**Joint Pathology Center**
**Veterinary Pathology Services**

# WEDNESDAY SLIDE CONFERENCE 2023-2024

## Conference #20 — 14 February 2024

**CASE I:**

**Signalment:**
12-year-old, female spayed Schipperke dog (*Canis familiaris*)

**History:**
A 12-year-old, female spayed Schipperke dog presented with multisystemic disease including severe enteropathy, hepatopathy, pneumonia, and dermatitis. The patient had been on long-term budesonide, prednisone, and dexamethasone for inflammatory bowel disease.

**Gross Pathology:**
Gross examination revealed severe ulceration of the mucosal surface of the colon, with abundant necrotic and fibrinous debris overlying the sites of mucosal injury. The medial/internal aspect of the right ear pinna was crusted with regionally extensive ulceration and mild hemorrhage. The skin, sclera, gingiva, and subcutaneous fat were mildly icteric. The liver was friable and homogenously pale tan and moderately enlarged, with slightly rounded margins. All liver lobes had disseminated pale tan foci ranging from 3-5 mm in diameter. The spleen was mildly enlarged with a meaty consistency. The lungs were diffusely mottled pink to purple, with similar disseminated pale tan foci as the liver. The bone marrow (left femur) was diffusely reddened.

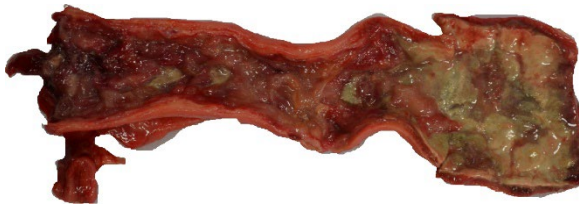

**Figure 1-1. Colon, dog. There is diffuse ulceration of the colonic mucosa with abundant adherent necrotic debris. (*Photo courtesy of:* Veterinary Diagnostic Laboratories, Colorado State University, Fort Collins, CO, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx)**

**Laboratory Results:**
*Neospora caninum* PCR: DNA detected in colon samples.
*Toxoplasma gondii* PCR: DNA not detected in colon.
*Leishmania* spp. PCR: DNA not detected in liver samples.

**Microscopic Description:**
Colon: There is marked multifocal to coalescing ulceration of the colonic mucosa. Overlying the injury are thick mats of necrotic cellular debris, fibrin, large numbers of histiocytes and degenerate neutrophils, entrapped mixed bacteria, and low amounts of hemorrhage. Inflammation, necrosis, and edema extend deep throughout the submucosa and muscularis, disrupting the mural architecture. Throughout areas of inflammation and within muscle fibers of the muscularis are myriads of unicellular tachyzoite parasitic organisms. Tachyzoites are 2-3 µm long by 1-2 µm wide, almond

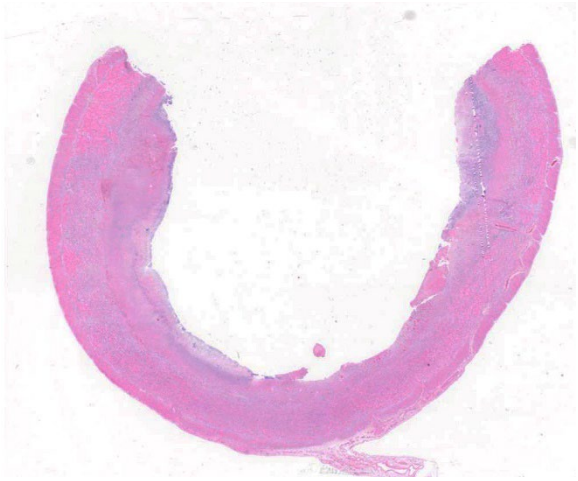

**Figure 1-2. Colon, dog. There is diffuse ulceration of the mucosa and multifocal transmural necrosis of the wall. (HE, 5X)**

shaped, with a round basophilic nucleus, and perinuclear clearing extending to both tapered ends. Rarely there are encapsulated clustered of tachyzoites (cysts). These organisms are also present within epithelial cells and endothelial cells.

Protozoal tachyzoites and cysts stain positive for Giemsa and negative for GMS and Gram stain.

**Contributor's Morphologic Diagnosis:**
Colon: Severe, multifocal to coalescing, necroulcerative colitis with myriad intralesional protozoa.

**Contributor's Comment:**
In addition to the lesions in the colon, tachyzoites were identified in the liver, spleen, and skin, and fewer numbers were identified in the bone marrow and lungs. There was variable necrotizing to histiocytic inflammation in each affected tissue. The morphology of the tachyzoites, along with the detection of *Neospora caninum* by PCR, are diagnostic of an unusual case of disseminated neosporosis. Differentials considered included *Toxoplasma gondii* and *Leishmania* spp. Toxoplasmosis cannot be distinguished from neosporosis histologically. Molecular diagnostics such as PCR have been instrumental in the diagnosis and differentiation of the two diseases. Leishmaniasis typically can be distinguished from the apicomplexan parasites due to the presence of the kinetoplast within amastigotes, but this is not always readily identifiable in standard H&E preparations.

*Neospora caninum* is an apicomplexan parasitic organism with a heteroxenous life cycle, for which canids are the definitive host.[4,7,8] In canids, disease is uncommon but typically presents as neuromuscular disease.[8,12,13] In puppies (<6 months) the typical clinical presentation is an ascending, progressive paralysis with rigidity and muscle atrophy, mostly of the pelvic limbs.[3,4,5,12] Adult dogs may experience recrudescence of latent infections, with multifocal CNS signs and polymyositis. The gross findings may include embolic foci of necrosis and hemorrhage in the brain and spinal cord and white streaking, corresponding to necrosis, in the muscles. Tachyzoites can be found free in the tissues or within histiocytes, endothelial cells, neurons, and epithelial cells, and are usually accompanied by marked necrosis and granulomatous inflammation.[1,2,5,6,12]

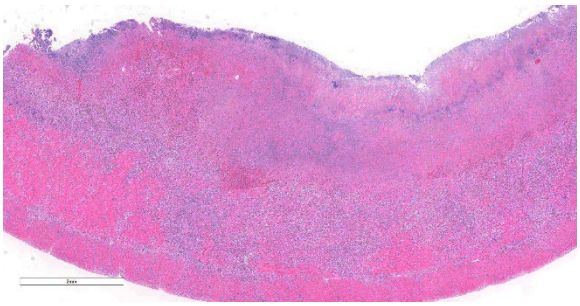

**Figure 1-3. Colon, dog. There is transmural necrosis of the wall. (HE, 15X)**

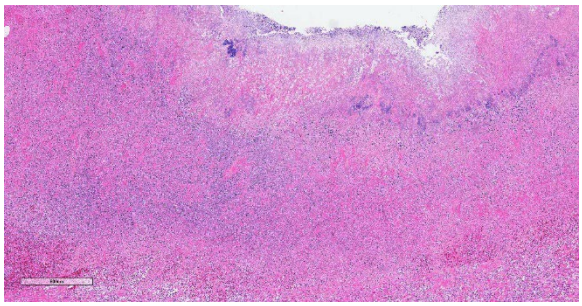

**Figure 1-4. Colon, dog. Higher magnification of the transmural necrosis. (HE, 25X)**

Cases of myocarditis, pneumonia, dermatitis, and dissemination are less common and have typically been associated with immunosuppression including steroid or chemotherapeutic administration.[4,9,10,11] In this case, the patient was on immunosuppressive doses of budesonide, prednisone, and dexamethasone for presumptive inflammatory bowel disease. Additionally, the patient had a history of coprophagia as well as regular exposure to cattle. Cattle are the most common intermediate host, for which mid-term abortion (at about 5-6 months gestation) is the most common clinical presentation.[3,4,5,7,8,] The absence of gross lesions is common in cattle; however, if present, the pathognomonic lesion in aborted fetuses is multifocal foci of necrosis in brain tissue.[5,6,14]

**Contributing Institution:**
Veterinary Diagnostic Laboratories
Colorado State University
Fort Collins, CO
http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx

**JPC Diagnosis:**
Colon: Colitis, necrotizing, multifocal to coalescing, transmural, with vasculitis, thrombosis, and numerous intramyocytic, intraendothelial, and free zoites.

**JPC Comment:**
*Neospora caninum* shares many histologic and biologic features with the closely related apicomplexan parasite *Toxoplasma gondii*; however, there are key differences between the two related to host range, virulence factors, and pathogenesis.[4] *N. caninum* follows a life cycle in which canids, including domestic and wild dogs, coyotes, wolves, and dingoes, serve as definitive hosts where sexual replication occurs, and a variety of intermediate hosts provide the ecological niche for asexual replication. As the contributor notes, the most common intermediate host is cattle, and it appears that the *N. caninum* life cycle is maintained globally through the close association between domestic dogs and cattle farms.[4] Evidence also exists for a sylvatic cycle, involving wild canids and ruminant species in North America, with a particular high seroprevalence noted among North American white-tailed deer.[4]

The *N. caninum* life cycle involves three main life stages: sporozoites within sporulated oocysts, rapidly dividing tachyzoites, and slowly dividing bradyzoites that are sequestered from the host immune system within tissue cysts.[4] The oocyst is the environmentally hearty form of the parasite and is formed by sexual replication in the intestinal epithelial cells of canids and subsequently expelled in their feces. The oocysts then sporulate within 24-72 hours in the environment and develop two sporocysts, each of which contains four sporozoites.[4] Intermediate hosts become infected by ingesting sporulated oocysts, which then release their sporozoites in the gastrointestinal tract. Sporozoites then infect intestinal epithelial cells, where they transform into tachyzoites that infect a variety of nucleated cells, including mononuclear cells that can traffic them throughout the body, and then replicate within

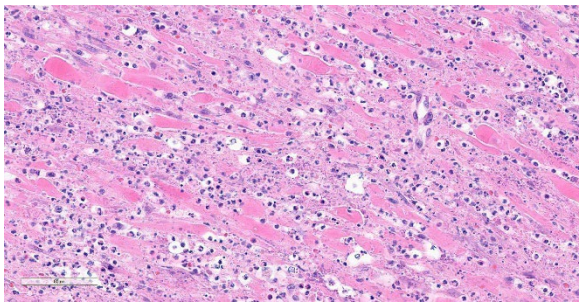

**Figure 1-5. Colon, dog. There is extensive necrosis within the smooth muscle of the inner longitudinal layer. Some fibers are shrunken and hypereosinophilic (atrophy) (HE, 300X)**

parasitophorous vacuoles within the host cell cytoplasm.[4]

During acute infection, tachyzoites may be found in almost all tissues of the body and repeated cycles of replication, host cell lysis, tachyzoite release, and infection of surrounding cells produce characteristic lesions and clinical signs. After approximately 20 cycles of replication, tachyzoites differentiate into bradyzoites within tissue cysts under pressure from the host immune response, and a quiescent, asymptomatic infection develops that can last for the life of the host.[4] This long-term persistent infection may recrudesce with changes in the host's immune status, such as pregnancy, immunodeficiency, or, as in this case, immunosuppressive therapy.[4]

Transmission of *N. caninum*, which can be vertical or horizontal, has been extensively studied due to the economic losses caused by abortions in infected cattle herds.[3,4] Vertical transmission is the predominant form of transmission in cattle, and can occur following ingestion of sporulated oocysts from the environment (exogenous transplacental transmission) or following recrudescence of infection of a persistently infected cow during pregnancy (endogenous transplacental transmission).[4] Endogenous transplacental transmission is the main mechanism by which *N. caninum* is maintained in the domestic cattle population, with fetal transmission rates as high as 95%.[4] Persistently infected cattle have an abortion risk between 1.7-7.4 times the risk of a naïve cow, though the risk diminishes with successive pregnancies, suggesting that some degree of host immunity sufficient to frustrate endogenous transplacental transmission develops over time.[4]

As illustrated by this case, a robust host immune response is critical to controlling *N. caninum* infection. Studies in cattle demonstrate that a Th1-type response is essential for restricting parasite replication and the conversion of tachyzoites to bradyzoites in tissue cysts.[4] Conversely, host immunosuppression tips the scales toward a Th2-type response, causing recrudescence characterized by the conversion of bradyzoites to tachyzoites and uncontrolled tachyzoite proliferation.[4]

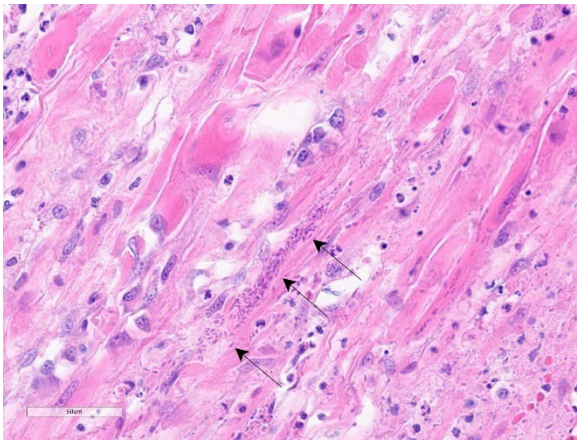

**Figure 1-6. Colon, dog. Attempts at regeneration of damaged smooth muscle are evidenced by large, often pleomorphic nuclei within smooth muscle cells. Leiomyocytes occasionally contain a cytoplasmic cyst containing numerous zoites (arrows). (HE, 400X)**

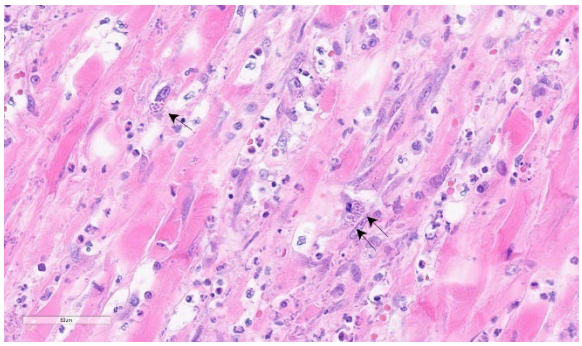

**Figure 1-7. Colon, dog. Another field in which smooth muscle cells contain cytoplasmic apicomplexan cysts. (HE, 400X)**

The role of humoral immunity is less clear, but it likely controls *N. caninum* infection by antibody neutralization of extracellular tachyzoites.[4]

This week's moderator was Dr. Francisco Uzal, Professor in the Department of Pathology, Microbiology and Immunology at the University of California Davis School of Veterinary Medicine and the California Animal Health and Food Safety Laboratory. Discussion of this case initially centered on the terms "ulceration" and "erosion," and whether either was appropriate to describe the mucosa in this case. Conference participants opined that the difference between the two was a matter of depth, with some participants using "ulceration" to indicate that the mucosal epithelium is lost, whereas others define the term to mean a lesion that extends deep to the muscularis mucosa. The term "erosion," for both camps, is reserved for more superficial mucosal loss, especially in areas in which the mucosa is composed of multiple layers of cells, such as the esophagus.

Participants next discussed the significant vasculitis observed throughout the section and whether the vasculitis was the cause or an effect of the massive, transmural necrosis present throughout the section. Participants felt that, while organisms were found in many endothelial cells, the vasculature was largely caught up in the inflammatory and necrotic milieu produced by *N. caninum*. Participants also noted the clinical history of inflammatory bowel disease and wondered if pathology associated with that condition was confounding histologic interpretation.

Discussion of the morphologic diagnosis was relatively straightforward in this case, though participants felt they would not have been able to determine whether the section was from the large or small intestine without the contributor's tissue identification. Participants also felt that the vasculitis and the tissue distribution of the organisms were notable histologic features and were thus included in the morphologic diagnosis.

**References:**


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12. Reichel MP, Ellis JT, Dubey JP. Neosporosis and hammondiosis in dogs. *J Small Anim Pract*. 2007;48(6):308-312.
13. Ruehlmann D, Podell M, Oglesbee M, Dubey JP. Canine neosporosis: a case report and literature review. *J Am Anim Hosp Assoc*. 1995;31(2):174-183.
14. Schlafer DH, Miller RB. Female genital system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 3. 2007; Elsevier Saunders:514–516.

**CASE II**:

**Signalment:**
Three month old female Quarter Horse (*Equus caballus*)

**History:**
A 3-month-old Quarter Horse filly presented to the Iowa State University Equine Internal Medicine Service for diarrhea of two weeks duration. The foal had been treated with omeprazole and sucralfate and several courses of trimethoprim sulfa and ceftiofur with no resolution of clinical signs.

**Gross Pathology:**
There were severe multifocal to coalescing, 0.1 cm to 5 cm pale nodules in all lung lobes. Some nodules were cream-colored with a fri-

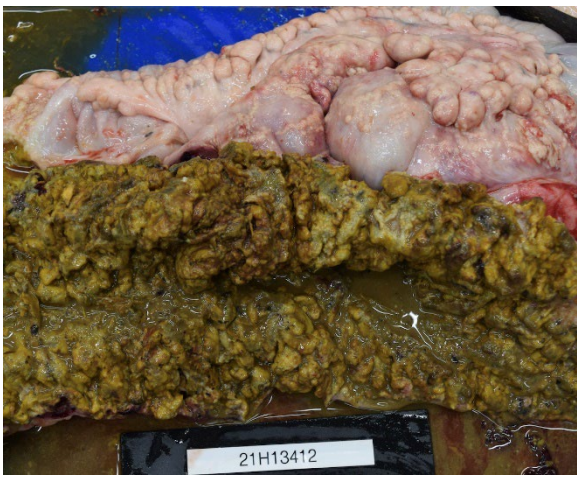


**Figure 2-1. Colon, horse. Throughout the large colon and cecum, there are multifocal to coalescing ulcerations of the mucosa. On the serosal surface, mostly along the mesentery, there are numerous multifocal to coalescing nodules containing caseous yellow material. (*Photo courtesy of*: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, https://vetmed.iastate.edu/vpath)**

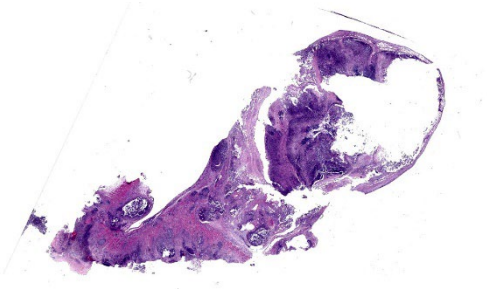

**Figure 2-2. Colon and lymph node, horse. A section of colon and adjacent lymph node is submitted. The mucosa and submucosa are effaced by abundant pyogranulomatous inflammation that multifocally extends into the underlying muscularis and serosa. The lymph node is effaced by the same inflammatory infiltrate. (HE, 5X)**

able consistency and were surrounded by a firm, white capsule. Others had a pale yellow liquid exudate exuding from the center of the nodule. The jejunum had multifocal, up to 0.5 cm in diameter ulcerations. Throughout the large colon and cecum, there were multifocal to coalescing ulcerations of the mucosa, which were covered by thick, yellow to dark green, friable material. On the serosal surface, mostly along the mesentery, there were numerous multifocal to coalescing nodules containing caseous yellow exudate. These nodules had a thick white capsule and ranged in size from 1 mm to 5 cm. The peritoneal cavity contained yellow, cloudy fluid.

**Laboratory Results:**

A fecal sample was submitted to UC Davis for foal gastrointestinal and diarrhea panel testing (*Clostridium difficile* toxins A and B, Equine coronavirus, *Lawsonia intracellularis*, *Salmonella* spp, *Cryptosporidium* spp, equine rotavirus, *Rhodococcus equi*, *Clostridium perfringens* antigen and toxins CPA, CPB, CPB2, netF, and CPE). The sample was positive for *Rhodococcus equi* and *Rhodococcus* VapA gene was confirmed by PCR.

**Microscopic Description:**

Colon, cecum, and mesenteric lymph node: Elevating the ulcerated mucosa, expanding the submucosa and muscularis with extension to the serosa and the nearby lymph node are multifocal to coalescing aggregates of hypereosinophilic coagulum surrounded by numerous degenerate neutrophils admixed with plump foamy macrophages, epithelioid macrophages and multinucleated giant cells that frequently contain clusters of basophilic 1 to 3 um coccobacilli (pyogranulomatous inflammation with liquefactive necrosis). The mucosa is partially or completely devoid of epithelium and is replaced by fibrinonecrotic debris and similar inflammatory cells surrounded by proliferated fibrous connective tissue. Multifocally, blood vessels are occluded by numerous degenerate inflammatory cells, foamy macrophages with intracytoplasmic coccobacilli, and hyalinized fibrinous meshes. Within the mesenteric lymph node, there is a complete loss of nodal architecture and the cortex and medulla are replaced by pyogranulomas.

Histochemical staining: The intrahistiocytic coccobacilli are highlighted by Gram stain (in blue color) and Fite's stain.

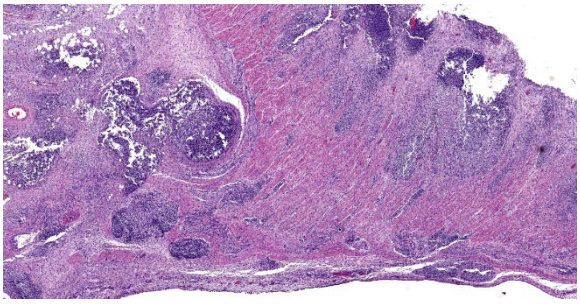

**Figure 2-3. Colon, horse. Higher magnification of pyogranulomatous inflammation within the colonic wall. (HE, 20X)**

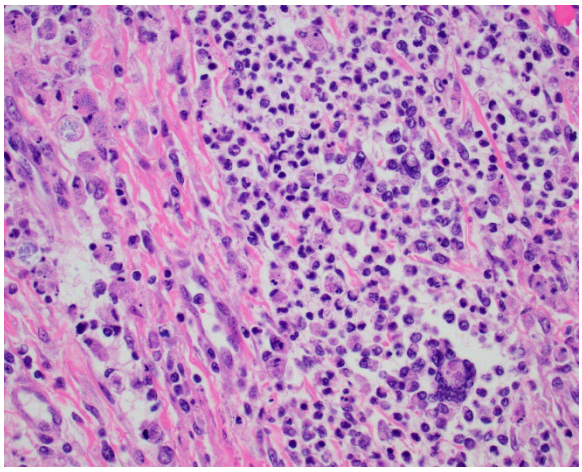

**Figure 2-4. Colon, horse. Pyogranulomas are composed of numerous variably degenerate neutrophils admixed with plump foamy macrophages, epithelioid macrophages, and multinucleated giant cells that frequently contain clusters of basophilic, 1 to 3 um coccobacilli. (HE, 400X) (*Photo courtesy of*: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University)**

**Contributor's Morphologic Diagnoses:**

1. Colon and cecum: Severe, multifocal to coalescing, chronic, pyogranulomatous, fibrinonecrotic typhlocolitis, with numerous intrahistiocytic coccobacilli.
2. Lymph nodes: Severe, multifocal to coalescing, chronic, pyogranulomatous lymphadenitis with numerous intrahistiocytic coccobacilli.

**Contributor's Comment:**

*Rhodococcus equi* is a gram-positive, facultative intracellular bacterium commonly found in soil and the gastrointestinal tract of herbivores.[6] The pathogen primarily causes diseases in foals and also affects cattle, sheep and goats, pigs, dogs, llamas, and immunocompromised humans.[1,3,6] Infections of *Rhodococcus equi* usually occur in foals between 1 and 6 months of age and usually present as pyogranulomatous bronchopneumonia and ulcerative enterocolitis.[6]

In horses, inhalation of the bacterium from the environment and swallowing of mucus-containing *Rhodococcus equi* from the airway are the major routes of infection.[3,6] *R. equi* is phagocytosed by macrophages and dendritic cells through the mannose-binding receptor, and the uptake of the bacteria is enhanced by complement and complement receptor 3.[9] With plasmid pathogenicity island (PAI)-encoded virulence-associated protein A (VapA) gene and positive regulators (orf4 and orf8), *Rhodococcus equi* can survive and replicate in macrophage phagosomes through inhibition of phagosome acidification and phagosome-lysosome fusion.[6,7,9] Hematogenous dissemination within macrophages to other sites in the body then occurs. Horizontally-acquired chromosomal virulence factors, such as a polysaccharide capsule, mycolic acid, lecithinase, phospholipase C, and cholesterol oxidase also play key roles in the development of systemic pyogranulomatous inflammation.[6,7,9] In goats and pigs, *R. equi* with VapN and VapB plasmids, respectively, are considered the virulent strains that cause systemic pyogranulomatous inflammation.[7] *R. equi* without the aforementioned Vap genes are classified as avirulent.[4]

In foals, multifocal to coalescing nodules with caseous centers usually develop in the cranioventral lung, but all lung lobes may be affected in severe cases.[6] Volcano ulcers covered by necrotic debris are seen over Peyer's patches in the small intestine, cecum, and large colon.[6] The nearby lymph nodes are enlarged with variably-sized abscesses. The histologic features of *R. equi* infection include suppurative to pyogranulomatous bronchopneumonia with numerous neutrophils, macrophages, and multinucleated giant cells. In the intestine, infiltration of macrophages and neutrophils in the lymphoid follicles and caseous necrosis that

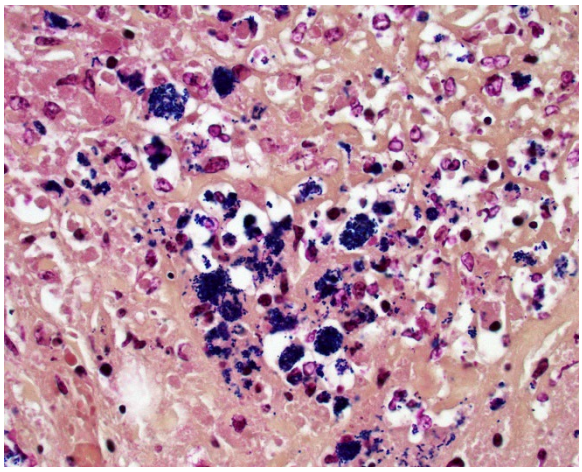

**Figure 2-5. Colon, horse. A Gram stain highlights bacteria within macrophages. (Gram, 400X) (*Photo courtesy of*: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University)**

extends to the covering epithelium are characteristic features. With time, the mucosa is covered with thick fibrinonecrotic debris. Some foals can also develop polyarthritis, osteomyelitis, and abscessation in multiple internal organs.[6,7] Infrequently, abortion caused by *R. equi* has been described in horses, with fetal lesions comparable to those in foals.[5] With special histochemical stains, *R. equi* is gram-positive and weakly acid-fast.

Differential diagnoses for systemic pyogranulomatous inflammation in horses include fungal and mycobacterial infections; however, concurrent pyogranulomatous pneumonia and ulcerative enterotyphlocolitis are characteristic of *R. equi*. Bacterial culture with positive detection of the VapA gene in isolated *R. equi* can confirm the diagnosis.

**Contributing Institution:**
Department of Veterinary Pathology
College of Veterinary Medicine
Iowa State University
https://vetmed.iastate.edu/vpath

**JPC Diagnoses:**

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

**JPC Comment:**
As the contributor notes, pathogenic *Rhodococcus equi* strains have the ability to replicate unimpeded within equine macrophage vacuoles.[2] The ability to persist inside these vacuoles is dependent on the rapid and abundant production of virulence-associated protein A (VapA), which is triggered primarily by temperatures above 33ºC, as occurs in mammalian hosts, and secondarily by low environmental pH.[2] Once expressed, VapA localizes to the bacterial membrane where it interacts with or is released into the vacuolar environment.[2,8]

VapA promotes the intracellular survival of *R. equi* by increasing phagosome and lysosome pH. Reserachers were initially surprised to find lysosomal acidification affected by VapA since *R. equi* multiply, and VapA is produced, within special phagocytic vacuoles that are distinct from host cell lysosomes; however, further research demonstrated that VapA is transferred from the phagocytic vacuole to lysosomes early in infection, possibly by vesicular transport.[8] In both the phagocytic vacuole and the lysosome, secreted VapA raises the pH of the compartment, leading to increased proliferation of *R. equi* and inactivation of acid hydrolases that would normally kill and digest the organisms.[2]

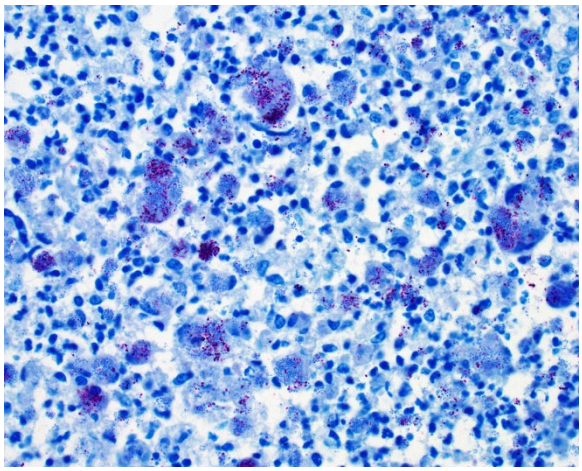

**Figure 2-6. Colon, horse. Coccobacilli are acid-fast, as demonstrated by a Fite-Faraco stain. (Fite-Faraco, 400X)(*Photo courtesy of*: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University)**

VapA raises the pH of intracellular compartments via two main mechanisms, the first of which is the exclusion of the proton-pumping ATPase (vATPase) from the phagosome membrane.[8] In normal cellular metabolism, lysosomal and phagosomal membranes are enriched with hundreds of vATPase complexes which acidify the lysosome by pumping hydrogen ions from the cytosol into the lysosome. In phagosomes and lysosome containing VapA, however, the plasma membranes have minimal, background levels of vATPase complexes, and this vATPase exclusion continues with prolonged infection times, preventing rapid acidification of lysosomes.[8] The exact mechanism of the vATPase exclusion remains unknown.

VapA also raises the pH of lysosomes and phagosomes by permeabilizing their lipid membranes, further dissipating the acidic proton gradient.[2,8] Studies have demonstrated that this permeabilization *in vivo* is sufficient to allow the release of protons or protonated water, but not complete enough to lead to lysis of the phagosomal or lysosomal membranes.[2] The end result of these two mechanisms is to create "leaky," semipermeable intracytoplasmic niches of relatively neutral pH where *R. equi* can thrive.

The intracellular thriving of *R. equi* is well illustrated in this slide, with scores of coccobacilli packed within macrophages and free throughout the examined tissue. The tremendous tissue damage wrought by *R. equi* once again made tissue identification difficult for conference participants, though most were able to narrow the tissue to either cecum or colon. Some participants remarked on prominent smooth muscle in the wall of many vessels in section; however, the moderator noted that this, along with occasional mineralization, is a common, incidental feature of equine vasculature.

The moderator noted that VapA production is necessary for pathogenesis and that *R. equi* strains without the plasmid-encoded gene are clinically inert. While an etiologic diagnosis of *R. equi* infection may technically require PCR for the VapA gene, the moderator noted that *R. equi* has a distinctive look (pyogranulomas, volcano ulcers) and distribution (lungs and colon) that make the diagnosis fairly straightforward if you have good old-fashioned horse sense. This is particularly true when, as in this case, the tremendous tissue destruction provides strong gross and histologic circumstantial evidence of pathogenicity.

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**CASE III**:

**Signalment:**
5-year-old female Holstein (*Bos taurus*)

**History:**
This adult cow was admitted to the clinic with sudden onset of weakness, abdominal pain, and bloody feces. In the preliminary report, an embryo wash was performed three weeks prior to submission. A suspected diagnosis of hemorrhagic bowel syndrome was made. The animal underwent abdominal surgery (manual blood clot dissolution) and symptomatic treatment; however, the cow spontaneously died the following night.

**Gross Pathology:**
The distal jejunum was distended and filled with coagulated blood. Multifocal intramural hematomas (approximately 3 x 2 x 1 cm) as well as blood coagula adherent to the intestinal mucosa (approximately 40 cm length, on average) were present in the affected segment.

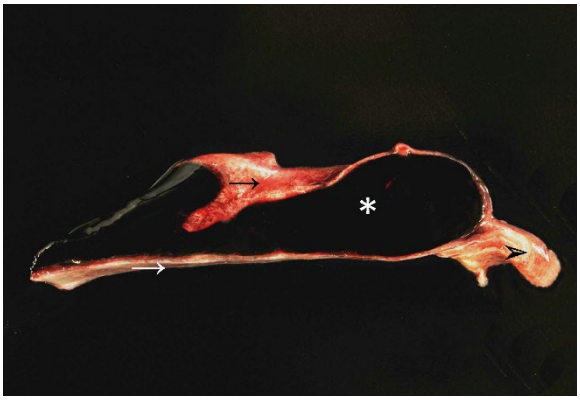

**Figure 3-1. Jejunum, ox. The distal jejunum is filled with clotted blood. (*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany, http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie)**

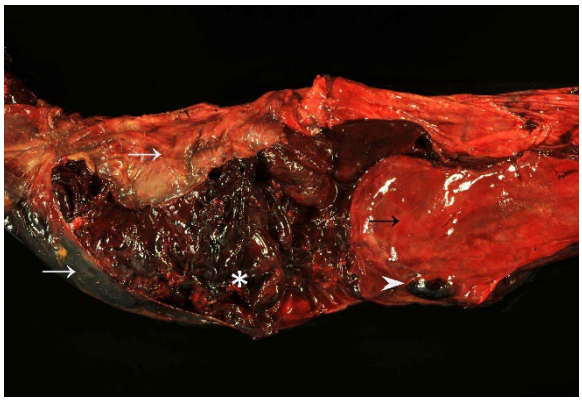

**Figure 3-2. Jejunum, ox. Multifocal intramural hematomas, as well as blood coagula adherent to the intestinal mucosa, are present in the affected segment. (*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany)**

Orally to the described lesion, the jejunal mucosa was diffusely bright red with multifocal dark red areas and multiple, up to 1 mm in diameter large, submucosal hematomas. Intestinal contents in the cecum and colon were dark-red, viscous, and admixed with large amounts of coagulated blood, whereas the rectum was empty. Additionally, there were multifocal petechial hemorrhages on the serosal surfaces of internal organs. A small amount of serous, red fluid was found in the greater omentum. Moderate pharyngeal edema was present. Additionally, the liver was diffusely soft and beige-yellow with mildly rounded edges. Moreover, the nose and lungs showed diffuse, moderate hyperemia and the lung also a mild, diffuse, alveolar edema.

**Laboratory Results:**

*Salmonella* spp. were not isolated from a small intestine sample. Bovine viral diarrhea virus and bovine herpesvirus-1 were not isolated in cell culture. PCR for Bluetongue virus and bovine herpesvirus-1 were negative as well. The test for the proteinase K-resistant prion protein was negative.

Immunohistochemistry on smooth muscle actin using the avidin-biotin peroxidase technique revealed a splitting of the lamina muscularis mucosae with accumulation of blood between the muscle layers.

**Microscopic Description:**

Approximately 90% of the tissue section shows a separation of the mucosa from the submucosa due to extensive submucosal hematoma formation. The overlying mucosal layer displays moderate necrosis but also autolysis with bright eosinophilic cellular debris and multifocal dilated villus lacteals. Furthermore, the mucosal layer is focally eroded. Also moderate numbers of rod-shaped bacteria can be observed on the luminal surface as well as mild fibrin formation in between the separated muscle layers.

**Contributor's Morphologic Diagnosis:**

Jejunum: Segmental mucosal necrosis and intramural hematoma formation, bovine.

**Contributor's Comment:**

Hemorrhagic bowel syndrome (HBS), first described in the early 90s, is a sporadic, often fatal disorder characterized by acute extensive segmental jejunal hemorrhage and intramural hematoma formation predominantly affecting adult dairy cattle.[2,3] A significant proportion of the affected animals are of Brown Swiss breed.[2]

Among other clinical symptoms, notable blackberry jelly-like blood clots within feces, decreased milk production, tachycardia and/or palor of mucous membranes are commonly reported.[3] Post-mortem findings usually include one or more short jejunal segments with severe intramural hemorrhage/hematoma for-

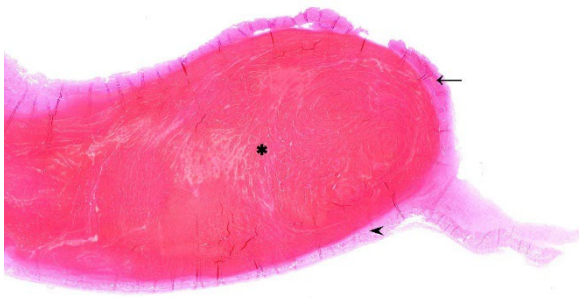

**Figure 3-3. Jejunum, ox. There is dissecting hemorrhage within the wall of the jejunum. (HE, 4X)(*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany)**

mation, which may completely or partially obstruct the intestinal lumen.[5] These findings were also observed in this case. Intraluminal blood clots were mostly observed at the areas of ruptured mucosa. The histologic findings in this case are consistent with previously described lesions.[1,2] A mild to moderate vasculitis, which has been described in some cases, was not observed in the presented case.[1,2,5]

The etiology and pathogenesis of this disorder is currently unknown. Several infectious factors, including the contribution of *Clostridium perfringens* or *Aspergillus fumigatus,* have been suspected, but no statistically significant relations have been detected so far.[5] Thus, the observed bacteria are more likely secondary to the progressing disease and autolysis.[5] Due to dilated villus lacteals and the moderate to severe submucosal vasculitis observed in other reported cases, it has been hypothesized that an initial vasculopathy or abnormal lymphatic function may be responsible for the prominent dissecting hemorrhages, but these theories have not been confirmed.[1]

Recent findings indicate that hemorrhages appear to originate within the lamina muscularis mucosa (Lmm) and lead to the eruption of the mucosa with severe intraluminal bleeding and blood clot formation within the jejunum.[2] The splitting of the Lmm potentially leads to the rupture of the many arterioles that course through the Lmm and supply blood from the submucosa to the subepithelial capillary beds.[2] This process is further accelerated by the arterial blood pressure, which results in a "zipper-mechanism" characterized by expanding, dissecting hemorrhages.[2]

In the present case, an immunohistochemical stain for α-smooth muscle actin (SMA) was performed which identified SMA-positive fragments of Lmm beneath the mucosa adjacent to the hemorrhage as well as on the contralateral side framing the tunica muscularis. Furthermore, immunohistochemical cytokeratin staining visualized the intestinal epithelium. Overall, the described findings correspond to previously reported cases and support the assumption that the fragmentation of the Lmm, which surrounds the hematoma, is a consequence of the intramuscular hematoma formation.[2] The cause of the spontaneous detachment of the Lmm layers remains unclear. Mucosal necrosis is considered as a change secondary to the resulting ischemia rather than a primary inflammatory entity such as described in hemorrhagic enteritis.[2]

Shock gut syndrome and hemorrhagic enteritis should be considered as differential diagnoses. The "shock gut" syndrome is most common in dogs and results in decreased perfusion of the intestine. It is morphologically characterized by congestion of intestinal mucosa, as well as reddish intestinal content.[5] Hemorrhagic enteritis in adult cattle can be caused by infectious (*Salmonella* spp., coronaviruses) and non-infectious factors (arsenic,

**Figure 3-4. Jejunum, ox. A smooth muscle actin stain demonstrates the elevation of the mucosa off of the underlying submucosa and smooth muscle layers. (anti-SMA, 4X)(*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany)**

oak, oleander poisoning) and manifests as reddish intestinal content, congested mucosa and diarrhea.[5]

Cattle affected by HBS often show acute onset of poor general condition, clotted blood in the feces, and sudden death.[1] The treatment can be symptomatic or surgical; however, in either case the prognosis is rather poor with most animals dying or being euthanised.[5]

**Contributing Institution:**
Department of Pathology
University of Veterinary Medicine
Hannover, Germany
http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/

**JPC Diagnosis:**
Small intestine: Mural hemorrhage, segmental, acute, diffuse, severe.

**JPC Comment:**
The typical gross and histologic lesions of hemorrhagic bowel syndrome (HBS), also known as jejunal hemorrhage syndrome, have been well described in the veterinary literature and are nicely summarized by the contributor.[1-3] Other common causes of intraluminal intestinal hemorrhage include intussusception, volvulus, salmonellosis, bovine viral diarrhea virus infection, coccidioisis, coagulopathies, and intestinal foreign bodies; however, HBS differs from these differentials by the presence of large intraluminal or intramural blood clots that result in small intestinal obstruction, most commonly in the jejunum.[2,3]

Hematologic parameters of HBS-affected cattle largely reflect the acute, hemorrhagic nature of the disease. There is usually a neutrophilia and hyperglycemia, both attributable to the release of inflammatory cytokines and endogenous, stress-related steroid release.[3] The physical obstruction of the proximal intestine results in sequestration of abomasal secretions, leading to electrolyte abnormalities, most commonly hypochloremia and hypokalemia.[3] Liver enzymes, such as sorbitol dehydrogenase and gamma-glutamyltransferase may be elevated due to gastrointestinal obstruction or stasis and subsequent absorption of bacteria and toxins from the affected area of intestine.[3] Other abnormalities include increased blood urea nitrogen, attributed to intraluminal digestion of blood, hyperlactatemia, and acidemia; inconsistent calcium and magnesium derangements have been reported.[3,4]

The acute nature of the disease and the poor prognosis despite treatment make HBS a source of high economic losses, and efforts are underway to determine the cause of the disease, the existence of any ante-mortem biomarkers, and the most promising treatments for acutely affected animals. Recent efforts have begun mining biosensor data, routinely collected from dairy cows to monitor estrus and milk yields, to determine if any clinically

silent premonitory signs can be identified. The first such report on these efforts found that decreases in rumination time and activity, rumen mobility, rumen temperature, and milk yields preceeded clinically evident signs, offering a potential early alert mechanism to identify and treat dairy cows early in the course of disease.[4]

Conference participants appreciated the unique, striking subgross histologic appearance of this case and discussion focused largely on whether the mucosal changes represent necrosis or autolysis. Participants believed that the changes were largely autolytic due to the lack of visible thrombi in section and the fact that tissue in less-affected, normal areas of the section had a similar histologic appearance. Participants also reviewed a smooth muscle actin immunohistochemical stain which illustrated the dramatically thin rim of muscularis mucosae lining the hematoma and nicely illustrated the splitting of this histologic layer as described in a recently published article characterizing this entity.[2]

The moderator noted that, though HBS cases have become rare recently, his diagnostic lab has historically seen multiple cases of HBS per week. The moderator shared a wealth of gross and histologic HBS images from this historic caseload that delighted conference participants. The moderator challenged the textbook clinical presentation of blood clots in the feces. In his experience, most animals die of intestinal obstruction caused by the intramural hematoma; it is only if the animal survives the acute period that the hematoma ruptures the mucosa and blood appears in the manure.

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## CASE IV:

**Signalment**:
3-week-old female suckling domestic piglet (*Sus scrofa domesticus)*

**History**:
The farm noticed increased morbidity and mortality of approximately 20-30% during the last three weeks. The piglets showed clinical signs of diarrhea, fever, lameness, tremor, and anemia. This animal was euthanized for diagnostic proposes shortly before necropsy.

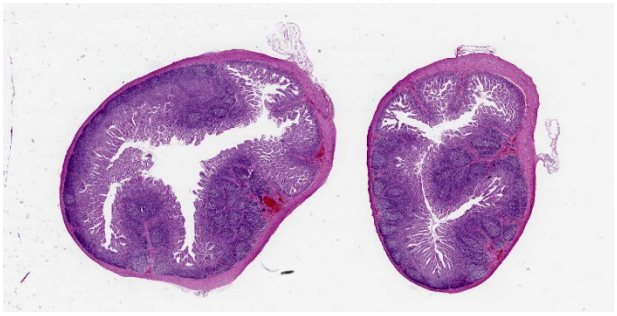

**Figure 4-1. Ileum. Two sections of ileum are submitted for examination. (HE, 5X)**

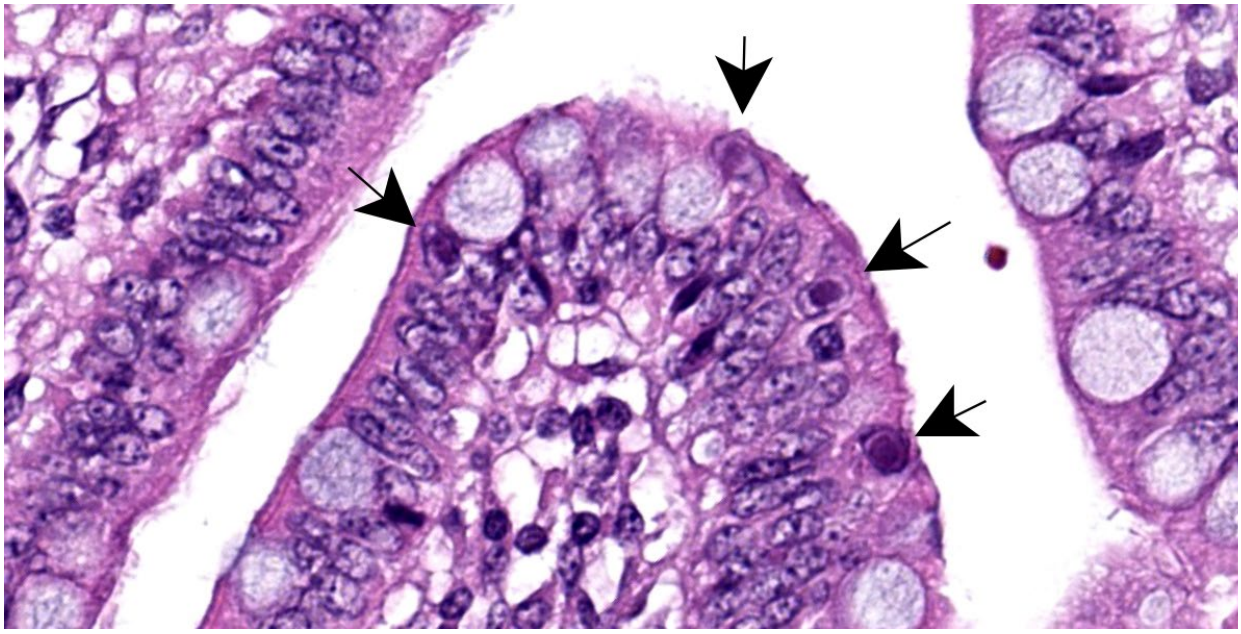

**Figure 4-2. Ileum, piglet. Scattered villar enterocytes have large intranuclear viral inclusions. (HE, 750X)**

**Gross Pathology**:
The animal was in poor nutritional condition. There was a small amount of milk in the stomach. The contents of all intestinal segments were yellow and watery. A high amount of turbid synovial fluid was found in all examined joints. The meninges were diffusely cloudy.

**Laboratory Results:**
Adenoviral particles were detected by transmission electron microscopic examination of the ileal mucosa.

In the bacteriological examination of an elbow joint (synovia) and of the brain meninges a small amount of *Streptococcus suis* was isolated. PCR serotyping identified *S. suis* Serotype 1.

No parasites were identified on fecal flotation.

**Microscopic Description**:
Multifocally, ileal villi are mildly to moderately fused and blunted with maintenance of normal enterocyte architecture. Enterocytes occasionally contain a single intranuclear inclusion body that leads to margination of the chromatin. These inclusion bodies are amphophilic, round to oval shaped, and 5-15 µm in diameter. In the lamina propria, a mild diffuse infiltration with lymphocytes, eosinophils, plasma cells, and granular lymphocytes is present. The vessels show an increased number of erythrocytes (congestion).

**Contributor's Morphologic Diagnosis**:
Mild, subacute, diffuse, lymphoplasmatic and eosinophilic enteritis (ileitis) and mild, multifocal, atrophic enteritis with villus fusion and intranuclear viral inclusion bodies (Adenovirus).

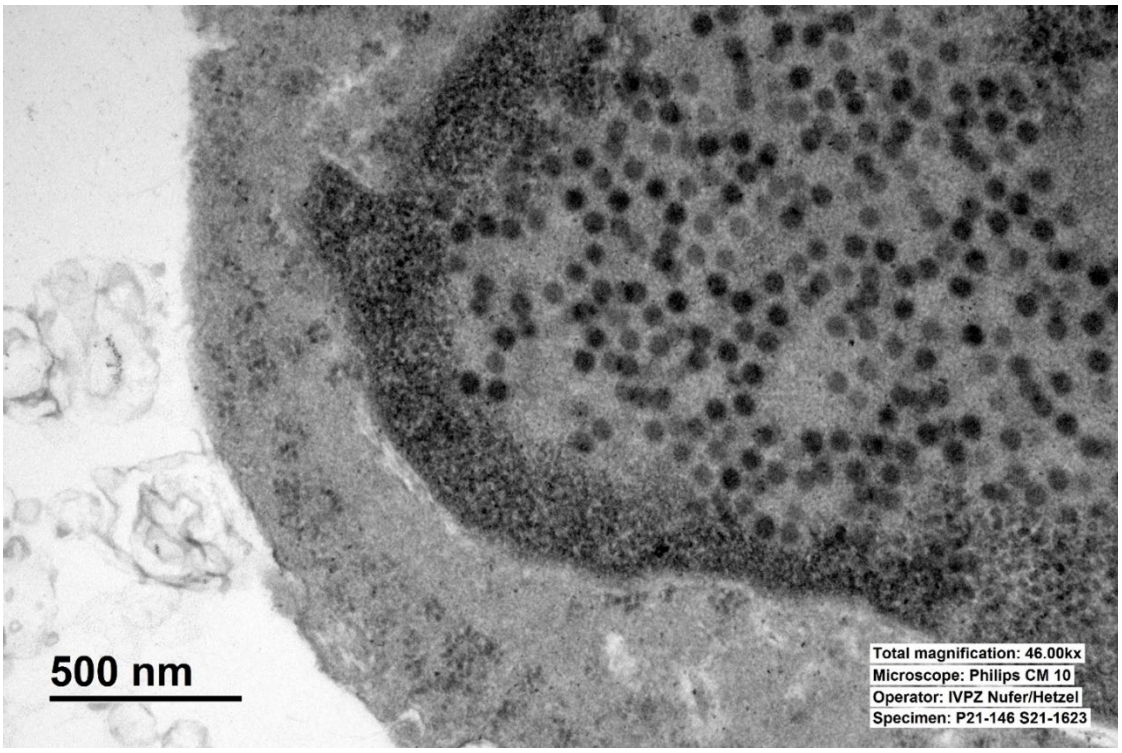


**Figure 4-3. Ileum, piglet. Electron microscopy reveals adenoviral particles in the nucleus of an enterocyte. The particles measure 75–80 nm in diameter. (*Photo courtesy of:* Institute of Veterinary Pathology Zurich (IVPZ), www.vetpathology.uzh.ch)**

**Contributor's Comment**:
Adenoviruses are non-enveloped, double-stranded DNA viruses with icosahedral symmetry. Adenovirus infections occur in a wide variety of animals inducing clinical signs ranging from subclinical to enteric or respiratory. Three species of Porcine adenoviruses (PAdVs), Porcine mastadenovirus A, Porcine mastadenovirus B, and Porcine mastadenovirus C, and five serotypes have been identified by virus neutralization assays.[1,3,7] Adenoviruses are considered host-specific and the pig is the only known species that is susceptible to PAdVs.[1]

Watery to pasty diarrhea, dehydration, and decreased weight gain are the most commonly observed clinical signs in pigs.[1] Cases of diarrhea caused by PAdVs are mostly seen in suckling pigs (1–4 weeks of age), but weaned and fattening pigs can also be affected.[1] Furthermore, PAdVs have been isolated from pigs with respiratory signs, encephalitis, nephritis, and abortions; they have also been isolated from animals without evident clinical history.[1,3-5] Transmission occurs mostly via the fecal-oral route or possibly by aerosol exposure. Mechanical vectors (tools, boots, vehicles, etc.) might have a possible role considering the high stability of the virus in the environment.[1]

Similar to this case, typical histological lesions include villous fusion, blunting, and shortening with intranuclear basophilic inclusion bodies in enterocytes of the distal jejunum and ileum.[1,8] To confirm an infection with PAdVs, the virus can be detected by electron microscopy, cytology from mucosal smears, or by the detection of viral antigens in

tissues by immunofluorescence or immunohistochemistry.[1,6] In general, PAdVs mostly lead to subclinical infections and are not known to have public health significance. Adenoviruses should nevertheless be considered as a differential diagnosis for gastrointestinal and possibly respiratory diseases in pigs.[1]

**Contributing Institution:**
Institute of Veterinary Pathology Zurich
www.vetpathology.uzh.ch

**JPC Diagnosis:**
Small intestine: Enteritis, lymphoplasmacytic, diffuse, mild, with few epithelial intranuclear viral inclusions.

**JPC Comment:**
Adenoviruses, so named due to their discovery in cultures of human adenoids, are notable histologically for their large, often basophilic intranuclear inclusion bodies. These inclusions represent newly assembled virions that form crystalline aggregates within the nucleus.[9] As flashy as these inclusions may be, as the contributor notes, adenoviral infection in pigs is typically asymptomatic, with the main presenting signs being yellow, watery diarrhea of three to four days' duration in 1-4 week old piglets.[2]

Once ingested, porcine adenovirus undergoes intranuclear replication, primarily in the enterocytes and lymphoid tissue located in the distal jejunum and ileum.[2] Viral antigen may be found 24 hours after infection and for up to 45 days post-infection, and infected enterocytes may be destroyed or may lose their villi during the acute phase of infection.[2] Interestingly, virus has been found in the tonsils after infection of enterocytes, raising the possibility of clinically silent viremia with certain isolates.[2] Porcine adenovirus has also rarely been associated with nephritis, raising the possibility of urinary-oral and urinary-nasal transmission.[2]

Gross lesions in cases of adenovirus-induced diarrhea include thinning of the wall of the distal small intestine, enlargement of the mesenteric lymph nodes, and the presence of yellow, watery contents in the small and large intestines.[2] In additional to the histologic features described by the contributor, the lamina propria is often infiltrated by histiocytes, plasma cells, and lymphocytes, as illustrated by this case. In cases of porcine adenovirus nephritis, the renal interstitium contains multifocal accumulations of lymphocytes and plasma cells, and renal tubules contain sloughed, necrotic tubular epithelial cells, some of which may contain characteristic intranuclear inclusion bodies.[9]

Aside from the eye-catching inclusion bodies, histologic lesions in the examined section were subtle, leaving participants rooting around for descriptive points. Conference participants discussed the length of the villi and, while the contributor described fused and blunted villi, these changes were not appreciated in the examined section. Participants also noted multifocal areas where crypt and villar epithelium appeared hyperplastic and piled up, though most felt these apparent lesions were simply function of cut. Similarly, the occasional lymphocytolysis observed in Peyer's patches were thought to be within normal limits as some lymphocytolysis is expected during normal lymphocyte turnover.

Discussion turned to the quantity and character of the inflammatory cells, which left some participants unimpressed; however, the majority felt that the number of lymphocytes and

plasma cells was mildly elevated and likely responsive to the obvious viral infection being telegraphed by the inclusion bodies.

The moderator noted that, while diarrhea can be experimentally induced by PAdVs, current veterinary literature is divided on whether these viruses cause porcine diarrhea in natural infections. While direct causation is not definitively established, PAdVs should be on differential lists of porcine diarrhea, particularly in the absence of other common etiologic agents, such as rotavirus or coronavirus, which can cause subtle histologic lesions similar to the examined case while causing significant to profound clinical diarrhea.

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