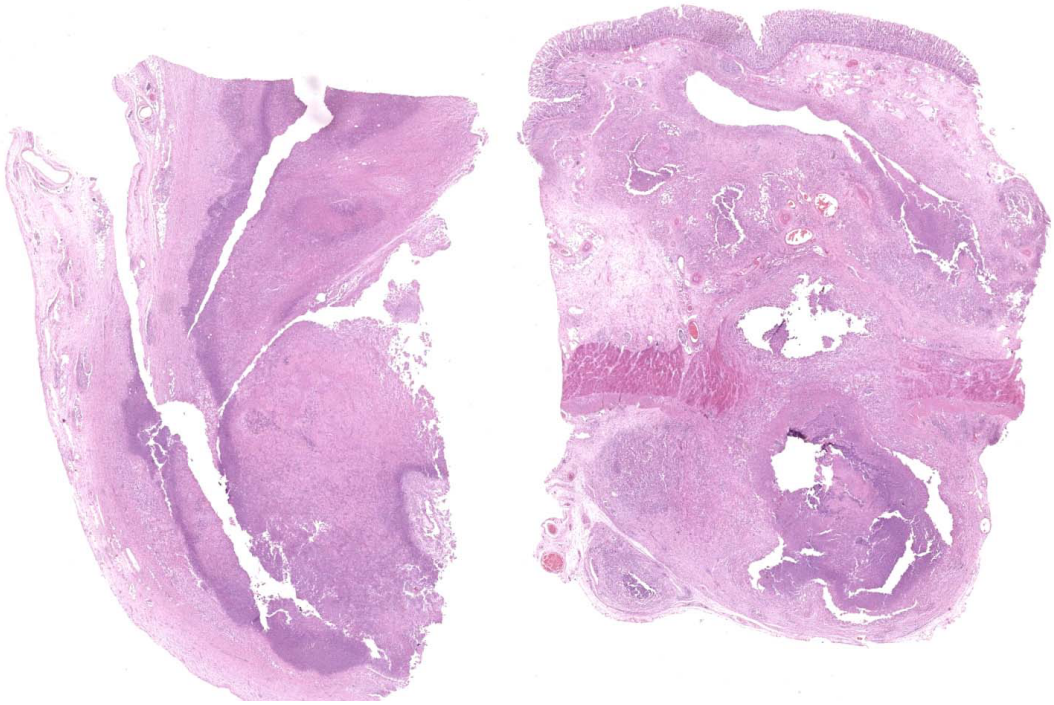
Gross Pathology:
The foal was in good body condition. The stifle joint of the left pelvic limb was moderately swollen. The subcutaneous tissue over the joint was expanded by moderate amounts of a yellowish and viscous fluid (purulent exudate), which also extended to and dissected adjacent muscles. The superficial lymph nodes (axillar and cervical superficial) were moderately enlarged, and their parenchyma was replaced by white to yellow and soft exudate. The joint capsule was markedly thickened, and the articular cartilage was irregular. There was a moderate amount of a yellowish and viscous fluid within the stifle joint (purulent arthritis). Scattered throughout all lung lobes 1.0 to 9.0 cm in diameter white to yellow, slightly salient soft nodules were observed. On cut surface, moderate amount of a creamy yellow material (purulent exudate) oozed from the nodules (pyogranulomatous pneumonia). Large colon mucosa was irregular and thickened with salient areas admixed with 2.0 to 10.0 cm in diameter, whitish to grayish depressed areas (ulcers). Within the intestinal wall there were multiple small, 0.5 to 2.0 cm in diameter, white to yellow soft nodules. Cecal, colonic, mesenteric and tracheobronchial lymph nodes were markedly enlarged (5.0-10.0 cm in diameter), white to yellow and soft. Nodal cut surfaces were irregular and yellowish, with loss of the corticomedullary distinction, due to accumulation of variable amounts of a creamy yellow material (purulent exudate).

Laboratory results:
Laboratory results are pending**.**

Microscopic description:
Large colon: locally extensive areas of the colonic submucosa, muscular layers and serosa are replaced and expanded by inflammatory nodules (pyogranulomas). These nodules are composed by a core of abundant eosinophilic cellular material and nuclear debris (necrosis), admixed with numerous degenerated and viable neutrophils, surrounded by a thick rim of moderate numbers of epithelioid macrophages and multinucleated giant cells (Langerhans type). Many of the macrophages contain intracytoplasmic bacteria (coccobacilli). These inflammatory nodules are further bounded by a thick layer of fibrous connective tissue admixed with numerous blood vessels (neovascularization - granulation tissue) infiltrated by moderate numbers of lymphocytes, plasma cells and macrophages. Collagen bundles in these areas and in the submucosa are separated by clear, non-staining spaces (edema). Several medium sized caliber blood vessels (arteries and veins) in the submucosa and in the serosa are partial to completely occluded by dense aggregates of an eosinophilic fibrillar material intermingled with



Colon, foal. Colonic lymph nodes are markedly enlarged, and the wall of the colon has multifocal areas of cellular infiltration. (Photo courtesy of: Veterinary School, Universidade Federal de Minas Gerais, www.vet.ufmg.br)



Colon, lymph node, foal. A section of colonic lymph node (left) and colon (right) are submitted for examination. At low magnification, normal nodal architecture is effaced by extensive necrosis and a dense cellular infiltrate. The wall of the colon is markedly expanded (particularly the submucosa and serosa) by nodular pyogranulomas that centrally efface the muscular wall. (HE, 6X)

small numbers of lymphocytes, neutrophils, erythrocytes and nuclear debris (fibrin thrombi). Rarely, the center of some thrombi is obscured by roughly granulated, hyperbasophilic and vitreous material (mineralization). Multifocally within the submucosa and serosa, there are prominent, dilated lymphatic vessels (lymphangiectasia), frequently filled with slight eosinophilic, fibrillar material (fibrin) with entrapped leukocytes. Few blood vessels present an irregularly shaped lumen, compressed by multifocal to diffuse subintimal deposits of granulated and hyperbasophilic material admixed with macrophages.

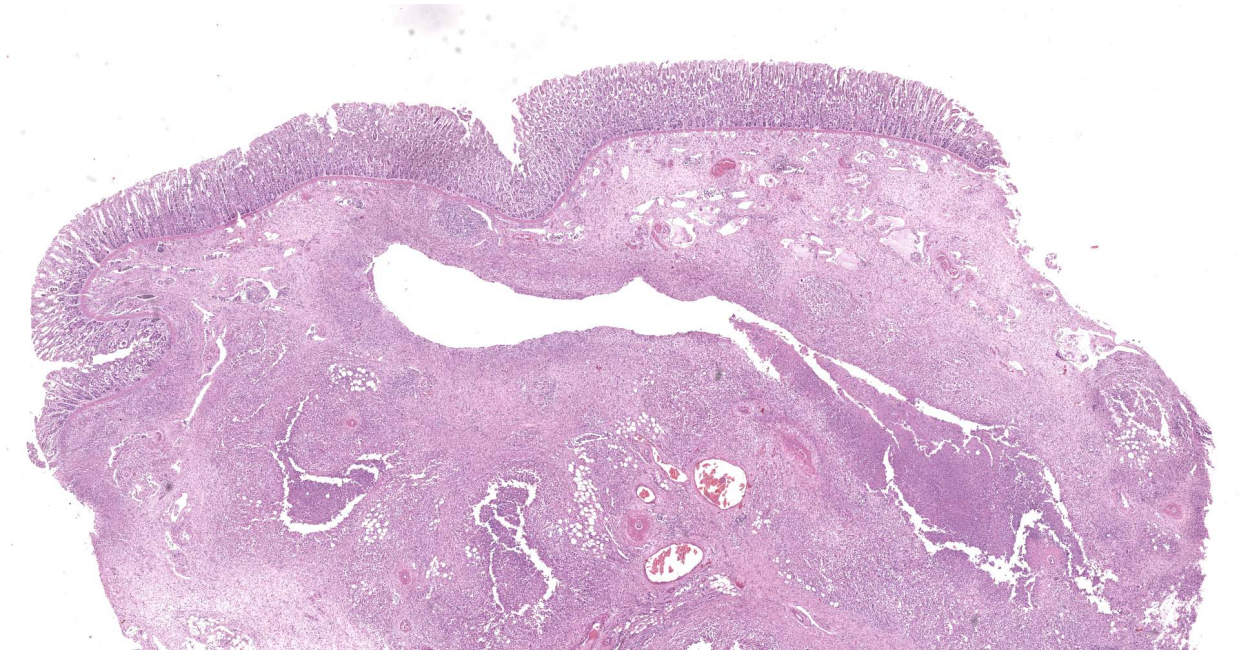
Mesenteric lymph node (per contributor): diffusely, nodal architecture is completely effaced and replaced by abundant eosinophilic cellular material and nuclear debris admixed with numerous degenerated and viable neutrophils. Surrounding this core are moderate numbers of epithelioid macrophages, occasional multinucleated giant cells (Langerhans type) and fewer lymphocytes and plasma cells. Rare macrophages and multinucleated giant cells contain intracytoplasmic bacteria (coccobacilli). Scattered within the cortical area and extending to the adjacent capsule, there are moderate numbers of fibroblasts along with collagen fibers (fibrosis). Rarely, in the cortical region, small aggregates of remaining lymphoid follicles are compressed (not present in all slides).

Tissues not submitted:
Lung present multiple pyogranulomas similar to those described in the intestine.

Contributor's morphologic diagnosis:
Large colon: Colitis, pyogranulomatous, multifocal to coalescing, transmural, marked, with fibrosis, thrombosis, lymphangiectasia and numerous intrahistiocytic coccobacilli

Mesenteric lymph node (per contributor): Lymphadenitis, pyogranulomatous, diffuse, marked, with rare intrahistiocytic coccobacilli

Contributor's comment:
The gross and histologic lesions in the lung, lymph nodes and intestine were typical of lesions

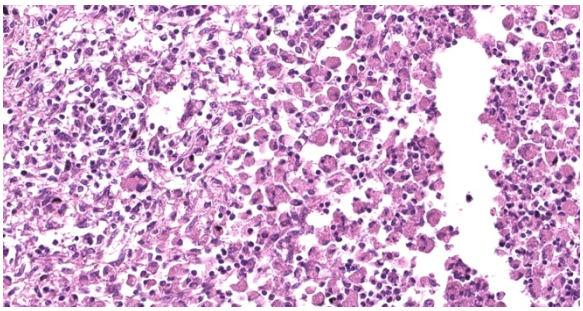


Colon, foal. Within the edematous submucosa, there are multiple large pyogranulomas with necrotic centers (and some dropout), as well as fibrinocellular thrombi within dilated lymphatics. (HE, 15X)

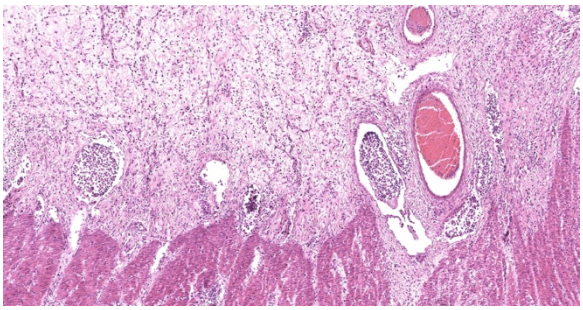
caused by *Rhodococcus equi*. The confirmation of Gram-positive coccobacilli within the cytoplasm of macrophages corroborates the presumptive diagnosis. Laboratory results (molecular analysis) are still pending. Nevertheless, *R. equi* colonies were not observed in the first attempt to isolate this bacterium from lung and intestine samples.

R. equi is a facultative, intracellular, Gram-positive bacterium that preferentially infects macrophages.[5] Virulent (and avirulent) isolates of this cosmopolitan pathogen may be found in the soil, air, equine feces and water, mainly in horse breeding farms. The disease occurs most commonly in foals up to 6 months of age and horses older than 1 year are rarely affected. When mature horses are affected, there is usually an accompanying immunodeficiency.[1] There are sporadic reports of the disease in other species, including cattle, goats, pigs, dogs, cats, and humans. *R. equi* is an important cause of pneumonia in HIV-infected or otherwise seriously immunocompromised humans.[5]

This pathogen has a pathogenicity island, likely acquired through horizontal gene transfer from a bacterial source of unknown origin, critical for virulence of the bacterium for foals. The 26 coding sequences of this pathogenicity island include the virulence-associated protein (Vap family), which are exclusive to *R. equi*. There are 6 full-length *vap* genes (vap A, -C, -D, -E, -G, -H) and 3-truncated *vap* pseudogenes (*vap*F, -I and –X). *Vap*A is the *vap* gene with demonstrated role in virulence and encodes an immunodominant temperature inducible and surface-expressed protein. *Vaps* C, D, E, F, G, I and X appear to be dispensable. *Vap*A is required for intracellular growth in macrophages and for the establishment of a persistent infection in severely immunodeficient mice. In macrophages, it aids in preventing maturation of the phagosome to the stage of fusion of *R. equi* containing vacuoles with lysosomes.[3]



Colon, foal. At the edge of a pyogranuloma, there are numerous macrophages and fewer multinucleated giant cells whose cytoplasm contains numerous 1-2um coccobacilli. (HE, 400X)



Colon, foal. Throughout the submucosa (here) and serosa, lymphatics are dilated and contain non-occlusive fibrinocellular thrombi. (HE, 86X)

Early signs of the usual chronic and progressive pulmonary form of the disease are fever (up to 41ºC), lethargy, increased respiratory rate with bronchovesicular sounds over large airways and wheezing over small airways, cough and, sometimes, bilateral nasal discharge.[1,3,4] As the lung lesions progress, foals present increases in respiratory rate and depth, and movement becomes increasingly distressful, leading to tachypnea, tachycardia and flared nostrils. Severe diarrhea might occur because of colonic mucosal invasion by the agent[4] or secondarily to antibiotic treatment.[1,3] Polysynovitis can occur in 40% or more of affected foals and result in no more than mild pain and decreased range of motion. Foals with septic polyarthritis are quite lame, as observed in this case. The most commonly affected sites include the tarsocrural, carpal, and fetlock joints.[6]

Intestinal lesions caused by *R. equi* are characterized by ulcerative pyogranulomatous enterocolitis. In this case, ulcers were evidenced grossly but were not present in the submitted slides, which had mainly multifocal pyogranulomas throughout the layers of the large colon. Intestinal infection seems to occur after the penetration of the specialized epithelium over Peyer's patches or intestinal lymphoid follicles by coughed up and swallowed bacteria. An initial neutrophilic response occurs leading to erosions of the epithelium (ulcers) with accumulation of macrophages and neutrophils in the lamina propria. The macrophages contain aggregates of *R. equi* but do not destroy them. Later necrosis of lymphoid follicles occurs with replacement by granulomatous inflammation, abscess formation and necrotic material. Infection then spreads to mesenteric lymph nodes with a similar result. Pyogranulomatous lymphangitis and mesenteric lymphadenitis characterize the chronic enteric disease.[8]

Classic form of the disease is pyogranulomatous bronchopneumonia, however, there are many extrapulmonary manifestations of *R. equi* infection. Abdominal manifestations of the disease include mesenteric lymphadenopathy, ulcerative pyogranulomatous enterotyphlocolitis, peritonitis, splenic granulomas or abscesses and large intra-abdominal abscesses. Nonseptic polysynovitis characterized by synovial effusion without lameness has been identified for a multitude of foals with *R. equi* pneumonia. Septic arthritis and osteomyelitis also have been reported, both with or without signs of pneumonia. Other less commonly reported EPD's include septic pleuritis, uveitis and hypopyon, pyogranulomatous hepatitis, intracranial abscesses, cellulitis and subcutaneous abscesses.[6]

Contributing Institution:
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JPC diagnosis:

1. Colon: Colitis, pyogranulomatous and necrotizing, chronic, multifocal to coalescing, severe, with pyogranulomatous lymphangitis and edema, and numerous intrahistiocytic coccobacilli.
2. Lymph node (per contributor): Lymphadenitis, pyogranulomatous and necrotizing, diffuse, severe, with numerous intrahistiocytic coccobacilli.

JPC comment:
The contributor provides a concise summary of *Rhodococcus equi*. First described in 1923 as the causative agent of "purulent bronchopneumonic disease in foals" by H. Magnusson in Sweden, it is currently one of 57 species within the *Rhodococcus* genus. Interestingly, only two species are currently considered pathogenic: *R. equi*, a significant pathogen in domestic species, and *R. fascians*, the causative agent of leafy gall in plants.[9]

As the contributor noted, the virulence associated proteins (*vap*A specifically) within the *R. equi* plasmid confer the ability to survive and replicate in macrophages. The full mechanism has not yet been discovered, but there is evidence that the bacterium shelters in an endosome within macrophages, called the *R. equi*-containing vacuole (RCV), the membrane of which has localized VapA.[9]

Separate from discussion of the *vap* plasmid of *R. equi*, the core genome also confers traits that allow for survival and pathogenicity in animals. Genes on the core genome help protect against desiccation and oxidative stress, and encode β-lactamases, aminoglycoside phosphotransferases, and multidrug efflux pumps. These genes augment this bacterium's ability to survive in the environment and resist antibiotic therapy and is highly conserved across strains.[9]

A recent description of six cases of *R. equi* in goats further characterized disease in this species. VapN was previously described in cattle samples, and all goats carried *vap*N positive strains, using PCR on all samples. An additional avirulent VapN-/VapA- sample was found in one animal. VapB is more common in pig isolates and was not found in these samples. The VapN plasmid is linear and is necessary for *R. equi*'s ability to replicate within macrophages.[7]

Previous reports of *R. equi* in cats has failed to fully characterize the plasmid profile of isolates. A recent study of 200 cats in Brazil found approximately 3.5% had a single pathogen infection with *R. equi*, and approximately 4% had coinfection with *R. equi* and *E. coli*. Interestingly, none of the *R. equi* isolates contained the plasmids VapA, VapB, or VapN, and were considered avirulent. While this further characterizes *R. equi* in cats, further research is warranted to determine the public health impact this may represent for immunocompromised or immunosuppressed populations.[2]

In the moderator's experience, this case varied from the typical presentation of colitis arising from *R. equi*, with the mucosa largely intact in these sections. Fibrin thrombi were noted by some conference participants but were not evident in all sections. Approximately 50% of foals with *R. equi* pneumonia will also have pyogranulomatous enterotyphlocolitis, pyogranulomatous lymphadenitis, intra-abdominal abscesses, or peritonitis. The typical portal of entry is respiratory, but then expectorate is swallowed and enters the gastrointestinal tract.

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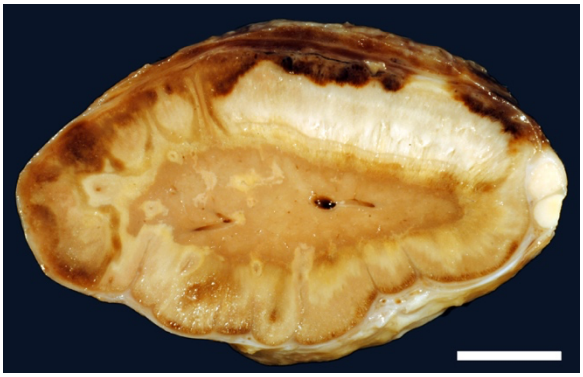
CASE 3: S 106/18 (4119806-00)

Signalment:
Eight month old, intact, male Hanoverian horse (*Equus ferus caballus*)

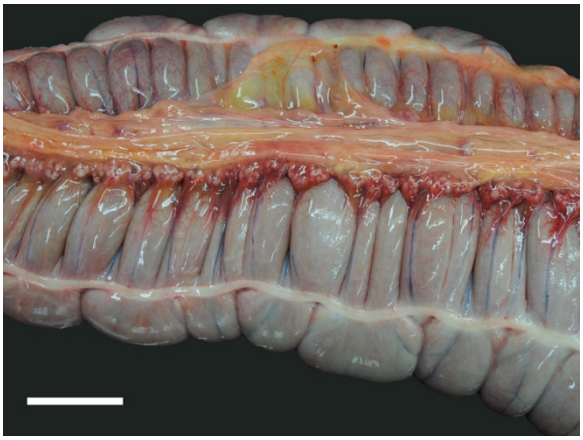
History:
The horse originally came from Poland. The colt was diagnosed with pneumonia due to suspected infection with *Rhodococcus equi* and received azithromycin, tulathromycin and rifampicin as antibiotic treatment. Shortly after treatment the animal presented in the equine clinic with signs of colitis including fever, profuse diarrhea, dehydration and severe hypoproteinemia, which was nonresponding to any kind of treatment. The colt received intensive medical care including plasma transfusions, constant infusion therapy (e.g. glucose, sodium bicarbonate, Ringer's solution), probiotic treatment. A fecal culture detected *Klebsiella pneumoniae* and *Clostridium difficile*. The colt was euthanized shortly afterwards due to therapeutic failure and with pure prognosis.

Gross Pathology:
The young stallion was in a very poor nutritional condition with signs of early serous atrophy of the cardiac, coronary, and the bone marrow fat. Severe subcutaneous edema affected the head, the ventral aspect of the neck and the ventral abdomen.

Two focal abscesses were detectable in the lung, which measured about 0.5 cm and 0.8 cm in size



Adrenal gland, foal. The adrenal gland had multifocal to coalescing areas of pallor (necrosis) and hemorrhage. (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)



Colon, foal. The colonic wall is edematous, and there are ecchymoses in the mesocolon. (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

respectively. In addition, there was severe acute diffuse alveolar edema and severe acute diffuse congestion of the lung.
In the abdominal cavity there was moderate serofibrinous ascites (6 liters).

The wall of the stomach was multifocal markedly thickened (1.8 cm) by a predominantly submucosal edema. The mucosa itself was hyperemic but revealed no signs of ulceration or erosion.

A moderate number of adult nematodes, morphologically consistent with *Strongylus* spp., was detected in the small intestine.
The large intestine was filled with green fluid with only little, finely structured particles. The intestinal wall was diffusely thickened and edematous (max. 1.8 cm) and there were multifocal mucosal erosions. In the mesocolon multifocal petechial and ecchymatous hemorrhage was present. The regional lymphatic tissue was moderately hyperplastic.
The adrenal glands had multifocal to coalescing areas of pale (necrosis) and reddish (hemorrhage) discoloration.

Laboratory results:
Microbiology:

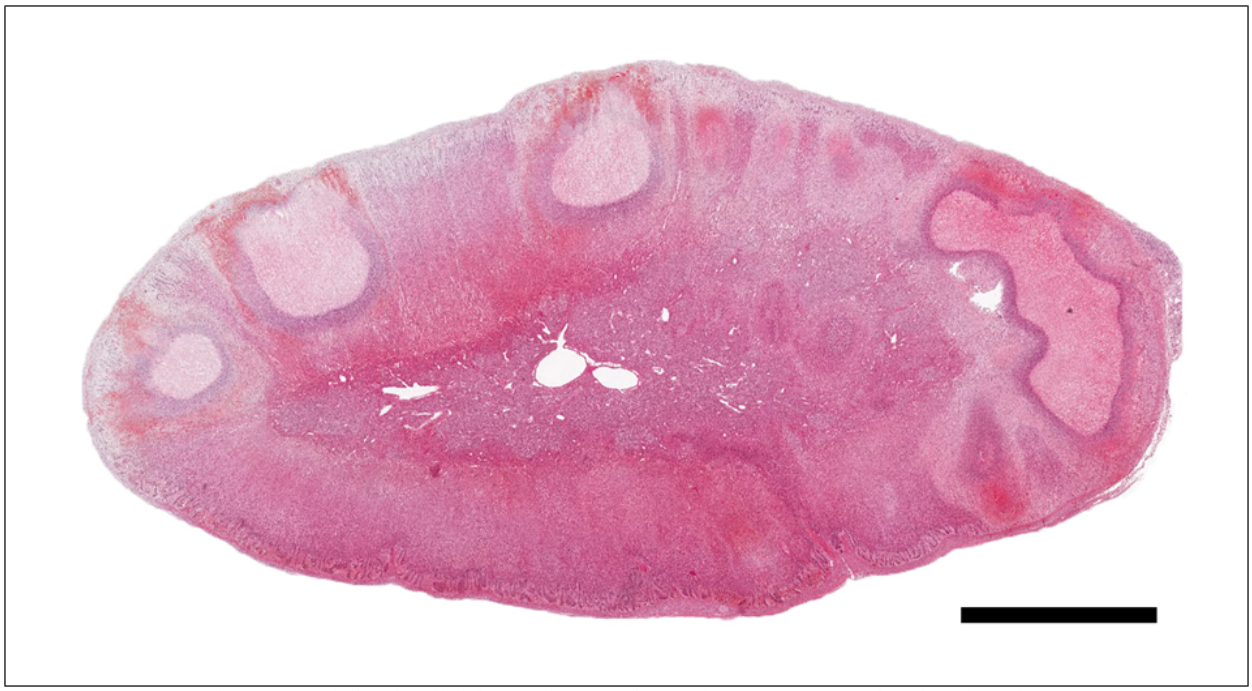
Tissue	Bacteria	
Abscess, lung	*Klebsiella pneumoniae*	+ + +
	Clostridium perfringens	+ + +
	Enterobacter cloacae	+ +
	Clostridium sordellii	+
Large intestine	*Klebsiella pneumoniae*	+ + +
	Escherichia coli	+ + +
	Escherichia fergusonii	+ + +
Instestinal lymphnode	*Clostridium perfringens*	+ +
	Klebsiella pneumoniae	+
	Escherichia coli	+
	Escherichia fergusonii	+

Microscopic description:
Adrenal gland: Within the adrenal cortex there are multifocal widespread areas with loss of cellular detail and differential staining (coagulation necrosis) surrounded by a rim of high numbers of degenerated neutrophils. In neighbored areas there are widespread accumulations of extravasated erythrocytes (hemorrhage) as well as multifocal small vessels occluded by homogeneous, eosinophilic material (fibrin thrombi). The adrenal medulla was unchanged.

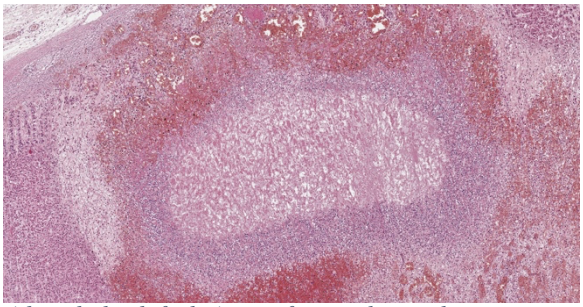
Contributor's morphologic diagnosis:
Adrenal gland: Cortical necrosis, marked, multifocal to coalescent, acute with hemorrhage, moderate, multifocal, acute

Contributor's comment:
Acute, severe adrenal hemorrhage is also termed adrenal apoplexy. It is a well- known condition in human and veterinary medicine and often associated with bacterial infections.[4] The most frequent clinical condition associated with infectious adrenal hemorrhage in human patients is an endotoxin-mediated septic shock.[3] Under the specific circumstances of adrenal hemorrhage in association with sepsis the condition is also named Waterhouse-Friderichsen Syndrome.[8] The most common and first described pathogens leading to Waterhouse-Friderichsen Syndrome in children were meningococci, but other bacteria such as *Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Klebsiella* spp. and others have been identified as possible agents.[3]

Especially in the adult horse, adrenal hemorrhage is often associated with acute, severe gastrointestinal diseases.[4] In this specific case of antibiotic-induced colitis, *Klebsiella pneumoniae* as well as *Clostridium difficile* were detected in different organs including the large intestine and intestinal lymph nodes. Within the adrenal gland, no bacterial structures were detectable using the Gram and the Giemsa stains, which supports the



Adrenal gland, foal. There are multifocal well-delineated areas of coagulative necrosis within the adrenal cortex. (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html) (HE, 1X)



Adrenal gland, foal. Areas of cortical coagulative necrosis (infarction) are surrounded by a dense band of cellular debris and hemorrhage. (HE, 51X)

hypothesis of endotoxin-induced adrenal hemorrhage.

In humans the arteries supplying the adrenal gland are branched 50-60 times. These smaller arteries open into relatively straight capillaries which are only passing through the zona fasciculata and abruptly form a vascular plexus around the zona reticularis.[2] This plexus seems predisposed to vascular damage, and the pattern of necrosis and the localization of the fibrin thrombi make such a pathogenesis probable, even in the present case.

Another predisposing factor, mentioned by several authors, is the hyperactivity of the adrenal cortex, particularly the increased synthesis of corticosteroids or at least an increased level of adrenocorticotropic hormone (ACTH) seems to raise the susceptibility of the adrenal cortex to endotoxin-induced hemorrhage.[3,8]

Contributing Institution:
Department of Veterinary Pathology
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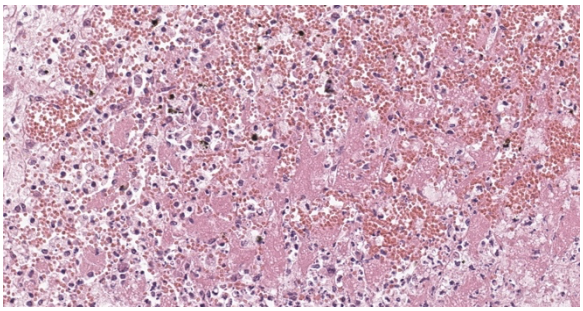
http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html

JPC diagnosis:
Adrenal gland, cortex: Necrosis, coagulative and lytic, multifocal to coalescing, with venous thrombosis and hemorrhage.

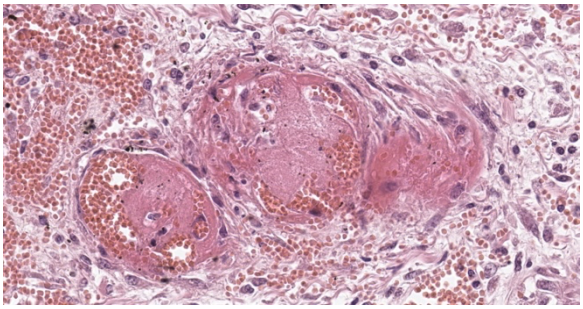
JPC comment:
This condition was described in calves more than 40 years ago, most often as sequela from bacterial sepsis. In these cases, there was multifocal hemorrhage and necrosis in the cortex, specifically in the zona glomerulosa and the inner parts of the zona fasciculata. In most cases, fibrin thrombi and platelet aggregates were observed in capillaries and sinusoid veins of the zona glomerulosa and the zona fasciculata. In areas of necrosis, large, individualized globules (10-20 μm) of fibrin were observed between cortical cells.[5]

In humans, this condition occurs most frequently in children, though this lesion is seen in approximately 1% of routine autopsies.[7] This syndrome has also been reported in nonhuman primates, including a female gang-housed baboon[1], as well as in a cynomolgus monkey with staphylococcal meningitis.[9] A recent investigation of systemic inflammatory response syndrome (SIRS) in pig tailed macaques (*Macaca nemestrina*) and yellow baboons (*Papio cynocephalus*) found bilateral adrenal hemorrhage (Waterhouse-Friderich syndrome) was a common sequela, in addition to multi-organ dysfunction (MOD) or failure, acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (DIC).[6]

The adrenal lesion presented here may be frequent in humans but is not a common diagnosis in horses. This case bears similarities with human cases but may have a slightly different pathogenesis. This syndrome was observed in nonhuman primates as a sequela to septicemia and did not develop during the initial insult. The fibrin thrombi observed in this case appear to be in veins, with no arteries in section occluded. While this appears to largely be the result of arteriolar compromise and ischemia, it is not evident in this section. With respect to the



Adrenal gland, foal. Smaller areas of necrosis contain individualized adrenocortical cells with granular eosinophilic cytoplasm and loss of nuclei admixed with hemorrhage. (HE, 380X)



Adrenal gland, foal. Fibrin thrombi are seen within vessels and sinuses throughout the gland (HE, 400X)

laboratory test results, the pattern of necrosis is considered less likely to be the result of *C. difficile* but may be consistent with the detected *Klebsiella pneumoniae*.

Accessory adrenal cortical tissue is an incidental finding seen in a large proportion of horses and does not play a role in the pathogenesis of this disease.

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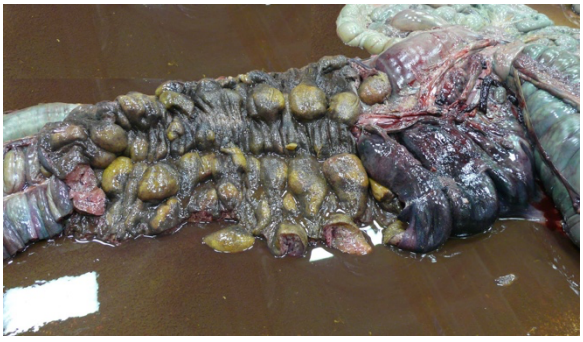
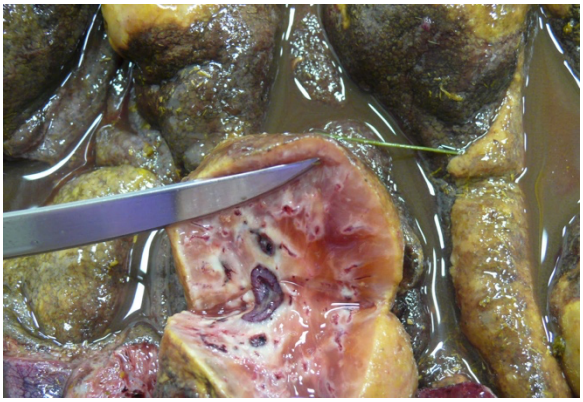
CASE 4: N13-1267 (4048726-00)

Signalment:
1 year old male Arabian horse (*Equus caballus*)

History:
This colt presented with a 24 hour history of watery diarrhea, fever, and colic with worsening signs as time went on despite Banamine therapy. The colt arrived down and thrashing within the trailer, with large amounts of diarrhea present. Physical exam showed severe dehydration, purple mucous membranes, CRT >3 seconds, heart rate 90 bpm, and no gastrointestinal sounds. This colt was sedated, an IV catheter was placed, and he was anesthetized with Valium for transportation into the veterinary medical teaching hospital. A quick scan of the abdomen showed very large, stacked loops of non-motile small intestine. Humane euthanasia was elected due to worsening signs and poor prognosis. There was a postmortem interval of 2 days before necropsy was performed.



Right ventral colon, horse. The right ventral colon is markedly thickened and the serosa is diffusely red-purple to black. The mucosa is thrown into markedly thickened folds and is necrotic. (Photo courtesy of: Department of Pathobiological Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive West, Madison, WI 53706, http://www.vetmed.wisc.edu/)



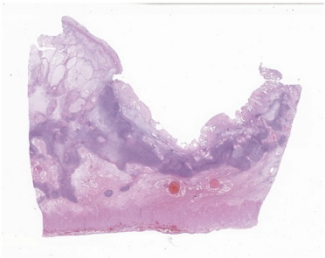
Right ventral colon, horse. The incised necrotic mucosa has cystic spaces scattered throughout. (Photo courtesy of: Department of Pathobiological Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive West, Madison, WI 53706, http://www.vetmed.wisc.edu/)

Gross Pathology:
There is approximately 2 liters of non-clotting serosanguineous fluid in the abdomen. The right ventral colon is markedly thickened, and the serosa is diffusely red-purple to black. The mucosa of the right ventral colon is thrown into markedly thickened and edematous, polypoid to nodular folds each measuring approximately 5 cm x 3.5 cm x 4 cm and are multifocally black mottled white (necrotic). The left ventral colon is also severely edematous (approximately 1 cm thick). The left ventral colon and cecum contain small numbers of tapeworms (*Anoplocephala perfoliata*) and approximately 10-15 proglottids. The serosa of the cecal apex is diffusely red-purple but is unremarkable on cut section. The colonic lymph nodes are moderately enlarged. There are two ulcers in the stomach located at the Margo plicatus. One is circular (approximately 5 cm x 3 cm) while the other is linear (approximately 9 cm x 0.5 cm).

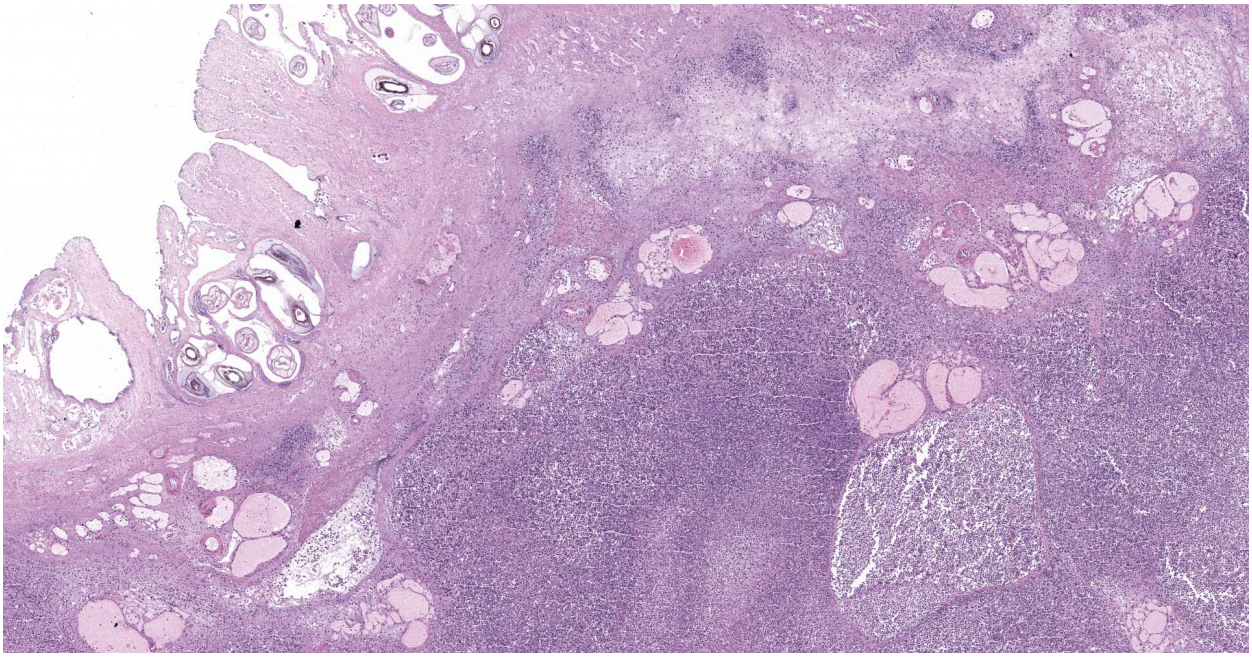
Laboratory results:
N/A

Microscopic description:
Right ventral colon: The submucosa is markedly thickened (up to 1.8 cm) by clear space (edema), abundant extracellular eosinophilic fibrillar material (fibrin), and multifocal areas of eosinophilic cellular and karyorrhectic debris (necrosis). Many macrophages intermixed with and surrounding large nodules to vast coalescing sheets of numerous of degenerate and non-degenerate neutrophils densely infiltrate the submucosa. The submucosal and tunica muscularis blood vessels are largely intact and congested, with a few scattered blood vessels containing partially occlusive fibrin thrombi. Often the thrombosed blood vessel walls segmentally contain brightly eosinophilic smudgy material with a loss of distinct wall layers (fibrinoid vascular necrosis). Incidentally, the tunica intima of muscular arteries often contains multiple irregular mineralized nodules that protrude into the lumen and are lined by endothelial cells (intimal bodies). There are multifocal foci of moderate amounts of hemorrhage, necrotic debris and inflammatory cells within the serosa. There is infiltration by numerous short and long bacilli bacteria, predominately in the mucosa but also scattered throughout the submucosa, tunica media, and serosa.

The overlying mucosa is multifocally elevated into wide polypoid folds. Diffusely the surface epithelium and multifocally the muscularis mucosa are effaced by necrotic material, with a few scattered remaining glands sometimes outlined by deeply basophilic granular material (mineralization). The mucosa is also diffusely infiltrated by numerous cavitated spaces containing 1-4 cross and longitudinal sections of larval nematode parasites. These larvae are ~200 um in diameter and up to 1.5 mm in length, have a thin to thick eosinophilic cuticle, platymyarian musculature, vacuolated lateral cords, and a large diameter intestine composed of a few multinucleated cells that occasionally contains brown iron pigment and are lined by a brush border. Rarely a muscular esophagus and part of



Colon, horse. A single section of markedly thickened colon is submitted for examination. (HE, 5X)



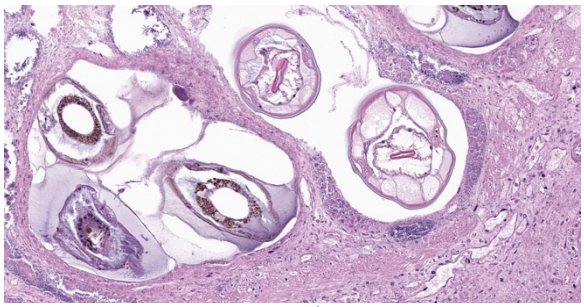
Colon, horse. The submucosa is markedly expanded by edema and Peyer's patches are effaced by necrotic neutrophils and cellular debris. Tangential and cross-sections of larval nematodes are present within the overlying autolytic mucosa. (HE, 35X)

a cuticularized buccal capsule are visible in longitudinal sections.

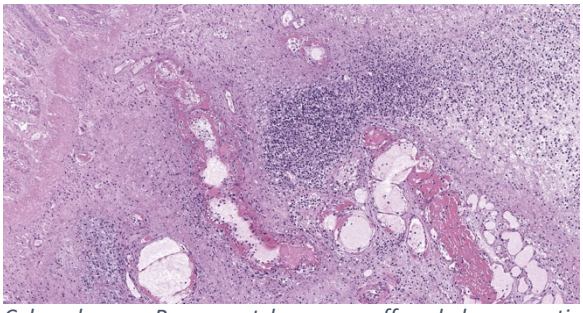
Similar, though less severe changes were found in the left ventral colon and the cecum (tissues not submitted). The colonic lymph node (tissue not submitted) showed moderate numbers of lymphoid follicles in the cortex and extending down into the medulla, as well as numerous degenerate and non-degenerate neutrophils and many macrophages occasionally exhibiting erythrophagocytosis expanding the subcapsular and medullary sinuses.

Contributor's morphologic diagnosis:
Right ventral colon: Severe necrosuppurative fibrinous colitis with submucosal edema, vascular thrombosis, and numerous intramucosal larval strongyles.

Contributor's comment:
The small strongyles of horses, also known as cyathostomins, are considered to be one of the most important pathogenic parasites in horses today. Over 40 species of cyasthostomins parasitize the cecum and colon of horses and individual hosts often are infected by 15 to 20 species at once during times of disease.[1] Clinical disease is often negligible in cases with a few worms; however, when there is infection by large numbers of hypobiotic larvae significant disease sometimes leading to death can occur as a result of mass emergence.[1,2] Emergence of larvae causes rupture of the muscularis mucosa and intense inflammation and edema.[2] This clinical syndrome is most commonly observed during late fall, winter, or early spring.[1] Clinical signs can be variable, but most commonly consist of persistent diarrhea, decreased levels of performance, ill thrift, edema, pyrexia, weight loss (sometimes to the point of emaciation), and colic.[1,6] There is often a marked hypoalbuminemia with a neutrophilia, and hyperglobulinemia.[1,4] Necropsy findings show inflammation of colon and/or cecum with mucosal hyperemia, hemorrhage,



Colon, horse. Cross sections of 4th stage larval cyathostomes have a thick cuticle, polymyarian-coelomyarian musculature, large lateral cords, and a uninucleate intestine which often contains blood pigment within epithelium. (HE 268X)



Colon horse. Peyer patches are effaced by necrotic neutrophils and cellular debris, and there is submucosal vasculitis and thrombosis. (HE, 110X)

ulceration, or necrosis in the acute stages. More chronic cases sometimes only have mucosal thickening due to edema, and irregular areas of congestion.[4] On close examination, there may be numerous mucosal nodules often only a few millimeters in diameter, which are raised red to black and can be umbilicated.[2] The nodules within the cecum and colon within the submitted horse are much larger than commonly reported with cyathostomins, likely due to the intense inflammation and edema.

Cyathostomins have a direct life cycle. Eggs containing embryos in the morula stage of development are shed within the feces of an infected animal. Within the feces the egg hatches into the 1st larval stage (L1). The 1st and 2nd larval stages (L1 & L2) stay in the feces feeding on bacteria. The 3rd larval stage (L3), the infective stage, migrates from the feces into the soil and is ingested by a host. Next, larva migrate and develop in the deep mucosa or submucosa of large intestines (mainly cecum and ventral colon). L3 larvae can remain within the intestinal wall for periods ranging from about 4 months to as long as 2 years.[4] Finally, they emerge to the lumen to develop into adults. Adults are mainly found in the dorsal and ventral colon and tend to cause few clinical problems.[1] New eggs may be passed in the feces onto the pasture within 5-6 weeks.[4]

There is debate about the significance of increased resistance to anthelmintics causing increased cases of cyathostominosis. Currently, there is widespread resistance to benzimidazoles, and to a lesser extent pyrantel, as well as an emerging resistance to macrocyclic lactones.[6] Anthelmintic treatment and specific husbandry were not reported in this case.

In the sections examined, there are large numbers of short and long bacilli bacteria that infiltrate the mucosa and submucosa. Given the extensive damage and inflammation within the intestinal wall, these may be opportunistic gastrointestinal flora causing a secondary bacterial infection or they may represent postmortem overgrowth given the 2 day postmortem interval.

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University of Wisconsin-Madison
School of Veterinary Medicine
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http://www.vetmed.wisc.edu

JPC diagnosis:

1. Colon: Colitis, necrosuppurative, multifocal to coalescing, with Peyer's patch necrosis, necrotizing vasculitis, fibrin thrombi, and severe submucosal edema.
2. Colon, mucosa: Cyathostome larvae, numerous.
3. Mesenteric and serosal arteries: Intimal bodies, multiple.

JPC comment:
The contributor provides a good review of cyathostomins in the horse. In addition to the horse, different species within the Cyathostominae subfamily parasitize the large intestine of elephants, pigs, marsupials, and turtles. While large strongyles may migrate beyond the mucosa of the cecum and colon, cyathostomin do not.[3] A heavy cyathostomin larval burden, along with tapeworms, has been associated with cecocolic intussusception.[5]

As stated by the contributor, a number of anthelminthics are no longer effective against cyathostomin, such as phenothiazine, thiabendazole, cambendazole, mebendazole, fenbendazole, oxfendazole, and febantel. There are rare reports of treatment success by combining anthelminthic treatment with corticosteroids to target inflammation.[3]

Intimal bodies occur primarily in the small arteries and arterioles of horses, most often in the brain, placenta, and submucosa of the intestine. All age horses are affected and arise from degeneration and mineralization of subendothelial smooth muscle cells and intercellular material. They are considered a background lesion and have no functional significance.[7]

Small cyathostomes rarely cause this severity of pathology, and there are extensive areas of inflammation not centered on larvae. We speculate that a component of the colitis may be due to pathogenic bacteria, such as *Clostridium* spp or *Salmonella* spp. The reported clinical history of colic and diarrhea may support an acute insult on top of the cyathostome burden. A culture of the gastrointestinal contents may have provided additional information in this case. This horse was also administered flunixin meglumine (Banamine) non-steroidal anti-inflammatory therapy. This section of tissue is from the right ventral colon, but NSAID injuries may present in locations other than the right dorsal colon.

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