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Conference Moderator: Dr. Fabio Del Piero, DVM, Diplomate ACVP
New Bolton Center
University of Pennsylvania

CASE I – POO-208 (AFIP 2739286)

Signalment: Piglet, six-weeks-old, mixed breed, castrated male.

History: This piglet was submitted from a 300 sow farrow to finish operation farm in southern Taiwan. Piglets were initially healthy when weaned at 4 weeks of age; at the early post-weaning period, 20% of the nursery piglets lost condition and became sick, showed poor growth rate, unthriftiness, dyspnea and pallor. Eventually piglets became emaciated with rough hair and some died or were culled; an increased mortality rate was reported in the weaner facility.

Gross Pathology: The piglet showed gauntness with poor nutritional state; pallor of skin and mucous membranes were noted upon external examination. At necropsy, enlarged lymph nodes were the most obvious lesion, particularly in inguinal, mesenteric, bronchial and mediastinal lymph nodes. Lungs were diffusely mottled and firm; few fibrinopurulent strands were adhered in the peritoneal cavity.

Laboratory Results: Positive for Type II porcine circovirus by polymerase chain reaction (PCR) and transmission electron microscopic examination of bronchial lymph node.
Negative for porcine reproductive and respiratory syndrome virus (PRRS) by pulmonary virus isolation.
Negative for swine influenza virus (SIV) by tracheal endothelial cellular virus isolation.
Negative for pseudorabies virus (PRV) by immunohistochemical staining of section of lymph node using pseudorabies monoclonal antibody.
Positive for *Streptococcus* sp. from peritoneal fluid.

Contributor's Morphologic Diagnoses:
1. Lymph node (mesenteric): Lymphadenitis, granulomatous, diffuse, moderate, with intrahistiocytic polymorphous eosinophilic to basophilic cytoplasmic inclusion bodies.
2. Tonsil: Tonsillitis with lymphocytolysis and rare intrahistiocytic basophilic cytoplasmic inclusion bodies.

Contributor's Comment: This case is consistent with post weaning multisystemic wasting syndrome (PMWS). In the submitted histologic sections of lymphoid organs, grape-like clusters of basophilic inclusion bodies are observed in epithelioid macrophages. Occasional multinucleated giant cells can be seen in the germinal centers of lymphoid follicles, paracortex and medullary and subcapsular sinusoids. There was evidence of mild lymphocytic depletion and increased pyknosis in lymphoid follicles. Variable eosinophilic and neutrophilic infiltration was also seen. Other lesions in this piglet were interstitial pneumonia, fibrinous peritonitis and lymphohistiocytic infiltrates within Peyer's patches.

Transmission electron micrography of cytoplasmic inclusions from the lymph node showed paracrystalline arrays of circovirus-like viral particles; PCR test from fresh lymph node also confirmed Type II porcine circovirus (PCV) in this tissue.

Porcine circovirus belongs to the family Circoviridae which also includes chicken anemia virus and psittacine beak and feather disease. Circoviruses are the smallest animal viruses using genome size and virion diameter as qualifiers. Another interesting feature of this family is that these are the only animal viruses that possess a circular single-stranded DNA genome. These three viruses do not share common antigenic determinants or DNA sequence homology.

PCV is a non-cytopathic virus that was isolated in 1974 from a pig kidney cell line (PK15). The line was shown to be persistently infected with porcine circovirus. At the time of its discovery, it could not be shown to cause disease in pigs. According to antibody titers, the virus has a high prevalence in both European and Canadian swine populations. Presently PCV is thought to play a role in postweaning multisystemic wasting syndrome (PMWS) in pigs. Postweaning multisystemic wasting syndrome was first descried in 1996 and to date, cases have been confirmed in the western provinces and the province of Quebec in Canada, in California, Indiana, Iowa, Spain, Ireland, UK, Japan, Korea and also in Taiwan.

Pigs with PMWS are generally affected two to three weeks post weaning. Signs may be exacerbated by stress. In acute outbreaks mortality may reach 10% but will be lower in endemically infected herds. The most common clinical findings of PMWS are wasting, dyspnea and enlarged lymph nodes. Pallor, jaundice and watery diarrhea are seen in some cases. Histopathological findings may include

interstitial pneumonia and basophilic intracytoplasmic inclusion bodies may be found in lymph nodes, tonsil, and spleen.

In Taiwan, postweaning multisystemic wasting syndrome (PMWS) characterized by progressive weight loss and dyspnea was first diagnosed in 1997. Since then the disease has become widespread here in Taiwan and is quite commonly found in nursery and pre-growers. The disease was first found in the major pig producing prefectures of southern Taiwan in weaning pigs, and is most often seen in pigs 5-10 weeks old. From the cases of Pig Research Institute Taiwan, we find that now it quite commonly exists in many pig farms. This disease is seen in herd sizes from small farms to large farrow to finish operations. In our cases, PMWS is commonly diagnosed in herds complicated with other respiratory diseases, including mycoplasmosis, salmonellosis, and PRRS which therefore have masked damages caused by the disease. In an acute outbreak, the postweaning mortality rate peaks at 10～15% (monthly calculation) and the worst case may reach 25%, especially during the winter.

The lack of specific gross lesions makes this disease syndrome extremely ambiguous, especially when farms experience this important disease concurrent with other infections such as salmonellosis and PRRS. Because damage was masked by the concurrent pathogens, pig producers here all seem to attribute the losses to other pathogens. As more information was found regarding the exact role of PCV in the pathogenesis of PMWS, which recently has been reproduced by co-infection with parvovirus, pig producers and veterinarians in Taiwan are just starting to be aware of the damage caused by the syndrome. Researches here are focused on the epidemiology and molecular features of PMWS.

AFIP Diagnoses: 1. Lymph node, mesenteric (per contributor): Lymphoid depletion, diffuse, moderate, with sinus histiocytosis, multifocal draining neutrophilia, and intrahistiocytic intracytoplasmic botryoid inclusion bodies, mixed breed, porcine.
2. Tonsil: Lymphoid depletion, diffuse, moderate, with intrahistiocytic intracytoplasmic botryoid inclusion bodies, mixed breed, porcine.

Conference Comment: The contributor provides an excellent review of porcine circovirus-2 (PCV-2) and postweaning multisystemic wasting syndrome (PMWS) and stresses the importance of the virus in co-infection with other common porcine viruses such as porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). Conditions currently associated with PCV-2 include PMWS, porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory

disease complex, reproductive failure, and most recently, granulomatous enteritis, necrotizing lymphadenitis and possibly exudative epidermitis (5).

A proposed pathogenesis for PMWS involves coinfection by PCV-2 and porcine parvovirus (PPV) which localize in the regional histiocytes of the oropharynx. In the host, PPV spreads via cell-associated viremia, replicates in lymphoid tissues, and stimulates lymphocyte and macrophage proliferation thereby resulting in PPV protective immunity. PCV-2 slowly replicates in macrophages but immunity to PCV-2 is not established. Due to the immunoproliferative response to the PPV infection, PCV-2 replication within macrophages is heightened and dissemination occurs as a macrophage-dominant pantropic granulomatous response recognized clinically as PMWS.

The clinical signs of PMWS include wasting and unthriftiness, dyspnea, lymphadenopathy and, less frequently, pallor, diarrhea and icterus.

Typical gross findings include a failure of the lungs to collapse and lobular atelectasis, lymphadenopathy (especially of the superficial inguinal lymph nodes), icterus, hepatic atrophy, and enlarged kidneys with cortical white foci and peripelvic edema.

The characteristic histologic lesions of PMWS are granulomatous inflammation with large, grape-like amphophilic or basophilic intracytoplasmic inclusion bodies within histiocytes and multinucleated giant cells (6).

Circoviruses of veterinary importance include: the agents of postweaning multisystemic wasting syndrome and psittacine beak and feather disease, chicken anemia virus and pigeon circovirus.

Contributor: Department of Pathobiology, Pig Research Institute Taiwan, P. O. Box 23, Chunan, Miaoli, Taiwan 350

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CASE II – 05-9006 (AFIP 2985005)

Signalment: 2 days old, gender not identified, quarter horse, *Equus caballus*, equine

History: On the affected farm 7 out of 8 neonatal foals died. One foal was necropsied by the submitting veterinarian. At necropsy, this 2 day old foal had no perirenal fat, no milk curd in the stomach, and ecchymoses in the lung, heart, and kidneys. The kidneys were described by the submitting veterinarian as being "trashed".

Gross Pathology: Both kidneys had 1-3 mm, coalescing suppurative foci that were almost entirely limited to the renal cortex (Fig. 1).

Laboratory Results: Foal serum IgG – 800 mg/dL
Actinobacillus equuli was isolated from the kidney, lung, liver, and spleen.

Histopathologic Description: Randomly distributed throughout the renal cortex and rarely in the medulla are accumulations of degenerating and intact neutrophils. These foci of neutrophils replace approximately 50% of the renal cortical parenchyma; occasional clusters of coccobacillary bacteria are present in the foci. Occasional glomeruli are partially obliterated by neutrophilic infiltrates. Hemorrhage and congestion is present in the remaining normal renal parenchyma.

Contributor's Morphologic Diagnosis: Nephritis, suppurative, multifocal, coalescing (embolic)

Contributor's Comment: In horses, the most common cause of embolic suppurative nephritis is *Actinobacillus equuli*, which is acquired *in utero*, during parturition, or shortly after birth. If foals survive for several days, microabscessation is seen in the kidneys and other organs, and polyarthritis is present.[1]

Actinobacillus equuli may cause mortality rates approaching 100% in neonatal foals. Infections occur worldwide, but appear to be declining. The infection in foals is sometimes referred to as sleepy foal disease, with foals that are sleepy or comatose at birth or soon afterwards. These so-called sleepers may be aroused but quickly revert to a comatose state. Foals infected with *A. equuli* often have low plasma immunoglobulin G.[2] In foals, serum IgG concentrations above 400 mg/dL are associated with protection against septicemic disease and concentrations above 800 mg/dL are sufficient to reduce the risk of infectious disease in most environments.

Actinobacillus equuli is a member of the Pasteurellaceae family. It has been associated with arthritis, bronchitis, pleuritis, pneumonia and peritonitis. The organism can be isolated from the oral cavity of healthy horses.[3] *Actinobacillus equuli* has recently been reclassified, with nonhemolytic isolates named *Actinobacillus equuli* subsp. *equuli*, and hemolytic isolates named *Actinobacillus equuli* subsp. *haemolyticus*.[4] Culture supernatants of hemolytic strains of *A equuli* are toxic to equine neutrophils. An RTX toxin/hemolysin from a hemolytic isolate of *A. equuli* has been characterized.[5]

Embolic suppurative nephritis occurs when bacteria alone or in small clumps, and small septic emboli lodge mainly in glomerular and peritubular capillaries. Larger emboli can lodge in afferent vessels of the kidney and produce septic infarcts, which may be unilateral. Developed abscesses are generally cortical rather than medullary but in bacteremia caused by Gram-negative enterobacteria, microscopic suppurative foci may be scattered in the medulla.[1]

AFIP Diagnosis: Kidney: Nephritis, suppurative, embolic, severe, with colonies of coccobacilli, Quarter Horse, equine.

Conference Comment: The contributor provides a thorough review of this classic disease of foals.

Like other Gram-negative bacteria, *Actinobacillus equuli* contains endotoxin, a lipopolysaccharide (LPS), which is a major component of the cell wall and has widespread effects, including those on macrophages, endothelium, platelets, Hageman factor, neutrophils, complement and mesenchymal cells. LPS-LPS binding protein complexes in the blood bind to CD-14 receptors and Toll-like receptor protein-4 (TLR-4) found on macrophages, neutrophils, dendritic cells, NK cells, mucosal epithelial and endothelial cells. Signals from TLR-4 initiate a number of cytokines and activate leukocytes and vascular cell walls (6). Macrophages become activated and produce interleukin-1 (IL-1) and tumor necrosis factor alpha

(TNF-A). Once activated, the endothelium synthesizes adhesion molecules, other cytokines, growth factors, eicosanoids, nitric oxide, and thrombogenicity is increased. Reactive oxygen species produced in the respiratory burst of activated macrophages have toxic effects, such as cell membrane lysis and extracellular membrane degradation. Endotoxin damages endothelium directly or indirectly. Damage to the endothelium and exposure of subendothelial collagen, will initiate the coagulation cascade by the intrinsic pathway. The extrinsic pathway is activated by thromboplastin-like procoagulant activity (PCA) expressed by endothelial cells and activated macrophages. Endotoxin stimulates platelets to make phospholipid more available for coagulation, and to synthesize and release thromboxane A2, which induces platelet aggregation. Endotoxin also activates factor XII (Hageman factor), and the alternate pathway of complement. Neutrophils are directly primed by endotoxin to increase their response to mediators; they are attracted by IL-1, complement and leukotrienes, and activated by TNF-A and complement. Endotoxin also stimulates mesenchymal cells to release proteolytic enzymes.

Besides the "sleepy foal" or comatose state, affected foals may present with fever, diarrhea and hot, painful, swollen joints.

Necropsy findings vary from no gross lesions in cases of acute death to severe enteritis, fibrinopurulent polyarthritis and polysynovitis, multiple small visceral abscesses and kidneys with multiple, grey, evenly distributed small cortical abscesses and medullary hemorrhage. In the conference, it was also pointed out that another target organ besides the kidney can be the adrenal gland. Additional information on a case of *A. equuli* causing adrenalitis can be found in the WSC archives; see Conference 28, Case 3, 1995.

Conference attendees briefly discussed other frequent causes of foal septicemia. A short list includes: *Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., and *Salmonella* spp. Additionally, failure of passive transfer is often associated in cases of neonatal septicemia; however, in this case the foal's immunoglobulin levels were at normal levels.

Contributor: Kansas State University
http://www.vet.ksu.edu/depts/dmp/index.htm

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CASE III – 05-21199 (AFIP 2988328)

Signalment: 4 year old female Thoroughbred horse

History: This mare had a 3 furlong workout, became wobbly while still on the track, collapsed, and died.

Gross Pathology: The mare was in good, athletic body condition. Approximately 1 liter of frank blood was free within the thorax and 0.5 liter of blood was present within the pericardial sac. Multiple poorly demarcated pale yellow linear subendocardial plaques were present in the left ventricle and on the left side of the interventricular septum. The liver had an enhanced lobular pattern.

Histopathologic Description: Multiple sections from the left ventricle and interventricular septum were examined and each had marked endocardial thickening due to fibrosis, endocardial fibrillar degeneration and mineralization, endocardial mucinosis, and infiltration of few foamy macrophages. Subendocardial Purkinje fibers were swollen and degenerate, with variably extensive cytoplasmic vacuolation. In the liver, few small (150-250 um) random to periportal foci of necrosis were infiltrated by low numbers of lymphocytes, plasma cells, and degenerate neutrophils.

Contributor's Morphologic Diagnoses: 1. Endocardial fibroelastosis with Purkinje cell degeneration
2. Mild necrotizing hepatitis and cholangitis (likely related to bacterial showering from intestine and of no clinical significance)

Contributor's Comment: Endocardial fibroelastosis (EFE) has been described in humans (1), cats (2), horses (3,4), and cattle (5). Primary EFE is unaccompanied by other cardiac lesions, whereas secondary EFE develops in conjunction with congenital cardiac anomalies, myocarditis, glycogen storage diseases, and carnitine deficiency (1,2,6). Primary EFE is an inherited disorder in humans and Burmese cats (6). In humans, both forms of EFE commonly result in sudden death in very young children (1). The pathogenesis of primary EFE is not clear, but may be related to impairment of drainage from myocardial lymphatics (6).

Equine endocardial fibroelastosis is an uncommon condition that has previously been associated with sudden death following exercise in 2 young Thoroughbreds (3). An additional case report described left-sided heart failure in a 4 month old Thoroughbred foal with similar endocardial lesions (4). No additional gross or histologic lesions were evident in heart in these previous cases, with the exception of mitral valve insufficiency in the foal that was assumed to have resulted from abnormal distensibility of the left atrium (4). This lesion in horses is presumed to be congenital, due to the young age of the 3 animals involved in these case reports and the absence of other predisposing inflammatory or degenerate lesions in endocardium or myocardium (3).

Entrapment and subsequent degeneration of Purkinje fibers within the fibroelastic tissue, with resultant conduction disturbances, has been hypothesized to be the cause of sudden death in humans and animals with endocardial fibroelastosis (6). Vacuolation and degeneration of Purkinje fibers was evident in the horse presented here and has been described in affected Burmese cats (6), although this lesion was not described in the other 3 equine cases described in the literature. Mineralization within foci of endocardial fibroelastosis, as identified here, was also not described in previously reported cases of EFE. Endocardial mineralization without proliferation of fibroelastic tissue has been reported in acute vitamin D toxicosis and lymphosarcoma in horses, although foci of metastatic mineralization were also described in other anatomic sites in affected animals (7,8).

In other species affected with EFE and in this case, hearts in the early stages of EFE may have a normal gross appearance (6). In advanced stages of the disease, the endocardium appears grossly thickened and opaque, with lesions mainly involving the left ventricular endocardium. Other cardiac-related causes of sudden death during or subsequent to exercise in equine athletes that present with anatomic lesions include rupture of great vessels (9), myocarditis, and myocardial interstitial fibrosis involving the cardiac conduction system (10). Both myocarditis and myocardial fibrosis are presumed to result in interference with normal conduction pathways, leading to potentially fatal arrhythmias. Conduction disturbances due to electrical or biochemical (electrolyte) aberrations generally

produce no specific morphologic lesions that can be identified at necropsy. In humans, an excess of circulating catecholamines during exercise, in combination with hypoxia-induced myocardial sensitization related to variations in heart rate and systolic blood pressure, is thought to promote potentially fatal arrhythmias (11).

AFIP Diagnosis: Heart: Endocardial fibroelastosis, diffuse, moderate, Thoroughbred, equine.

Conference Comment: Conference attendees agreed that bundles of Purkinje fibers were completely surrounded by fibrous tissue but debated whether the vacuolated and lacey appearance of the Purkinje fiber's cytoplasm indicates they are undergoing degeneration or if the vacuoles indicate glycogen storage and are a normal finding. There was additional debate over whether the lesion was predominantly fibrotic or fibroelastic. Haphazardly arranged aggregates of deeply eosinophilic fibers were suspected to be elastic fibers. Subsequent staining with modified Russell-Movat pentachrome, confirms they are elastic fibers as they are black and are surrounded by yellow collagen fibers.

In addition to an excellent review of EFE, the contributor also provides a list of other cardiac-related causes of sudden death in equine athletes. Although many questions still remain to be answered concerning equine EFE, increased reports of the disease warrants inclusion in the differential diagnosis for unexplained sudden death, especially in young equine athletes.

Contributor: Animal Health Laboratory, University of Guelph, P.O. Box 3612, Guelph, Ontario, Canada N1H 6R8
http://ahl.uoguelph.ca

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CASE IV – 05-2007 (AFIP 2984970)

Signalment: Six-week-old, male, Jersey calf, bovine

History: Sudden death in pen raised calves in a dairy beef operation.

Laboratory Results: *Salmonella dublin* was isolated from lung, and small intestine

Histopathologic Description: Random foci of hepatocellular necrosis occur throughout the liver section. The foci are roughly nodular in outline and contain variable numbers of macrophages and neutrophils. In the lung, there is expansion of the interstitial space by mononuclear cells and a few neutrophils. Alveolar spaces contain fibrillar eosinophilic material consistent with fibrin commingled with erythrocytes, neutrophils and macrophages in small numbers. Scattered foci of hemorrhage involving large numbers of adjacent alveoli and a few bronchioles are present. The interlobular septa are widened by proteinic fluid and contain dilated vessels with similar fluid.

Contributor's Morphologic Diagnoses:
Acute, multifocal, severe, necrotizing hepatitis (Etiology: *Salmonella dublin*)
Acute, diffuse, moderate, interstitial pneumonia (Etiology: *Salmonella dublin*)

Contributor's Comment: *Salmonella typhimurium* and *Salmonella dublin* are the most common agents causing salmonellosis in the bovine. Both adults and calves

may be infected. Enteritis and septicemia may occur in both calves and adults and cows may abort. Clinical signs include, fever, inappetence, and depression. In calves with *Salmonella dublin* infection clinical signs of pneumonia and septicemia predominate. When enteritis is present, there is the production of foul smelling, liquid feces that may contain blood and mucus.[1]

Salmonella typhimurium has been reported as the most common serotype recovered from sick cattle. However, *Salmonella dublin* is a common isolate in Europe. In the United States, *Salmonella dublin* was once common only west of the Rocky Mountains. However, in recent decades it has been moving eastward and isolated with increasing frequency from veal and dairy beef operations.[2]

Carrier animals are an important source of infection. Carrier animals harbor the infection in lymph nodes and visceral organs and shed bacteria intermittently in feces and milk. A carrier state appears to be more common in heifers infected between one-year-of-age and first calving and for cows if infected around parturition. In Denmark, the risk was higher in late winter to early spring. The risk of a carrier state was highest in herds currently experiencing an outbreak of clinical disease.[3]

The *spv* gene is a virulence factor commonly found in Salmonella isolates that are host adapted to animals including *S. dublin*, *S. choleraesuis*, *S. gallinarum-pullorum* and *S. abortusovis*. Salmonella species with a broad host range such as *S. typhimurium* and *S. enteritidis* have only a variable proportion of isolates that carry this virulence factor. The gene encodes a MetR/LlysR-type transcriptional activator. The gene is strongly expressed following uptake into eukaryotic cells. It apparently does not affect colonization or invasiveness in the bowel. Rather, it increases the growth rate of the bacteria in the intracellular compartment.[4]

The lesions of *Salmonella dublin* infection in calves are characteristic of an acute septicemia. Random foci of hepatocellular necrosis with infiltrates of macrophages (typhoid nodules) are seen in the liver. Histologic changes in the lung are characterized by acute injury to the interstitium with exudation of fibrin and inflammatory cells into the alveolar spaces.

AFIP Diagnoses: 1. Liver: Hepatitis, histiocytic and lymphocytic, necrotizing, random, moderate, Jersey, bovine.

2. Lung: Edema and hemorrhage, diffuse, acute, marked, with intraalveolar fibrin.

Conference Comment: There are over 2,300 serovars of *Salmonella* belonging to the genus *Salmonella*, family *Enterobacteriaceae*. *Salmonella* are Gram-negative,

flagellated, facultatively anaerobic bacilli that possess three major antigens: H antigen or flagellar antigen; O or somatic antigen; and Vi antigen, a superficial antigen present in very few serovars (e.g. *S. typhi*) (5). *Salmonella* live in the intestinal tract of vertebrates and are passed into food, water and the environment through fecal contamination. In animals, salmonellosis manifests itself in three basic forms: 1) the enteric form characterized by watery diarrhea which may contain mucous, fibrin or blood and has a characteristic "septic-tank" odor; 2) the septicemic form characterized by fever, inappetence and depression and 3) the abortion form. Some serovars, such as *S. dublin* in cattle, have a higher incidence of causing abortion than others. Most species of *Salmonella* are host adapted to either humans, specific animals or to both (e.g. *S. typhimurium, S. enteritidis*).

Salmonella infection often results in a carrier state where the host does not suffer from the disease but sheds large numbers of the bacteria into the environment. Active carriers are oftentimes animals that have recovered from the disease and continue to shed bacteria in their feces.

Salmonella are ingested by the host and subsequently adhere to and penetrate intestinal enterocytes, multiply intracellularly, and invade deeper tissues where they are phagocytosed by macrophages and neutrophils. They are transported within phagocytic cells via the lymphatics to regional lymph nodes and ultimately to the reticuloendothelial system of the spleen and liver.

Intestinal infection results in mucosal degeneration, neutrophilic inflammation of the mucosa and lamina propria, thrombus formation within vessels of the lamina propria and shortening of intestinal villi. Young animals are more susceptible to infection due to immature intestinal microflora, which normally inhibit *Salmonella* growth in mature animals (1).

Virulence factors associated with *Salmonella* include: a heat-labile enterotoxin that is related to cholera toxin; at least three cytotoxins which induce intestinal cell damage, lipopolysaccharide (LPS), variably active flagella which affect functional motility and invasiveness, siderophores or iron chelators which bind to host iron, and both plasmid and chromosomal virulence genes (1).

Typical gross necropsy findings include enteritis which can range from catarrhal to fibrinous and hemorrhagic with characteristic Peyer's patch necrosis. Differentials for Peyer's patch necrosis and fibrinohemorrhagic enteritis include *Salmonella* sp. enteritis, bovine pestivirus infection (BVD) and bovine morbillivirus infection (Rinderpest).

Contributor: Arizona Veterinary Diagnostic Laboratory

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Signature Authenticated by Approve
Approved by: Carl I Shaia,
:Monday, 05 December, 2005 at 12:51:

Carl I. Shaia, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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