WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 5

5 October 2011

CASE I: SDSU-2 (JPC 3105833).

Signalment: 12-month-old crossbred calf (*Bos taurus*).

History: Out of a group of 1200 from Canada, this was the 3rd calf to become ill and die after treatment. Calves were reported to be depressed and off feed and water. This calf presented to the field veterinarian "terminally ill in lateral recumbency" and was euthanized.

Gross Pathology: Necropsy findings noted by the field veterinarian included: esophagus contained non-hemorrhagic erosions. Small and large intestine contained multiple hemorrhagic ulcers.

Laboratory Results: Florescent antibody tests on lung, esophagus, and small intestine were positive for BVD virus. Cytopathic-BVD virus was isolated from lung and kidney. BVD ear notch ELISA test was positive on a fresh ear notch. Fecal flotation was negative for parasites.

Histopathologic Description: Within sections of small intestine, there is multifocal moderate to severe suppurative, mucohemorrhagic enteritis with attenuation and necrosis of crypt epithelium primarily confined to areas overlying Peyer's patches. Peyer's patches are severely depleted and their normal architecture has been destroyed; dilated crypts containing degenerate epithelial cell, neutrophils,

mucous and hemorrhage are herniated into the submucosa previously occupied by Peyer's patches. Moderate infiltration of the lamina propria by lymphocytes and plasma cells is also present. Within mesenteric adipose tissue, there is mild accumulation of perivascular lymphocytes and plasma cells; vasculitis is not present.

Contributor's Morphologic Diagnosis: Small intestine: Enteritis, mucohemorrhagic, neutrophilic, acute to subacute, moderate to severe, with crypt epithelial necrosis and destruction of Peyer's patches due to BVD virus infection.
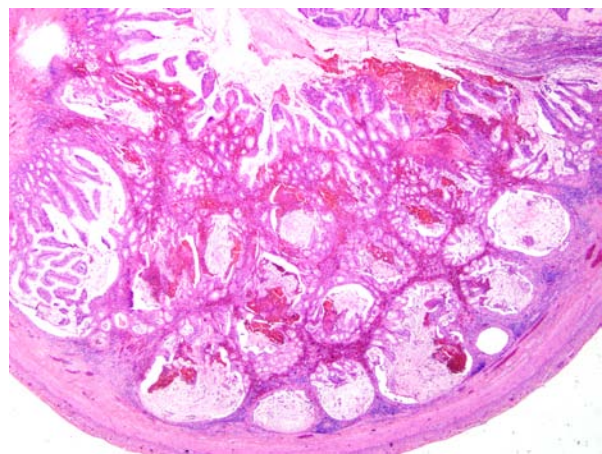
1-1. *Small intestine, cow. Peyer's patches have collapsed with herniation of intestinal villi through the submucosa. The intestinal lumen is filled with mucin, cellular debris, and hemorrhage. (HE 20X)*
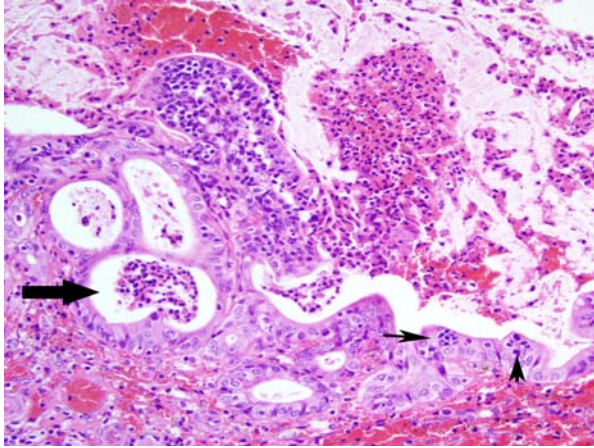
1-2. Small intestine, cow. Villi are markedly shortened and denuded of epithelium. Intestinal crypts are widely dilated, lined by attenuated epithelium and filled with degenerate neutrophils, necrotic epithelium, and cell debris (crypt abscesses). Multifocally mucosal epithelium is infiltrated by clusters of lymphocyte and neutrophils. (HE 200X)

Contributor's Comment: BVD virus is an RNA virus belonging to the genus *Pestivirus*. Infection of pregnant cattle can result in fetal abortion or birth of persistently-infected (PI) calves. PI calves typically are unthrifty and do not live beyond 2 years of age. PI calves can succumb to fatal mucosal disease if infected with cytopathic BVD virus (mutation of non-cytopathic BVD virus can also cause mucosal disease in these PI calves).[1]

Infection of healthy, immunocompetent, non-pregnant cattle by BVD virus usually results in only subclinical to mild clinical disease; however, primary infection by very virulent strains of BVD virus can result in severe clinical disease with mortality which cannot be differentiated from mucosal disease by clinical signs and necropsy findings.[1]

In this particular case, it is uncertain whether this calf was persistently infected and died as a result of mucosal disease, or if this was a healthy calf which became infected by a particularly virulent strain of BVD virus. The low morbidity, high mortality of the group suggests this was a PI calf, which died of fatal mucosal disease. Molecular characterization of the isolated virus may have aided in the differentiation of these two syndromes.

JPC Diagnosis: Small intestine: Enteritis, necrotizing, diffuse, moderate with focally extensive Peyer's patch necrosis and crypt herniation.

Conference Comment: Bovine viral diarrhea (BVD) is an acute, highly contagious disease of cattle with worldwide distribution that results in enteric disease, respiratory disease and reproductive loss. BVD virus can also infect sheep, goats and pigs and has been isolated in many wild and captive African species. BVD is separated into two biotypes, cytopathic (CP) and noncytopathic (NCP), based on cytopathic effects in vitro, and separated into two genotypes based on antigenic variation. NCP strains are associated with acute bovine virus diarrhea, BVD-induced thrombocytopenia, abortions, and are teratogenic, and CP strains produce mucosal disease. BVD is immunosuppressive and predisposes animals to secondary infections.[1,2]

Virus is shed in body fluids, and primary replication occurs in the tonsils and oropharyngeal lymphoid tissues. Virus then enters circulating monocytes and is transported to lymphoid tissues and the subepithelial connective tissue of the dermis and GI tract, where it spreads locally to overlying epithelial cells. In addition to the subclinical form in immunocompetent adults as mentioned by the contributor, BVD manifests in two other forms. Transplacental infections during days 50-100 of gestation result in fetal death, abortion, or mummification; infection at day 100-150 of gestation results in congenital defects such as microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, microphthalmia, thymic aplasia, hypotrichosis, alopecia, brachygnathism, growth retardation, and pulmonary hypoplasia. If a calf survives infection prior to 125 days of gestation, it may develop immunotolerance and lifelong, subclinical infection, which is the most pervasive source of infection of other cattle. A second form is mucosal disease, which occurs when a calf is infected with a noncytopathic genotype prior to 125 days of gestation and becomes immunotolerant, and is then infected with a cytopathic strain. Mortality in calves with mucosal disease approaches 100%.[1,2,3]

Gross lesions with subclinical BVD are seen as mild erosions or shallow ulcerations of the oral cavity. Mucosal disease manifests with erosions and ulcerations of mouth, tongue, esophagus, oral and ruminal papillae, abomasum, cecum, and colon; linear esophageal ulcerations ("tiger-stripe"); swollen, necrohemorrhagic Peyer's patches with diphtheritic membranes; and erosive or ulcerative interdigital dermatitis and coronitis. The virus causes widespread vasculitis, epithelial necrosis, and lymphocytolysis.[1,2,3]

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CASE II: 10L-0087 (JPC 3170668).

Signalment: 2-year-old male Thoroughbred horse (*Equus caballus*).

History: The colt initially presented with a short-term history of recurrent diarrhea and mild colic episodes. The animal had been losing weight for one month, appeared depressed and had a mildly elevated body temperature of 39°C.   After initial treatment (prednisolone, worming with fenbendazole) but progressive deterioration it was referred. At referral it presented dull and depressed with a heart rate of 72bpm, a weak pulse, congested mucous membranes and normal gut sounds on auscultation.   Rectal examination revealed a large fluid filled colon. Despite extensive fluid therapy the animal became acidotic (pH 7.21), hypoglycemic and failed to respond adequately to potassium supplementation and glucose/ insulin administration.   The colt subsequently developed PU/PD, showed mild colic signs, which failed to respond to fluxinin and was euthanized two days after admission.

Gross Pathology: The animal was in good to moderate body condition with mild reduction of subcutaneous and mesenteric adipose tissue.   Apart from mild ascites (1 litre), the cecum and colon were moderately filled with watery to viscous brown-green digesta and exhibited moderate to focally severe mucosal acute hemorrhages. Within the mucosa were large numbers of dark red to brown, 1 mm to 5 mm diameter, partly raised, centrally convex nodules containing small metazoan parasites (nematodes). Very few, red ~ 1 cm long nematodes were observed within the large intestinal content. A mild activation of cecal and colonic mesenteric lymph nodes was present.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.)**:**
Clinical pathology:

PCV	48%
TP	56g/l
Albumin	21g/l
Globulin	35g/l
WBC	14.10×10^9
Lymphocytes	11%
Neutrophils	86%
Na	111 (126-146) mmol/l
K	1.2 (3.0-5.0) mmol/l

2-1.   Colon, horse.   The colon is filled with watery, brown ingesta. Similar fluid was seen in the cecum. Photograph courtesy of Veterinary Pathology, University of Liverpool, www.liv.ac.uk
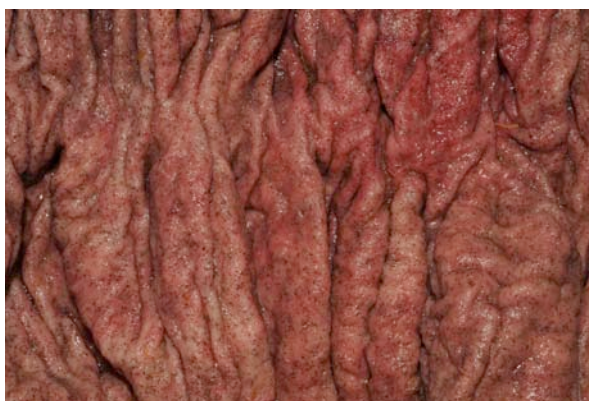

2-2.   Colon, horse.   Numerous 1 mm to 5 mm dark red nodules containing small nematodes are present within the colonic mucosa. Photograph courtesy of Veterinary Pathology, University of Liverpool, www.liv.ac.uk

Parasitology: Colonic content was subjected to a parasitological worm egg screen examination. No eggs were detected.

Histopathologic Description: Colon, mucosa: There is focal erosion and ulceration of villus tips; enterocytes exhibit a mild goblet cell hyperplasia. Multifocally enterocytes contain 20-60 μm large, basophilic, PAS negative fine granular intracytoplasmic structures (goblet cell hyperplasia/ apicomplexan protozoa?). The lamina propria contains moderate to large numbers of metazoan parasites (nematode larval stages) consistent with small strongylid larvae.

Smallest larvae are 20 μm in diameter, up to 100 μm long and crescent shaped with pointed tail tips (L_3) with mild peripheral histiocytic infiltration. Fewer L_3 stages exhibit very mild or no inflammatory reaction (hypobiotic early third larval stages, EL_3).

A population of further matured up to 100 μm diameter nematode larvae with an eosinophilic, thick, smooth cuticle, vacuolated lateral cords, prominent
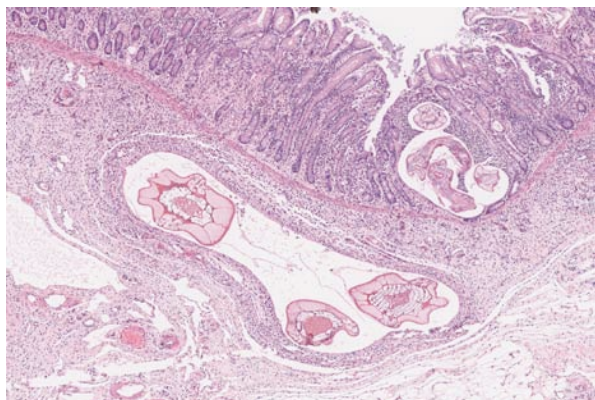
2-3. *Colon, horse. Mucosal epithelium is eroded and there are numerous cyathostome larva of varying maturity within the lamina propria as well as the submucosa. (HE 40X)*

platymyarian musculature and intestinal as well as genital tract is observed throughout the lamina propria mucosae (L_4). The intestine often exhibits prominent brush borders and these stages multifocally contain large amounts of dark red to dark brown pigment (iron pigment). The inflammatory response ranges from an acute, densely cellular neutrophilic and partly eosinophilic cuffing (emerging larvae?), to a capsule-like formation composed of macrophages and fibroblasts.

Few migrating L_4 stage larvae (not present in all sections) associated with superficial epithelial erosion and ulceration, acute haemorrhage and mild to moderate neutrophilic and eosinophilic infiltration is observed.

A mild to moderate diffuse infiltration by lymphocytes, plasma cells, lesser histiocytes and neutrophils and focal acute hemorrhages and few crypts containing cellular debris as well as neutrophils (crypt abscesses) is present in the mucosa.

Submucosa: Parasitic nematodes within the submucosa are much larger, 200 µm in diameter, with nematode specific external and nematode intestinal structures often containing erythrocytes, indicating blood uptake. A moderately dense histiocytic infiltration admixed with proliferating fibroblasts is surrounding the majority of these stages. The submucosa is generally expanded by clear spaces (moderate edema) as well as a multifocal to confluent mixed cellular (lymphocytes, plasma cells, macrophages, lesser neutrophils and occasional eosinophils) infiltrate.

Contributor's Morphologic Diagnosis: Moderate chronic ulcerative and hemorrhagic mixed cellular (histiocytic, lymphoplasmacellular, neutrophilic and eosinophilic) colitis with parasitic granuloma formation with intralesional, partly encapsulated nematode larval stages ($L_{3/4}$), consistent with small strongyles, "larval cyathostominosis", Thoroughbred horse (*Equus caballus*).

Contributor's Comment: Small strongyles (*Nematoda*, *Strongylida*), also known as cyathostomins, are extremely prevalent amongst the equine population worldwide and constitute frequent and highly pathogenic invaders of horses today.[5] Though horses can carry large worm burdens without displaying clinically significant symptoms, the clinical syndrome of larval cyathostominosis (cyathostomiasis), which occurs as a result of mass emergence of hypobiotic intestinal stages, can be associated with high fatality rates.[9]

More than 50 nematode species from subfamilies of *Cyathostominae* (~44 species), *Strongylinae* (minimum 11 species), and *Gyalocephalinae*, popularly known as trichonemes, cyathostomes or cyathostomins are embraced by the group 'small strongyles'.[10] Small strongyles range from 0.4 to 1.5 cm length in male animals and 0.5 to 2.0 cm length in female animals and are overall smaller than large strongyles, although some species may reach 2 cm to 3 cm in length.[9] Adults of some species inhabit the cecum or dorsal and ventral colon, with others inhabiting just one of these intestinal compartments. Adult small strongyles feed on enterocytes / enterocytic cellular debris or penetrate the epithelial barrier, open small vessels and digest blood.

In consistency with large strongyles, small strongyles have a direct life cycle with no intermediate hosts. Eggs are passed through the feces onto pasture. Depending on environmental conditions, eggs may hatch and develop from the first stage larvae (L_1) to infectious L_3 stages in as few as three days. Once ingested by the equine host, they continue to mature, and, in a rapid life cycle, new eggs may be passed onto the pasture in as few as 5-6 weeks. Larval development of most species takes place entirely in the mucosa of the cecum and colon, but few penetrate the muscularis mucosae and develop within the submucosa.[3]

In the present case, large nematode larvae containing ingested erythrocytes were observed in the submucosa. Due to their size and ingestion of blood, larval migration of large strongyle species has to be considered. It has been shown that L_3 of large strongyle species exsheath in the small intestine and penetrate the mucosa and submucosa of the small intestine, caecum and colon within 1–3 days where they moult to the fourth stage larvae (L_4) by about day 7.[4] Larval stages of different small and/or large
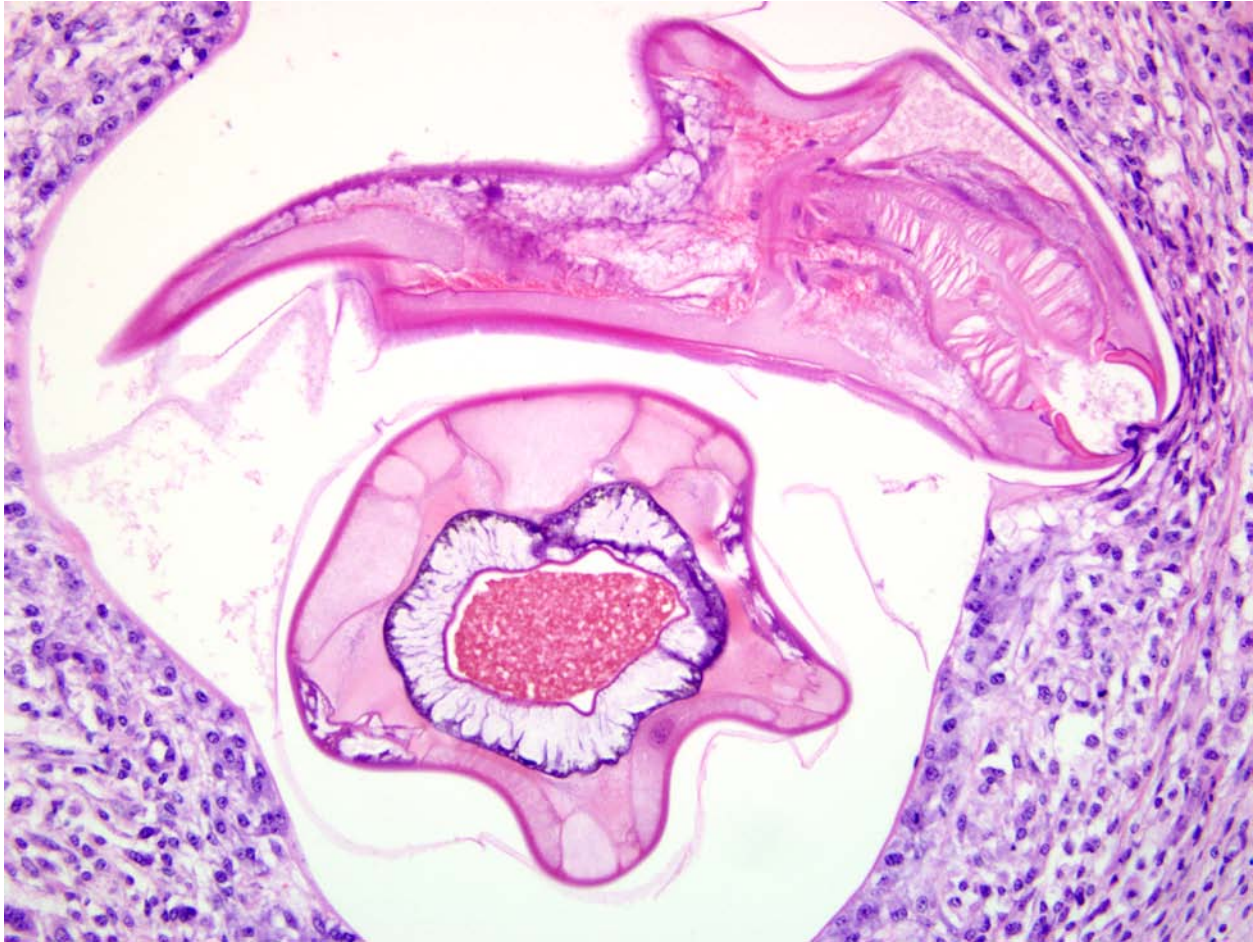
2-4. Colon, horse. Mature (L4) larval cyathostomes have an eosinophilic cuticle, vacuolated lateral chords, and a digestive tract containing blood. In longitudinal section (above) the chitinized buccal cavity and muscular esophagus are prominent. (HE 400X).

strongyle species are not distinguishable histologically and therefore, co-infection cannot be ruled out.

The entry of small strongyle larvae into tubular gland lumina generally provokes a chronic inflammatory response and marked goblet cell hyperplasia. Emergence of L_4 can be associated with an acute eosinophilic infiltration. Cyathostomins differ from other worm species in that the maturation of early third stage larvae (EL_3) can be arrested for prolonged periods of time (hypobiosis). After ingestion, L_3 exsheath and invade the large intestinal mucosa. Here, larvae protect themselves by becoming encysted and the host generally only reacts with a minimal inflammatory response to those stages. Up to 90% of an equine worm burden may become hypobiotic and EL_3 stages can remain within the intestinal walls for 4 months up to as long as 2 years.[3]

The majority of clinical signs associated with heavy infections in horses up to 2-3 years of age are unthriftiness, anemia and sometimes diarrhea. A typical clinical picture can include neutrophilia, hypoalbuminemia, hyperglobulinemia, low total serum protein as well as slightly high total protein, possibly due to dehydration, findings most consistent with protein-losing enteropathy.[7] Marked clinical signs are less common in older animals, though protective immunity never develops. However, the most significant damage can arise from simultaneous mass emergence of encysted L_4 larvae ('larval cyathosominosis') continuing their development to the adult stage in the intestinal lumen. Catarrhal and/ or hemorrhagic enteritis with severe diarrhea, potentially serious colic, leading to emaciation and in some cases death (mortality rate up to 50%) can be caused by severe damage to the gut wall. The climate is responsible for the occurrence of hypobiotic stages. In temperate climates larvae will accumulate during grazing season, encyst during the cooler months and re-emergence may occur en-masse as spring sets in. Reversed timing is observed in tropical climates where larval hypobiosis generally occurs during hot, stressful summer months with larval emergence in autumn.[1,7]

The antemortem diagnosis of larval cyathostominosis can, due to presentation of nonspecific clinical signs, be challenging. Depending on clinical findings, a variety of gastrointestinal diseases (e.g. salmonellosis, nonsteroidal anti-inflammatory drug-induced colitis, intestinal lymphosarcoma, inflammatory bowel disease, infections with *Lawsonia intracellularis* or *Clostridium* spp.) as well as other causes of ill-thrift and hypoproteinemia in nondiarrheic animals (e.g. renal disease, peritonitis, malnutrition) have to be considered as differential diagnoses.[8] Diagnosis in the live horse can be attempted by the fecal flotation method. However, this method can be unreliable because of variation in larval survival rates under certain culture conditions, is time-consuming and labor intensive and can be falsely negative or low because of the absence or paucity of adult nematodes. Therefore, definitive antemortem diagnosis is often not possible.

However, a presumptive diagnosis can be made based on signalment, clinical signs and exclusion of other possible causes. Post mortem diagnosis generally reveals strongly indicative findings. Additionally, transillumination is an easily employable method to detect intramucosal larvae and provides a fast and definitive diagnosis. Recently, investigations into internal transcribed and intergenic spacers of nuclear rDNA have been undertaken and these markers have proven to be useful for the identification of *Strongylinae* and *Cyathostominae* species. Importantly, the PCR-based methods developed using these markers can be sensitive and specific for diagnosis, and therefore might, via the detection of parasitic DNA extracted from intestinal biopsies, provide a useful diagnostic tool for *intra vitam* diagnosis of larval cyathostominosis.[4,6]

JPC Diagnosis: Colon: Colitis, granulomatous, with numerous mucosal and submucosal L_3 and L_4 stage larvae.

Conference Comment: The contributor mentioned large PAS-negative cells in the mucosal epithelium which contain fine intracytoplasmic granules. Conference participants attributed this to goblet cell hyperplasia. When we repeated the PAS stain at our institute, the cytoplasm of some of these cells was PAS positive, consistent with goblet cells. Conference participants also noted intimal bodies - irregular mineralized masses covered by endothelium which protrude into the lumen of small arteries and arterioles of horses, especially in the intestinal submucosa. These are considered to be normal findings.

The moderator discussed the importance of careful preparation and examination of the luminal surface of the affected intestine, because the characteristic gross appearance of cyathostomiasis with multiple red to black pinpoint nodules diffusely covering the mucosa can easily be missed if the digesta or feces is not carefully washed from the mucosal surface. Also, the mucosa and submucosa become very edematous and congested but not hemorrhagic, and will easily tear if palpated carelessly.

The process of massive emergence of hypobiotic larvae causing disease in otherwise clinically normal horses is similar to the phenomenon in cattle with "type II" disease as a result of *Ostertagia* spp. Ostertagiosis is the most important parasitic disease in grazing cattle and sheep in temperate climates, resulting in production loss and death. Lesions include severe abomasal mucous metaplasia, epithelial hyperplasia, and interstitial inflammation. This results in abomasal alkalosis, edema, and significant protein loss.[2]

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CASE III: SL-10-1298 (JPC 4002887).

Signalment: One male and one female, eight-month-old Holstein calves (*Bos taurus*).

History: Out of this herd of 100 animals, 25 are sick, and seven have died. Clinical signs include sudden onset of respiratory disease characterized by open mouth breathing and recumbency that occurred over a period of 24 hours. The affected animals all share a common feed bunk.

Gross Pathology: None provided.

Laboratory Results: PCR detected bovine respiratory syncytial virus RNA in pooled samples of lung. General bacterial cultures from each animal obtained many colonies of *Mannheimia hemolytica*.

Histopathologic Description: Lung: There is multifocal, segmental necrosis of bronchiolar epithelium and multifocal fibrinosuppurative bronchopneumonia. Multifocally affecting bronchioles is segmental to complete necrosis of bronchiolar epithelium with formation of respiratory epithelial syncytia. The syncytia are closely associated with the airway surface, present within the bronchiolar lumina, or occasionally found within adjacent alveoli. The syncytia rarely contain 5-8 micron in diameter, homogeneous, eosinophilic intracytoplasmic inclusion bodies. Airways contain sloughed cells and abundant necrotic cellular debris.

Affecting the pulmonary parenchyma is multifocal to coalescing, fibrinous and necrotizing bronchopneumonia. Alveoli contain variable amounts of viable and degenerate neutrophils, round "oat cells" with streaming nuclear contents, mats of fibrin, eosinophilic proteinaceous edema fluid, and intact alveolar macrophages. Occasional colonies of small coccobacilli are admixed with necrotic debris within alveoli. Alveolar septa are markedly expanded by fibrin, edema, and neutrophils. There are rare fibrin thrombi. The pleura and interlobular septa are markedly expanded by fibrin, edema, and suppurative infiltrates. Mats of fibrin, degenerate cellular debris, and viable and degenerate neutrophils line the pleural surface.

Contributor's Morphologic Diagnosis: Lung: 1) Marked, multifocal to coalescing, fibrinosuppurative bronchopneumonia.
Lung: 2) Marked, multifocal, necrotizing bronchiolitis with intralesional syncytia.

Contributor's Comment: Bovine respiratory disease complex (BRD), also known as shipping fever, is a common disease process in feedlot cattle and of major
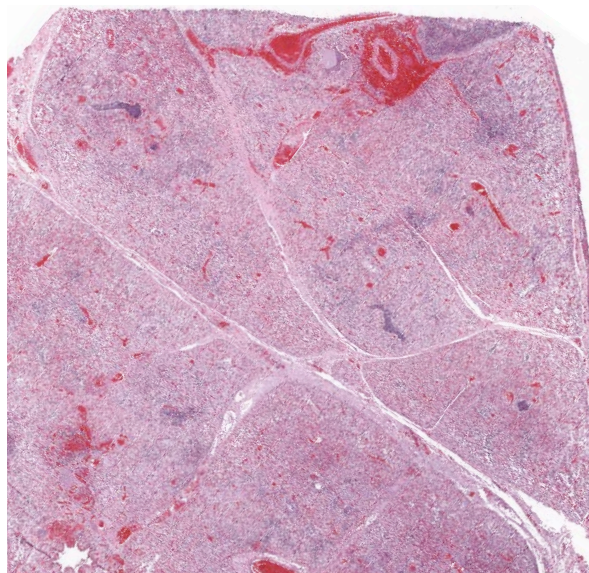

3-1. Lung, cow. At low magnification, there is marked lobular consolidation, outlines of airways with necrotic cellular debris, and expansion of interlobular septa with edema and emphysema. (HE, 63X)

economic importance in North America. BRD is an infectious respiratory disease caused by numerous viral and bacterial agents. Viral agents include bovine herpes virus-1, bovine viral diarrhea virus, bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus-3 (PI-3), and are considered key elements in the pathogenesis of BRD.[7] Bacterial pathogens contributing to BRD include *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*.[7] *M. haemolytica* is considered to be the predominant bacterial pathogen associated with this disease complex, and it plays a more significant role in adult dairy cattle than in dairy calves.[6]

The classic presentation of BRD in beef animals is pneumonia affecting multiple calves three days to three weeks after shipment from the farm of origin to the feedlot.[5] Risk factors in this group include: mixing of naïve calves from various sources; stress due to weaning, transport, crowding, handling, vaccination, dehorning, and food/water deprivation; metabolic acidosis due to diet change; and exposure to adverse or rapidly changing environmental conditions. In one-to-four-month-old dairy and veal calves, cases may be sporadic, enzootic, or epizootic.[5] In this group, risk factors include: poor air quality due to indoor housing, crowding, poor ventilation, and high humidity; reduced defenses due to failure of passive transfer of immunoglobulin; exposure to viral or other pathogens from co-housing with adults; and stress due to concurrent diseases or weaning.[5,6] Clinical signs include depression, pyrexia, tachypnea, dyspnea, and anorexia. While animals may respond to early antimicrobial therapy, relapse is common.[5]
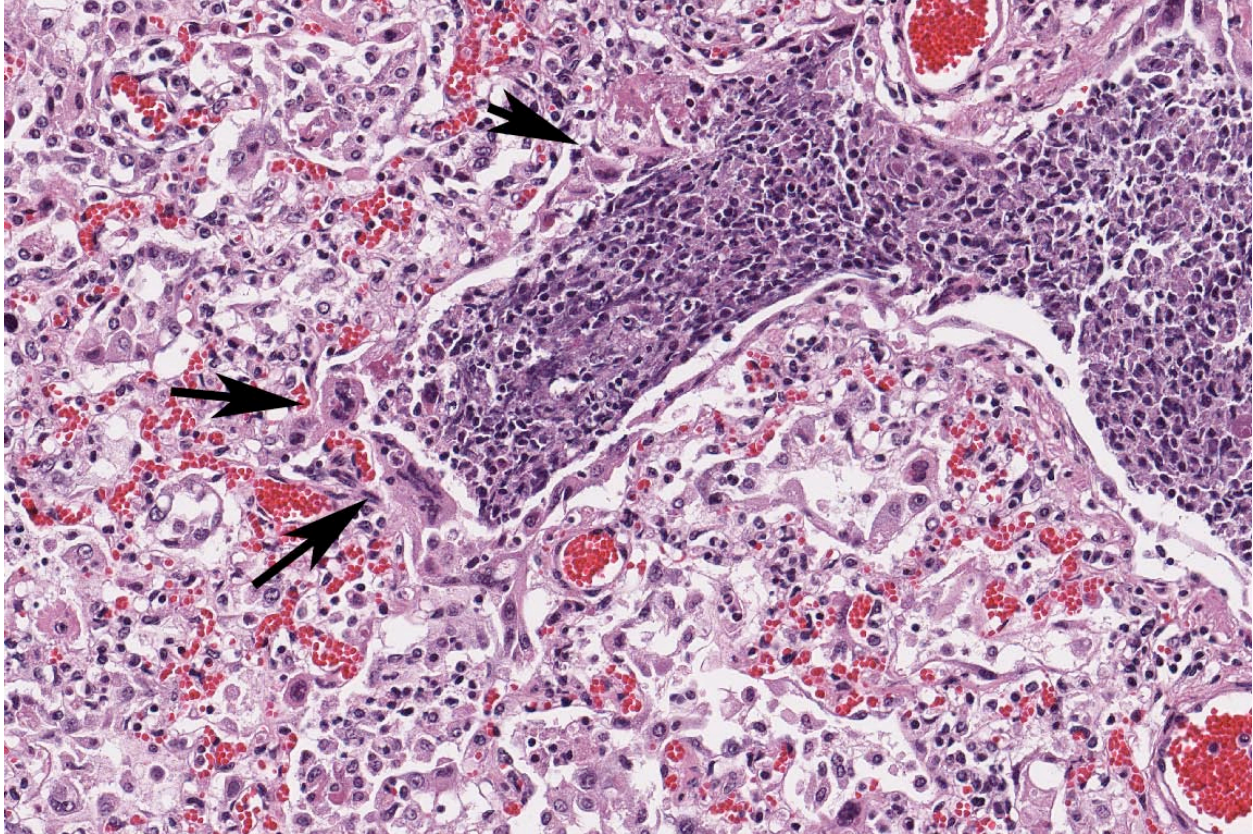
3.2. Lung, cow. Airways are filled with necrotic cellular debris. Alveolar walls are markedly thickened and alveolar lumens contain a fibrinocellular exudate with numerous macrophages and neutrophils. There are numerous multinucleated viral syncytia within remaining airway epithelium.

This case includes lung from one of two submitted eight-month-old Holstein calves that demonstrated open mouth breathing and acute onset of respiratory disease. The histologic features are consistent with a viral and bacterial etiology. Differentials for the bronchiolar epithelial necrosis and epithelial syncytia formation included BRSV and PI-3. Polymerase chain reaction detected BRSV nucleoprotein. The severe fibrinosuppurative bronchopneumonia, coagulative necrosis, and leukocyte necrosis ("oat cells") is suspicious for *Mannheimia haemolytica* or *Histophilus somni*. Many colonies of *M. haemolytica* were obtained via general bacterial cultures of lung.

Bovine respiratory syncytial virus (BRSV) is of the genus Pneumovirus in the family Paramyxoviridae. It is a major cause of respiratory disease and a major contributor to BRD.[1] The infection is common in North America and Europe, within both beef and dairy operations. The seroprevalence of BRSV in adult dairy and beef cattle ranges from 40-95%.[1,2] BRSV is an important cause of respiratory disease in two-week to five-month-old beef and dairy calves, and epidemics involving adult cattle have been reported.[3]

The severity of disease caused by BRSV depends on the age and immune status of the calf, route of infection, and strain of the virus.[3] Aerosol spread is the most common route of infection. The virus replicates in the epithelial cells of the upper respiratory tract, including the nasal cavity, pharynx, trachea, bronchi and bronchioli, and type II pneumocytes and alveolar macrophages. Significant effects include loss of cilia, necrosis of bronchial and bronchiolar epithelial cells, and depressed opsonization and phagocytosis by alveolar macrophages.[3] While specific serum and mucosal antibody responses occur in response to BRSV, the antibody is nonneutralizing and serum titers are poorly correlated with protection from disease.[2] A biphasic clinical course that is observed in some natural infections may represent a worsening of the disease following an immune response; however, the enhancement of the disease process by the immune system is controversial.[2] Viral shedding following experimental infection of naïve calves occurs between two and eight days post-infection. Viral antigen is most abundant in the cranioventral lung.

Most BRSV infections are subclinical. Mildly to severely affected dairy calves may demonstrate fever, cough, nasal/oral/ocular discharge, and tachypnea.[3] Severely affected animals may exhibit severe respiratory distress, including dyspnea, forced grunting upon expiration, and increased abdominal effort.
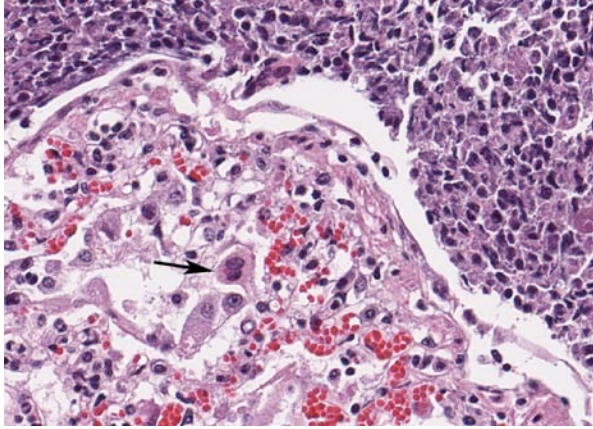
3-3. *Lung, cow. Airways are filled with necrotic cellular debris, and remaining epithelium is flattened and attenuated. A multinucleated viral syncytia contains a prominent round eosinophilic viral inclusion (arrow). (HE 200X)*
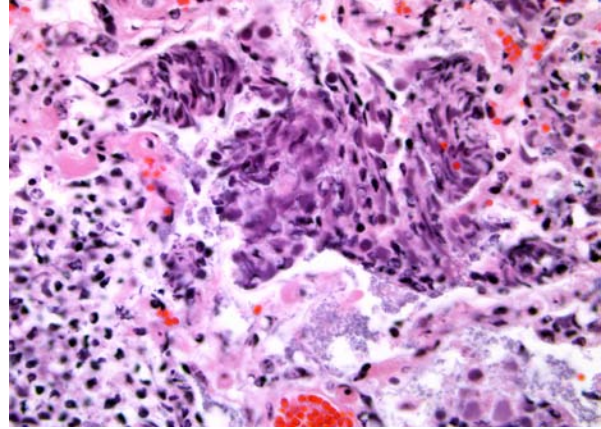
3-4. *Areas of necrosis contain many degenerate neutrophils with streaming, often smudgy nuclei (oat cells). Numerous colonies of coccobacilli are admixed with necrotic cellular debris. (HE 400X)*

Gross findings of BRSV infections differ between cranial and caudal portions of lung. The cranioventral lung is grossly deep red to mottled, atelectatic, collapsed, and rubbery in texture.[1,2] The caudodorsal lung may be heavy, edematous, or more firm than normal, with or without emphysema.[1,2] These gross lesions are widely variable, and may vary with concurrent bacterial bronchopneumonia. Lesions in other organs are not usually observed.[2]

Histologically, BRSV is characterized by necrotizing bronchiolitis, formation of bronchiolar epithelial syncytia, and exudative or proliferative alveolitis; these lesions are due to the cytopathic effects of the virus on epithelium, as previously described. Bronchioles are lined by attenuated epithelium, and multinucleated syncytial cells are frequently closely associated with these areas or found free in the lumina. Occasionally, alveoli may contain syncytia.[2] Bronchioles contain necrotic epithelial cells and neutrophils, and alveoli contain neutrophils and macrophages. Hyaline membranes may occur but are infrequent.[1,2] Intracytoplasmic eosinophilic inclusion bodies are occasionally present in syncytial cells.[2]

Differential diagnoses include bovine parainfluenza virus-3 (PI3) infection. In both diseases, syncytia with intracytoplasmic inclusion bodies occur. Generally, PI3 is associated with fewer syncytia located within the alveoli. Ancillary diagnostics are usually necessary for definitive diagnosis of BRSV, due to the confounding nature of multiple pathogens in the bovine respiratory disease complex. Diagnosis may be confirmed using fluorescent antibody tests, immunohistochemistry, antigen-detecting ELISA, or real time reverse transcriptase PCR(qRT-PCR).[1,2] qRT-PCR is considered the gold standard, based on its speed and sensitivity.[1]

Mannheimia haemolytica is a member of the Pasteurellaceae family. *M. haemolytica* is a small gram-negative coccobacillus that exists in the upper respiratory tract of healthy ruminants, primarily within the nasopharynx and tonsillar crypts.[4,5] Twelve serotypes exist, and serotypes A1 and A5 are known to colonize the upper respiratory tract of cattle and sheep as part of the normal commensal flora4. Serotype A1 quickly becomes the predominant organism once the commensal relationship is disrupted, and this serotype is also frequently isolated from pneumonic tissue.[4,5] Stress, exposure to cold, and concurrent viral illness and decreases in lung defenses are possible causes for the shift from a commensal relationship to pathologic one. The host response to the infection is the primary cause of clinical disease.[5] Virulence factors include leukotoxin, lipopolysaccharide, capsular polysaccharide, transferring-binding proteins A and B, O-sialogylcoprotease, neuraminidase, IgG1-specific protease, outer membrane proteins, and fimbriae.[5] These virulence factors allow *M. haemolytica* to evade clearance, avoid host defenses, and impair leukocyte function.[4,5] The result is massive recruitment of neutrophils into alveoli that are ineffective at killing bacteria but cause significant endothelial damage to capillaries and small vessels resulting in vascular leakage of protein, fluid, and fibrin into alveolar and interstitial spaces.[4,5,8]

Gross lesions caused by *M. haemolytica* include cranioventral lobar or lobular fibrinous bronchopneumonia, foci of coagulative necrosis, and variable fibrinous pleuritis.[5] Variable amounts of straw-colored thoracic fluid may be present.[4] On cut surface, the lung may be described as "meaty," being dark purple to red, heavy, firm or hard, and moist.[4,5]

Histopathologic findings of *M. haemolytica* pneumonia include fibrinous and suppurative bronchopneumonia

with necrosis of leukocytes.[5] Necrotic leukocytes, including neutrophils and macrophages, are lytic, and exhibit a streaming pattern of pale basophilic chromatin; as this pattern resembles oats, the term "oat cells" is commonly used.[5] Alveoli and airways contain variable amounts of viable and degenerate neutrophils and macrophages, fibrin, edema, erythrocytes, colonies of bacteria, and necrotic debris. Fibrin thrombi are frequently present, and interlobular septa are distended by fibrin, edema, and inflammation. Sharply demarcated regions of coagulative necrosis are often present.[5] Differential diagnoses for the gross and histopathologic lesions include *H. somni* and *P. multocida*. Evidence of bronchiolar necrosis is suggestive of an underlying viral infection; however, neutrophil-mediated damage may also cause such lesions.[5]

In the diagnostic setting, definitive diagnosis is determined by aerobic bacterial culture. However, prior treatment of the animal with antimicrobials frequently results in failure to culture bacterial agents. As well, opportunistic and secondary bacteria, including *Arcanobacterium pyogenes* and *Mycoplasma* species, may be present and confound the results.

JPC Diagnosis: 1. Lung: Bronchopneumonia, necrotizing, diffuse, moderate, with epithelial viral syncytia and intracytoplasmic viral inclusion bodies.
2. Lung: Bronchopneumonia, fibrinosuppurative, diffuse, moderate, with oat cells, numerous bacteria, and fibrinosuppurative pleuritis.

Conference Comment: The contributors provided an excellent synopsis of bovine respiratory disease complex, an economically important syndrome in cattle. The moderator emphasized that although the classic presentation of BRSV infection is with a gross appearance of cranioventral consolidation and caudoventral emphysema, as mentioned by the contributors, BRSV infection may also present as diffuse scattered edema and emphysema, without the typical gross demarcation. Additional differential diagnoses for enzootic pneumonia were discussed, such as bovine herpesvirus-1 and bovine adenovirus, which produce intranuclear viral inclusion bodies instead of inclusions in the cytoplasm.

The contributor mentioned leukotoxin, a primary virulence factor produced by *M. haemolytica*, which is a repeats in toxin (RTX) exotoxin. RTX is named for the presence of a tandemly-repeated nine-amino acid residue sequence in the protein, specifically glycine-rich nonapeptides, which bind Ca2+ on the C-terminal half of the protein. RTX toxins are pore-forming protein toxins produced by a wide range of pathogenic gram-negative bacteria that result in necrosis or apoptosis and are generally classified as hemolytic,

cytotoxic or both. Other important RTX toxins in veterinary medicine include hemolysin produced by *Escherichia coli*, ApxI and ApxII produced by *Actinobacillus pleuropneumoniae*, leukotoxin produced by *Pasteurella multocida*, and adenylate cyclase toxin produced by *Bordetella bronchiseptica*.[2,5]

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CASE IV: TAMU-01 (JPC 4003049).

Signalment: 20-year-old American Paint mare (*Equus ferus caballus).*

History: This icteric horse presented with anemia, and renal failure and hemoglobinuria, were noted on clinicopathologic evaluation. Despite transfusion of eight liters of blood, supportive care, and diuresis, renal values continued to increase and the animal's clinical condition continued to decline. Twenty-four hours prior to euthanasia, the animal was anorectic and had no fecal output.

Gross Pathology: All tissues were discolored tan/yellow (icterus), with pallor of all organs (anemia). The kidneys were swollen and bulged on section (N11-296A, nephrosis). Compared to other pale organs, the kidneys were dark and brown urine was in the urinary bladder (hemoglobinuria).

Laboratory Results:
Hemogram
Leucocytosis with absolute mature neutrophilia (28975 {2260-8580})
Mild absolute monocytosis
Mild lymphoctosis
2+ eccentrocytes

Serum = 4+ hemolysis
Creatinine kinase 4683U/L (73-450)*
Aspartate aminotransferase 2246U/L (134-643)*
Total protein 11.6 g/dl (5.3-7.3)*
BUN 58mg/dl (7-28)
Creatinine 4.3mg/dl (1.1-2)
Total bilirubin 10.3mg/dl (<4.1)*
* tests can be falsely elevated when hemolysis >2+

Histopathologic Description: Kidney: Segmentally, proximal tubules are variably distended and lined by attenuated, pale, basophilic epithelium with variably sized nuclei that are large and vesiculate with a prominent nucleolus. Some tubule are lined by necrotic and apoptotic cells, a few tubules have proliferation of cells with many nuclei/cells, and a few scattered tubules are lined by a simple, tall/cuboidal epithelium with occasional mitoses (tubular degeneration, necrosis and regeneration). Many distended lumens contain granular casts (Perl's negative) that sometimes have admixed, smooth hyalinized foci and often contain degenerating neutrophils (heme pigment casts). Many less affected proximal tubules are lined by swollen epithelium and contain a pale eosinophilic acellular content. In these tubules the content stains pale blue with Fe stain (Perl's/Prussian –blue reaction), and the cells contain Fe-positive stippling. The interstitium and perivascular areas are pale and expanded (edema) with foci that are

4-1. Kidney, horse. The kidneys are swollen, bulge on cut section, and are dark red. Photograph courtesy of Texas A&M University, Dept of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, http://vetmed.tamu.edu/vtpb.


4-2. A leaf from Acer rubrum, the red maple. Photograph courtesy of Texas A&M University, Dept of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, http://vetmed.tamu.edu/vtpb.

hyalinized and eosinophilic (fibrin, presumed) and large, reactive stromal cells. Many sloughed cells are in lower nephron tubules and collecting ducts with granular casts, fewer degenerating neutrophils and acid hematin (Perl's negative) clumping, and interstitial edema is obvious.

Contributor's Morphologic Diagnosis: Subacute, segmental tubular nephrosis with tubular degeneration necrosis and regeneration, karyomegaly, and granular casts with edema.

Contributor's Comment: This slide has most of the changes imaginable with tubular nephrosis, especially those of a nephrosis from hemoglobinemia. The horse had exposure to red maple limbs with leaves that had fallen after a wind storm over a week before presentation, and identifiable pieces of red maple leaves (N11-296B) were found in the colon.[1] Contact with wilted red maple (*Acer rubrum*) leaves and the presentation with hemolytic anemia, hemoglobinuria, icterus, Heinz bodies or eccentrocytes (methemoglobin) and some degree of renal failure are

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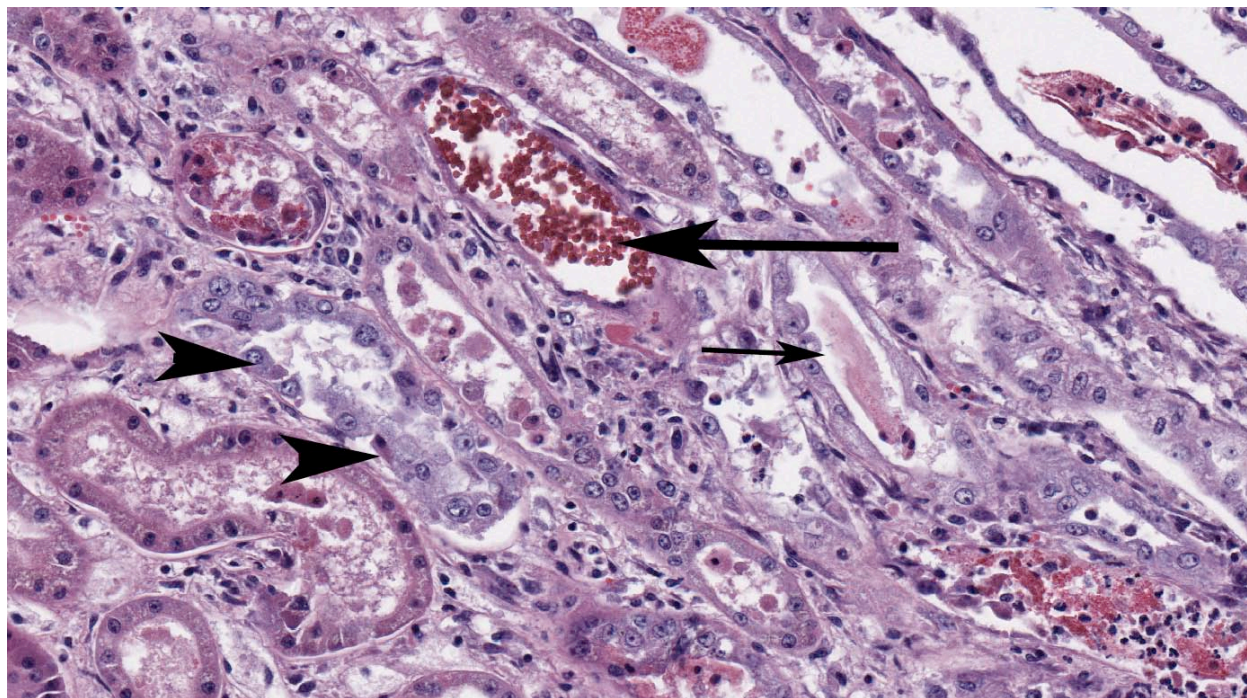
4-3. Kidney, horse. Necrotic tubules contain bright granular eosinophilic hemoglobin casts (large arrow) and protein casts (small arrow). Numerous tubules are lined by hypertrophic basophilic regenerative epithelium (arrowheads). (HE 200X)

characteristic.[1-14] Apparently, methemoglobin is not often quantitated to show defective oxygen carrying capacity, and our lab did not do new methylene blue staining, so Heinz bodies were not described. Many cases are published and most do reference renal failure, and although some report deaths, many horses survive (dose response, presumably). Our horse could not be stabilized and clinically was considered to be in renal failure. The renal lesion certainly is a joy for the pathologist. Features of interest to pathologists in red maple toxicity are:

- the kidney was/is remarkably preserved despite autolysis in other tissue
- note where the iron metal stains
- it is a segmental nephrosis
- the karyomegaly often is not emphasized.[6]

The toxic principle is in wilted leaves, and its identity is unknown. Gallic acid is one of the usual suspects as an oxidant, and Vitamin C (and steroids) is recommended for treatment.[1,8] The combination of intravascular hemolysis, hemoglobinemia and hypoxemia make the renal lesion. Reasonable differential diagnoses include: onion toxicity (we have cases of wild onion toxicity in horses but it is milder), nitrite intoxication (iatrogenic), equine infectious anemia (fever), babesiosis (fever and parasites visible) and immune mediated hemolytic anemia (agglutination positive). We have had cases associated with silver maple.

JPC Diagnosis: Kidney: Tubular degeneration, necrosis, and regeneration, diffuse, moderate with hemoglobin and cellular casts and scattered tubulorrhexis.

Conference Comment: This condition is only described in equids, and is probably due to their decreased capacity to reduce methemoglobin. Damage to the kidney results from acute tubular ischemic necrosis secondary to hemolysis. Neither hemoglobin nor myoglobin are nephrotoxic by themselves, and neither will cause injury when injected into a healthy kidney; however, in an ischemic kidney either may increase tubular necrosis. Gallic acid contributes to the oxidative stress of erythrocyte membranes resulting in Heinz body formation and subsequent anemia and methemoglobinemia. Heinz bodies are 1-2μm round protuberances on the erythrocyte membrane composed of denatured, precipitated hemoglobin.[1,3,5,7,13]

There are two forms of *A. rubrum* toxicosis: a peracute form that typically occurs late in the autumn and a hemolytic form which occurs in early autumn. The peracute form results in massive methemoglobinemia, tissue anoxia and sudden death. Gross lesions include brown discoloration of tissues and blood, and cyanotic mucous membranes. The hemolytic form causes Heinz body formation, and subsequent intra- and extravascular hemolysis, in combination with methemoglobinemia. Clinical signs include weakness, lethargy, depression, anemia, icterus, hemoglobinuria and hemoglobinemia. Gross lesions include icterus,

serosal petechia and ecchymosis, brown discoloration of the kidneys, splenomegaly, pulmonary congestion and edema, and mild to severe centrilobular hepatic degeneration and lipidosis.[5,7,13]

Differential diagnoses for hemolytic anemia in horses include *Leptospira bratislava*, *Babesia caballi* and *B. equi*, and wild onion (*Allium* sp.), which cause intravascular hemolysis; equine infectious anemia and *Anaplasma plasmaphagocytophilum* cause extravascular hemolysis. Other causes of methemoglobinemia include nitrate poisoning, chlorate toxicosis, and drugs such as phenacetin and acetanilide. Additional causes of Heinz body anemia include onion consumption in cattle, horses, dogs, and cats; rape, kale, and turnip consumption in cattle and sheep; chronic copper toxicosis in sheep, cattle, and swine; zinc toxicity in dogs; selenium deficiency; and the urinary acidifier methylene blue in cats. Heinz bodies can also occur spontaneously in cats.[5,7,13]

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