CASE 1: 11857 (JPC 4033346).

Signalment: 5-month-old male crossbreed calf (Bos taurus).

History: This was one of 38 calves with ages varying from 1-6 months out of a herd of 78. The affected animal was acutely dyspneic and febrile. Additionally, it showed lethargy, tremors, bruxism, open mouth breathing, dehydration, rapid and noisy breathing, coughing, serous or mucopurulent nasal discharge, recumbency and finally, death.

Gross Pathology: There were areas of consolidation, edema and emphysema in craniocentral regions of the apical and cardiac...
lung lobes, which were red-brown and rubbery to firm.

**Laboratory Results:** Immunohistochemistry was positive for BRSV (polyclonal anti-BRSV, VMRD Inc., Pullman, WA) and negative for parainfluenza type-3 virus (VMRD Inc., Pullman, WA). No bacterial growth on microbiological cultures.

**Histopathologic Description:** Bronchiolar lumens contain a copious quantity of necrotic epithelial cells and neutrophils. Some lymphocytes and plasma cells encircle bronchioles and blood vessels. Mononuclear cells also thicken alveolar septa. Alveoli contain numerous neutrophils and macrophages and eventually some fibrin. Syncytial cells are prominent and appear as multinucleate cells closely associated with the bronchiolar epithelium, and in the alveoli. Some proliferation of type II pneumocytes appears as scattered tombstone-like cells or complete cuboidal epithelialization of the alveoli.

**Contributor’s Morphologic Diagnosis:** Lung: Bronchiolitis, necrotizing and pneumonia bronchointerstitial, suppurrative, subacute, multifocal, moderate, with marked hyperplasia of type II pneumocytes and epithelial syncytia, crossbreed, bovine.

**Contributor’s Comment:** The bovine respiratory disease (BRD) pneumonias constitute a significant proportion of feedlot diseases. Bovine herpesvirus-1 (BHV-1) (infectious bovine rhinotracheitis virus); parainfluenza virus-3 (PI-3); and bovine respiratory syncytial virus (BRSV) are recognized as primary respiratory pathogens. Bovine viral diarrhea virus 1 and 2 (BVDV-1–2), bovine adenovirus A–D (BAdV-A–D), and bovine coronavirus (BCoV), bacteria (Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni), and Mycoplasma spp. have also been recognized as additional agents associated with severe BRD.

Among those etiologic agents, bovine respiratory syncytial virus (BRSV) seems to be a major contributor to the bovine respiratory disease of BRD complex. This virus belongs to the *Pneumovirus* genus within the subfamily *Pneumovirinae*, family *Paramyxoviridae*, and is an enveloped, non-segmented, negative-stranded RNA. By electron microscopy, the morphology of the RSV virions appears to be either very pleomorphic, with a shape roughly rounded and a diameter between 150 and 35 nm, or filamentous with a length that can reach 5μm and a diameter between 60 and 100 nm.

The bovine respiratory syncytial virus (BRSV) has been recognized as a pathogen in cattle responsible of an acute respiratory disease syndrome in beef and dairy calves since the early 1970s. The impact of BRSV infection on the cattle industry results in economic losses due to the morbidity, mortality, treatment and prevention...
costs that eventually lead to loss of production and reduced carcass value. BRSV causes acute outbreaks of respiratory disease in 2-week to 5-month-old dairy and beef calves. This virus could also predispose to bacterial pneumonia in feedlot beef cattle and occasionally cause respiratory disease in naive adult dairy cows.

The virus replicates predominantly in ciliated respiratory epithelial cells but also in type II pneumocytes. It appears to cause little or no cytopathology in ciliated epithelial cell cultures in vitro, suggesting that much of the pathology is due to the host’s response to virus infection. RSV infection induces an array of pro-inflammatory chemokines and cytokines that recruit neutrophils, macrophages and lymphocytes to the respiratory tract resulting in respiratory disease.

Microscopic lesions in BRSV infections consist of bronchointerstitial pneumonia, characterized by necrotizing bronchiolitis, formation of bronchiolar epithelial syncytia, and exudative or proliferative alveolitis. The subacute lesions of BRSV represent early repair of the previous lesions and additional lymphocyte-mediated lysis of virus infected cells. Bronchiolitis obliterans may occur as early as 10 days after infection.

JPC Diagnosis: Lung: Bronchointerstitial pneumonia, necrotizing and suppurative, diffuse, with rare multinucleated viral syncytia.

Conference Comment: This is an excellent example of bovine respiratory syncytial virus (BRSV) infection, containing all the pertinent histologic features as dutifully described above by the contributor. There is some slide variation in terms of the numbers of viral syncytia contained within several sections. Additionally, the presence of suppurative inflammation led many to speculate on the additional presence of one of the many secondary bacterial pathogens associated with this entity.

BRSV is one of many components of the commonly described bovine respiratory disease complex (BRDC), a pathologically complex and economically important disease which costs the
U.S. cattle industry up to $1 billion per year. While complex in its pathogenesis, the disease is ultimately a manifestation of the stress which cattle experience during weaning, processing, shipping and commingling. For this reason, it is logical that clinical signs of BRDC generally occur 7-10 days following this series of events resulting in the leading cause of morbidity and mortality in U.S. feedlots.

BRSV is the largest player in the BRDC, with seroconversion estimates in some geographic areas of over 70% in calves under 12 months old. With the addition of the aforementioned primary viral pathogens and the bacterial pathogens *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Histophilus somni*, *Pasteurella multocida*, *Mycoplasma bovis* and *Trueperella pyogenes*, assigning a specific etiologic diagnosis to gross or histopathologic lesions can prove to be an arduous task for the aspiring pathologist. Conference participants compared and contrasted the gross pathology of two other readily identifiable bacterial pathogens - *M. haemolytica* was described as resembling a “brush fire” histologically due to the “advancing front of coagulative necrosis” caused by bacterial toxins, while lesions of *M. bovis* often appear as “golf balls” due to its predilection for causing suppurative bronchiolitis with bronchiectasis.

A recent study compared the susceptibility of bovine airway epithelial cells to three different respiratory pathogens associated with the BRDC, with the differences between them perhaps due to direct correlation to the variation in disease manifestation. PI-3 readily entered the apical membrane of ciliated epithelial cells. These cells were largely resistant to BHV-1; however, when tight junctions were opened or the epithelial monolayer was damaged, BHV-1 infected basal cells. BRSV, in contrast, was unable to infect either epithelial or basal cells; however, the submucosal cells were susceptible though not specifically identified. The results led the authors to speculate on how BHV-1 and BRSV are able to cross the epithelial barrier as is apparently necessary to incite infection, even suggesting such bacterial pathogens as *M. haemolytica* are actually primary initiators. This is in contrast with well-established data elsewhere of reduced ciliary clearance by epithelial degeneration and necrosis as a result of BRSV infections leading to secondary bacterial invasion.

In large part thanks to its human relative, RSV, much has been described in regard to the immunopathogenic mechanisms of BRSV. The nonstructural proteins of RSV, NS1 and NS2, are instrumental in mediating resistance to IFN-stimulated responses, specifically by blocking phosphorylation and activation of IRF-3. This interruption of the MyD88-independent pathway, which occurs exclusively through TLR 3 or TLR 4 signaling, reduces activated interferon thus limiting the innate immune response. While TLR 3 recognition of double-stranded, viral RNA utilizes only MyD88-independent signaling, TLR 4 has the additional option of mediating NF-κB activation in concert with MD2 and CD14 when it recognizes the F protein of BRSV. It is this fusion protein that is also responsible for mediating fusion of the viral envelope and the formation of viral syncytia seen histologically in both BRSV and PI-3 cases. The persistence of the virus in the face of widespread vaccination and in spite of the advances in the understanding of its pathogenic mechanisms has led to researchers developing improved technologies. Some promising examples include live attenuated vaccines which are devoid of either NS1 or NS2 and are capable of inducing robust antibody responses.

Given the endemicity of these prominent respiratory pathogens and their considerable economic impact, significant opportunity for discovery remains in the complex pathogenesis of BRDC; though we leave the reader to speculate where in the process of beef production the most impactful of those opportunities lie, whether it is management considerations, preventive therapy, or treatment.

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**References:**


Signalment: Four-week-old male crossbred pig (Sus scrofa).

History: A respiratory disease in a pig herd quickly spread from the finishing unit to the growing and breeding units. The affected animals showed prostration with respiratory signs including sneezing. The disease disappeared two weeks after onset, except in the breeding unit. The presently examined pig was one of two in the breeding unit submitted for necropsy.

Gross Pathology: Patchy, mottled, dark-red, and consolidated foci were found in the cranial, middle, and caudal lobes of the lung. Affected pulmonary lobules were well demarcated from normal areas. No gross lesions were detected in other organs.

Laboratory Results: Virology: Influenza A virus (subtype; H1N2) was isolated from the samples of nasal discharge and lung tissue. No pathologic bacteria were isolated from the lungs. Immunohistochemistry: Type A influenza virus matrix antigen was detected in the lung. Antigens of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), Mycoplasma hyopneumoniae and Mycoplasma hyorhinis were negative in the lung.

Histopathologic Description: Necrosis and desquamation of the bronchial epithelial cell layer was multifocally found in the affected lobes of the lung. The lumen of the involved bronchioles was extended, and some bronchioles were obstructed by necrotic cell debris, macrophages, and neutrophils. Necrotic cell debris and the inflammatory cells were also found in bronchia, alveolar ducts, and adjacent alveolar lumina. Hyaline membranes, formed by cellular debris accumulation and exudative proteins, were occasionally observed in some sections of alveolar wall. Slight lymphocyte accumulation was noted around bronchioles and adjacent vessels. Some bronchioles were found to have a hyperplastic epithelial cell layer. Alveolar septa were slightly thickened with lymphohistocytic infiltration and hyperplasia of type II pneumocytes.

Immunohistochemical analysis to detect type A influenza virus revealed presence of influenza virus matrix antigens in the epithelial cells, with necrotic changes in some bronchioles and adjacent alveolar walls. Other major pathological findings in this case were basophilic intranuclear inclusion bodies (cytomegalic inclusion bodies) in

2-1. Lung, pig: There are patchy areas of atelectasis scattered throughout the section centered on small airways. (HE 0.63x)
epithelial cells of nasal mucous glands with slight lymphocytic infiltration in the lamina propria and slight inflammatory exudates in the nasal cavity.

**Contributor’s Morphologic Diagnosis:** Lung: Pneumonia, bronchointerstitial, necrotizing, multifocal, moderate, subacute.

**Contributor’s Comment:** Influenza virus infections are a common cause of swine pneumonia worldwide. Swine influenza viruses are type A viruses, and major epidemic subtypes—defined by the nature of the hemagglutinin (H) and neuraminidase (N)—are H1N1, H1N2, or H3N2. Influenza virus infections in swine cause acute respiratory disease with high morbidity and function as a major etiology of porcine respiratory disease complex (PRDC), as the virus infections act synergistically with other viral and bacterial infections in the respiratory tract. Further, swine influenza virus infections are not limited in significance to merely swine production but represent major public health concerns, as pigs are susceptible to infection of avian and human influenza viruses and often result in appearance of reassortment viruses in pigs.

The common microscopic findings in swine influenza infections are necrotizing bronchitis and bronchiolitis. The influenza virus primarily infects the epithelial cells lining the surface of the respiratory tract, and infection induces cytolysis of infected cells. Cell death induced by the viral infection also occurs through apoptosis induced by the viral components. The term ‘bronchointerstitial pneumonia’ is used in veterinary pathology to describe cases with pulmonary lesions showing histologic features of both bronchopneumonia and interstitial pneumonia. This combined type of pneumonia is frequently seen in many viral infections in which viruses replicate within and cause necrosis of bronchial, bronchiolar, and alveolar cells. The term has also frequently been used in microscopic examination of influenza virus infections in animals. Although histopathologic features of swine influenza are considerably characteristic, immunohistochemical evaluation with sections of formalin-fixed tissue can prove nevertheless useful in differential diagnosis. Similar histological lesions are necrotizing bronchopneumonia induced by PCV2, interstitial pneumonia induced by PRRSV, or mycoplasmal pneumonia. Of note, findings on immunohistochemistry were negative for all three diseases in the present case.

**JPC Diagnosis:** Lung: Pneumonia, bronchointerstitial, necrotizing, multifocal, moderate, with type II pneumocyte hyperplasia.

**Conference Comment:** In swine, influenza viruses specifically target airway epithelial cells, leading to its hallmark histopathologic lesion of necrotizing bronchitis and bronchiolitis. We typically reserve the diagnosis of bronchointerstitial pneumonia for those cases in which pathogens target both airway epithelium as
well as either pneumocytes or vascular endothelium within alveolar septa. This seems to occur exclusively with viral infections, and this case represents an excellent example. Of note is the affinity for the terminal airways in this case, as many bronchioles are barely evident among the extensive inflammation and necrosis in the periphery of the section with relative sparing of the larger airways.

All influenza viruses of significance in swine are type A viruses, with the subtypes H1N1, H1N2 or H3N2 being most common. Influenza is an orthomyxovirus, a single-stranded RNA virus which is well known for its ability to constantly adapt through spontaneous mutations of its hemagglutinin and neuraminidase proteins (antigenic drift) or via recombination of genes with those in strains infecting other species (antigenic shift). It is this talent of recombination which enables cross-species transmission and earns it the distinction of being the deadliest virus known to man, with the 1918 pandemic of H1N1 killing up to 40 million people worldwide.

Early studies demonstrated influenza receptors of both avian and human viruses within the trachea of swine, leading to the labeling of swine as a “mixing vessel” from which pandemic influenza may arise. It has since been determined that avian influenza can infect humans just as readily as swine, negating the need for the pig as an intermediate host. Thus it is the avian viruses which have been given the most attention as of late, the most noteworthy being the H5N1 strain known as highly pathogenic avian influenza. (see WSC 2012 conf 14, case 4 or WSC 2013 conf 7, case 4 for two examples).

Influenza is capable of cross-infection between swine and people, with the 2009 H1N1 pandemic being the most well-known example. Triple reassortments, which are influenza viruses circulating in swine that possess both avian- and human-origin genes, have been identified which add to the circulating pool likely resulting in the increased incidence of newly reassorted viruses. Newly reassorted viruses pose a significant biosecurity and management problem for the swine industry, with the need for protection against the increasingly antigenically diverse viruses being of significant concern.

In addition to hemagglutinin (used for attachment to and internalization of host cells) and neuraminidase (which prevents viral progeny aggregation), the proteins that influenza is classified by, there are several others important for disease pathogenesis and diagnostics. The polymerase PB2 directs cell processes toward virus replication and is considered most significant of the polymerases with regard to pathogenicity. The nonstructural protein PB1-F2 also contributes to virulence through four mechanisms: inducing apoptosis, IFN suppression, increasing viral replication rates or delaying viral clearance, and increasing inflammation. NS1, another nonstructural protein, interferes with antiviral response and exhibits both proapoptotic and antiantiapoptotic activities. It also is only expressed in infected cells, making its antibodies a useful naturally occurring DIVA (Differentiating Infected from Vaccinated Animals) tool. The nucleoprotein (NP) is a highly-conserved, internal protein in all type A influenza viruses and thus an appropriate target for ELISA. This could potentially alleviate the demonstrated problems associated with antigenic variation when detecting hemagglutinin antibody.

Early detection of different subtypes of influenza virus, most notably H5N1 which experimentally only induces subclinical disease in swine, is a significant public health concern. New techniques utilizing oral secretions collected by the suspension of absorbent ropes within the pens of swine may be the way forward for rapid screening in large facilities, though antibody-based diagnostics from oral fluids are not yet a proven technology.

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References:
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CASE III: 09N2904 (JPC 4019370).

Signalment: 11-year-old, castrated male domesticated alpaca (Vicugna pacos).

History: This adult male alpaca was presented to the VMTH for partial anorexia and lethargy with drooling. He was recently switched to new grass hay in which alfalfa and weeds were present. He was allowed access to fresh grass in a pasture for a few hours but developed diarrhea that resolved. He shared his enclosures with goats (all healthy).

Over the course of a 3-day hospitalization, the alpaca was noted to be anxious, depressed, and colicky with decreased gastrointestinal motility and intermittent recumbency (both sternal and lateral). He was treated intensively with fluids, antibiotics and supportive care. Facial and pulmonary edema, oliguria and azotemia developed over the 3 days with initial partial response to diuretics followed by apparent anuria. Sinus tachycardia was present initially, but by early on day 3 arrhythmias were noted. Ventricular tachycardia (up to 190 bpm) with runs of premature ventricular contractions developed and worsened in spite of I.V. lidocaine. The alpaca went into cardiac arrest and could not be resuscitated.

Gross Pathology: There was diffuse subcutaneous edema, mild abdominal effusion, and pulmonary congestion and edema. On the epicardial surface of the right ventricle there was a focally extensive area of petechiation. The heart silhouette, weight, and measurements were considered normal. The mucosal surface of the pylorus and entire duodenum was diffusely discolored dark red to purple and thickened, and serosal surfaces of the entire bowel were reddened. The cortical surfaces of the kidneys were mottled dark red to purple, and in the right kidney there were wedge shaped areas of red-purple discoloration which tapered into the medulla. The bladder contained urine.

Laboratory Results: Abnormal lab work included: elevated BUN (62 increasing to 116); elevated creatinine (4.8 increasing to 8.4); hyperglycemia (410 to 690); leukocytosis with neutrophilia, lymphocytopenia and monocytosis (11,620 with 73% neutrophils, 10% bands, 4% lymphocytes and 13% monocytes). Serum collected on day 2 and analyzed at the California Animal Health and Food Safety Lab was positive for oleandrin.

Histopathologic Description: Heart (left ventricular myocardium): Multiple foci of hemorrhage are present in the subendocardial and subepicardial myocardium. Myofibers in these foci and in scattered foci throughout the sections are pale, granular to fibrillar or fragmented and the endomysium and perimysium are expanded by a combination of edema and cells with variably pyknotic to plump nuclei. Adjacent myofibers
occasionally have centrally located hypertrophied nuclei. The endocardial hemorrhage in two sections surrounds Purkinje fibers which are vacuolated and fragmented. Scattered individual myofibers are hyalinized and are brightly eosinophilic (Zenker’s necrosis) or lightly basophilic (early mineralization). Rarely, necrotic fibers are surrounded by neutrophils or mononuclear cells (myophagia).

Kidney: There are large wedge shaped areas of acute infarction characterized by hemorrhage and coagulative necrosis of the cortex and medulla. Cells of the infarcted glomeruli and tubules have loss of cellular detail and karyolytic nuclei. Glomeruli multifocally contain abundant fibrin within capillaries. Small numbers of renal tubules exhibit acute tubular necrosis in which epithelial cells are hyper eosinophilic, have pyknotic nuclei, and slough into the lumen (cellular casts). Surrounding tubular epithelium is multifocally attenuated. There is multifocal mineralization of necrotic tubules.

**Contributor’s Morphologic Diagnosis:** 1. Heart (left ventricle): Moderate multifocal subacute myocardial degeneration and necrosis with associated hemorrhage compatible with oleandrin intoxication.
2. Kidneys: Severe multifocal acute infarction and multifocal acute tubular necrosis.
ADDITIONAL DIAGNOSES (TISSUES NOT ON SLIDE):

1. Stomach (C3), small intestine and colon:
   Moderate diffuse congestion and multifocal transmural hemorrhage with multifocal superficial necrosis.

2. Adrenals: Severe multifocal subacute medullary hemorrhage and necrosis.

3. Pelvic limbs: Mild multifocal myonecrosis with hemorrhage and multifocal myofiber regeneration.

Contributor’s Comment: Oleander intoxication in this alpaca was confirmed by finding oleandrin in the serum antemortem and the fact that oleander was present on the property. Pink oleander (Nerium oleander) is a popular showy flowering ornamental shrub that was widely planted in California in median strips of freeways, verges of country roads, parks, farmsteads and urban homes. Unfortunately, all parts of the plant are highly toxic with over 35 bio-active compounds having been isolated from this plant.\(^{14}\)

The most studied of the toxic principals are cardioactive glycosides including oleandrin. The heart and GI tract are the primary targets of intoxication, though neurological signs have been reported and renal tubular degeneration has been seen in some cases. South American camelids (llamas and alpacas)\(^6\) and horses account for the majority of cases in our VMTH pathology archives, although much of the literature is concerned with intoxications in humans, cattle, and other commercial livestock.\(^1\)-\(^4\),\(^8\),\(^15\) Birds and rodents are fairly resistant experimentally, as are primates, though experimental or natural intoxication has been reported.\(^10\),\(^13\)

The cardioactive glycosides of oleander exert a positive inotropic effect, caused by interference with normal calcium channel mechanisms and eventual intracellular accumulation of calcium.\(^10\) The underlying mechanism for renal failure is undetermined at this time, but is a common finding with oleander toxicity in New World camelids.\(^6\) Two possible mechanisms include inhibition of the Na\(^+\)K\(^+\) ATPase pump in the renal tubules causing direct tubular injury, or infarction...
due to hypoperfusion secondary to cardiac damage. It is also possible that it is a combination of both mechanisms, as in this case both infarct and acute tubular necrosis were observed.

Other cardiotoxic plants, zoo or wild animals that might be exposed include: foxglove (Digitalis lannata or D. purpurea), yellow oleander (Thevetia peruviana), rhododendrons and azaleas (Rhododendron spp.), mountain laurel (Kalmia latifolia), kalanchoe (Kalanchoe blossfeldiana and its hybrids), milkweeds (Asclepias species), lily of the valley (Convallaria majalis), Dogbane (Apocynum cannabinum), avocado (Persea Americana Guatamalan variety). Other cardiotoxins include ionophores such as monensin and salinomyocin, which was responsible for large alpaca mortality event through contamination of a commercial feed.5


Conference Comment: Arriving at a precise etiologic diagnosis proved challenging for conference participants in this case, whom at best could consider oleander on a differential list which included other cardiac toxins such as monensin (ionophore used as coccidiostat that also acts on Na+/K+ pumps), coffee senna or western water hemlock (plants known to cause muscular & myocardial necrosis in livestock), yew (Taxus sp.), gossypol (toxic alcohol from cottonseed meal), locoweed (plants better known for “star gazing” in cattle but which cause myocardial degeneration), or cantharidin (toxic principle from blister beetles that causes gastric, cystic and myocardial necrosis) in addition to the aforementioned cardiotoxic plants listed by the contributor.9

To further complicate the picture, ischemic myocardial necrosis also occurs in disseminated intravascular coagulopathy, vitamin E/selenium deficiency, and inflammatory diseases such as periarteritis nodosa. Subendocardial necrosis is observed following acute brain injury (“brain-heart syndrome”) or in catecholamine excess from a functional pheochromocytoma.9 The presence of renal necrosis does not assist in narrowing down the list, as the potential candidates for inducing renal infarcts or toxic tubular necrosis is equally extensive with significant overlap from the cardiotoxic differentials. As the contributor points out, identifying the geographic location as California in the present case significantly elevates oleander from others on the list. The gross and microscopic findings of endocardial hemorrhage, while considered non-specific at best, have been reported as commonly occurring in oleandrin toxicity, is prominent in the distributed sections, and may have yielded some assistance in narrowing this relatively long list of ruleouts.

Some discussion among participants revolved around the presence of apparent vascular necrosis of some larger arteries and whether to attribute those to vasculitis, injury due to hypertension or the renal infarct, with the most favorable opinion being secondary injury from a period of hypertension.

Oleandrin toxicity is most often associated with horses, though it is known to be toxic to many species. Participants briefly contrasted the manifestations of toxin ingestion as reported in horses with the current case. In the horse, mortality appears to be more common, with a rate of 50% (30 cases) versus 25% in camelids (12 cases). The clinical signs between species are similar, with the triad of gastrointestinal, cardiac and renal disease consistently present in each species.7,12

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References:


CASE IV: 12-12710 (JPC 4048081).

Signalment: 8-month-old Angus heifer.

History: This was the sixth calf to die from a group of approximately 225. The first calf died in September, just before weaning and had shown bloody diarrhea that did not respond to treatment with oxytetracycline. The group was treated with a coccidiostat in the water. Calves had been vaccinated for clostridial agents plus IBR, BVD and PI3. Several other calves died in the course of a few days in late October.

This heifer was found sick and treated with penicillin and banamine, but died. It was submitted for necropsy to the Washington Animal Disease Diagnostic Laboratory at Washington State University.

Gross Pathology: Multifocally, throughout the jejunum, ileum, cecum, and colon, were widely scattered, linear to elliptical areas of mucosal ulceration that ranged from 0.5 to 8.0 cm in length, with the ileum being most severely affected. The edges of the ulcers were raised and there were multifocal areas of hemorrhage within the surrounding mucosa. Throughout the small intestines were segmental regions of hyperemia. Within the colon there was a single dark red, blood-stained fecal ball. Other minor lesions included mesenteric lymphadenopathy and mild, mucopurulent tracheitis.

Laboratory Results: Feces from this heifer were cultured for Salmonella spp. and were negative. Anaerobic cultures of feces isolated Clostridium perfringens type A.

The sera from two herdmates of this heifer were tested by antigen ELISA for persistent infection with BVDV. Both were positive.

Histopathologic Description: In a section of ileum, Peyer’s patches were moderately to markedly hypocellular and were partially to completely replaced by ectatic intestinal crypts (crypt herniation) that were often filled with karyorrhectic cellular debris admixed with many degenerate neutrophils. The overlying mucosa was extensively ulcerated. The ulcers were covered by large coagula composed of numerous degenerate neutrophils admixed with abundant necrotic cellular debris and mixed bacterial colonies. Numerous neutrophils infiltrated the adjacent lamina propria and submucosa. Remaining crypts in the adjacent, less severely affected mucosa, were mildly to markedly ectatic and filled with abundant mucus admixed with necrotic cellular debris and many degenerate...
neutrophils. The lamina propria in these regions was moderately expanded, and crypts elevated by increased numbers of lymphocytes, plasma cells, neutrophils, and varying amounts of edema.

A replicate section immunostained for the presence of bovine viral diarrhea virus (BVDV) revealed positive immunoreactivity in the crypt epithelial cells and macrophages. Positive controls had appropriate immunoreactivity and a replicate slide stained with a non-specific antibody (isotype control) had no immunoreactivity in those areas.

**Contributor’s Morphologic Diagnosis:** Enterocolitis, necrotizing and ulcerative, multifocal, severe with Peyer’s patch necrosis and crypt herniation associated with BVDV infection.

**Contributor’s Comment:** Lesions present were compatible with mucosal disease caused by persistent infection with bovine viral diarrhea virus.

Bovine viral diarrhea virus (BVDV) is an RNA virus of the genus *Pestivirus* that causes a wide variety of clinical disease primarily in cattle which is its natural host. A highly mutable virus, BVDV has developed two primary genotypes, 1 and 2, with both cytopathic (cp) and noncytopathic (ncp) forms within each genotype.

Type 1 genotypes are considered to be more prevalent but less virulent than type 2 genotypes, although exceptions occur. Cytopathogenicity in vitro does not correlate with virulence.

In naïve, immunocompetent non-pregnant cattle, infection with BVDV can cause mild clinical disease characterized by fever and leukopenia, or they may develop classic bovine virus diarrhea, including ocularonasal discharge, oral erosions and enteritis. Highly virulent strains (usually type 2 genotypes) may cause acute, severe disease with high morbidity and mortality with lesions indistinguishable from mucosal disease (described later). Transiently infected or vaccinated cattle develop neutralizing antibodies that are protective against reinfection.

Infection of naïve pregnant animals can result in fetal loss, congenital defects or birth of live, persistently infected (PI), immunotolerant calves. Infected fetuses may be aborted or may develop hydrancephaly, cerebellar hypoplasia, thymic atrophy, osteosclerosis or cataracts and other ocular lesions. PI status requires infection of the fetus early in gestation (40 -125 days). PI cattle are immunotolerant and do not develop antibodies to BVDV. They shed large amounts of virus and are an important source of infection for the herd. Detection and removal of PI animals is an
important management strategy on cattle ranches. PI status can be detected by PCR for BVDV on buffy coats, but this does not distinguish from transiently infected cattle. Antigen ELISA on serum or ear notch supernatants, however, detects animals with high viral shedding and is the preferred method for screening for BVDV PI animals. PCR on pooled samples from ear notch supernatants, with subsequent individual testing on positive pools is an efficient way to screen large herds.

PI calves are often unthrifty and most succumb to mucosal disease before 2 years of age. Mucosal disease occurs when a calf persistently infected with an ncp strain of BVD becomes infected with a similar cp strain, or when the ncp resident strain mutates to become cp. The classic clinical presentation of mucosal disease is a small calf with oculonasal discharge and severe diarrhea. Gross lesions include coronitis, nasal and oral erosions, and ulcerative lesions throughout the gastrointestinal tract, including esophageal ulcers and characteristic, severe enteric ulcerations overlying Peyer’s patches. The epithelial necrosis and herniation of crypts into severely depleted Peyer’s patch lymphoid follicles is characteristic of BVD; rinderpest causes similar lesions but is distinguished by characteristic multinucleate syncytial cells with morbillivirus cytoplasmic inclusions. Diagnosis of BVD can be confirmed by immunohistochemical demonstration of viral antigens within ulcerative lesions in the skin and throughout the gastrointestinal tract.

**JPC Diagnosis:** Small intestine: Enteritis, necrotizing, diffuse, severe, with focally extensive Peyer’s patch necrosis and crypt abscessation.

**Conference Comment:** In an era in which ivermectin has virtually eliminated a multitude of picturesque lesions in domestic animals, veterinary pathologists can find consolation in the persistence of bovine pestivirus, or BVDV, and its ability to induce a tremendous variety of pathology. This case is an exceptional example of the classic presentation of ulceration and Peyer’s patch necrosis within the gastrointestinal tract associated with the form of infection known as mucosal disease (MD), or which can be seen in acute infections of highly virulent strains. Linear ulcerations within the alimentary tract are classic, but one must also consider lesions from the brain to the reproductive tract, to include cerebellar hypoplasia, leukomalacia, retinitis, retinal atrophy, cataracts, myocardial necrosis, metaphyseal osteosclerosis, orchitis, oophoritis and thymic atrophy as possibly being associated with BVDV infection. Additionally, there are reports of disease in other species to include white-tail deer, sheep, goats, mouse deer, mountain goats and alpacas.

The economic impact of the BVDV is significant, with the bulk of the expenses due to the acute transient infections which can compromise the immune system and exacerbate other bacterial or viral infections. Eradication efforts have been underway throughout Europe and in areas within the U.S. with restricted animal movement such as in the Upper Peninsula of Michigan. These efforts focus on identifying the beating heart of the disease, the reservoir and source of infection known as the persistently infected (PI) calf.

As the contributor describes in detail, the PI calf develops in-utero following exposure to the virus, specifically a non-cytopathic (ncp) strain. For the calf to become immunotolerant to BVDV, the virus must avert the immune system by infecting the fetus before the humoral immune system is fully developed (~125 days gestation). Also, the virus must suppress the innate immune response through inhibition of type I IFN signaling. Type I IFN signaling is normally stimulated through the recognition of viral double-stranded (ds) RNA via TLR 3 (as discussed in case I) or RIG-1. Non-cytopathic strains, which already have less dsRNA to begin with compared with cytopathic strains, achieve this suppression through the expression of Ems glycoprotein. Ems exhibits RNAse activity and degrades extracellular RNA, thus decreasing stimulation of TLR 3 and RIG-1. Similarly, the glycoprotein Npro degrades IRF-3 which is also involved in IFN signaling, though in conjunction with IRF-7 through TLR 7, 8, and 9. Expression of both Ems and Npro is necessary to induce persistent infection. These underpinned mechanisms of disease pathogenesis elaborately correlate with the ncp strain’s ability and cp strain’s inability to produce a PI animal.

The difference between cp and ncp strains was demonstrated in vitro as the ability to induce apoptosis in bovine turbinate cells. This corresponds with the continual expression of NS3 protease in cp strains versus little to no expression.
in ncp strains. NS3 induces apoptosis through both intrinsic (caspase 9) and extrinsic (caspase 8) pathways. Activated caspase 8 incites apoptosis of B- and T-lymphocytes in ileal Peyer’s patches, which may correlate activation of the extrinsic pathway with the Peyer’s patch necrosis observed in this case. Additionally, the antiapoptotic BCL-2 protein is upregulated by ncp BVDV, which may also relate to the difference in disease manifestation between the two strains.4

Bovine pestivirus shares its genus with other prominent diseases of domestic animals such as Classical swine fever virus and Border disease virus of sheep. Additionally, a closely related virus that causes similar clinical presentations as BVDV has been circulating in Brazilian cattle with reports of cases in Europe and Asia as well indicating possible widespread dissemination. To date, this virus has been called “Hobi-like”, “BVDV-3” or “atypical pestivirus”2, and in time may add itself to the list of economically important Pestiviruses.

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References: