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

## WEDNESDAY SLIDE CONFERENCE 2019-2020

# C o n f e r e n c e 16

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**CASE I:** S1809996 (JPC 4135077).

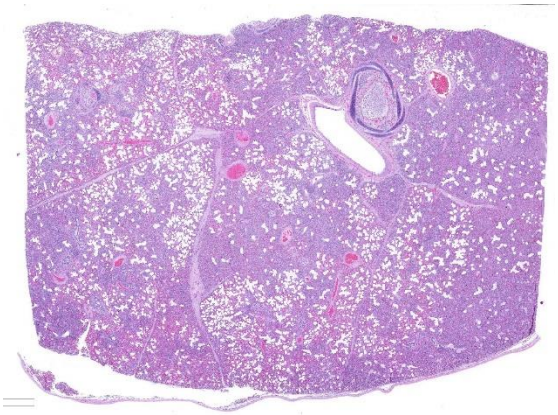
**Signalment:** A 3-month-old, male, mixed-breed pig (*Sus scrofa*)

**History:** This pig had no previous signs of illness, and was found dead.

**Gross Pathology**: Approximately 70% of the lungs, primarily in the cranial regions of the lobes, were patchy dark red, and firm compared to the more normal areas of lung.

**Laboratory results:** Porcine reproductive and respiratory syndrome (PRRS) PCR was positive from splenic tissue, and PRRS IHC was strongly immunoreactive within the cytoplasm of macrophages in the affected lung tissue. Porcine influenza virus PCR, porcine circovirus – 2 IHC, and *Mycoplasma hyopneumoniae* IHC were all negative. Small numbers of *E. coli* were isolated from the cranioventral lung with aerobic culture.

**Microscopic Description:** The interstitium within the section is diffusely infiltrated by moderate to large numbers of predominantly mononuclear cells along with edema. There is abundant type II pneumocyte hyperplasia lining alveolar septae and many of the alveolar spaces have central areas of necrotic macrophages admixed with other mononuclear cells and fewer neutrophils. Occasionally there is free nuclear basophilic

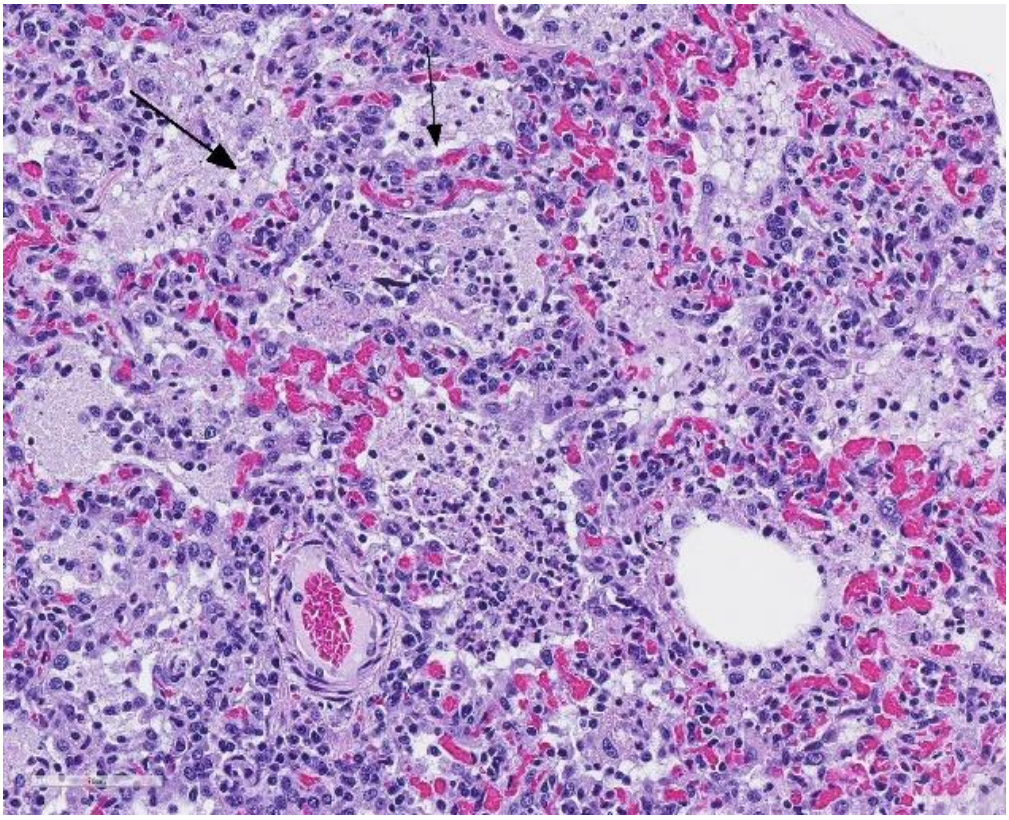


***Lung, pig (HE, 6X). There is diffuse consolidation of the lung. At low magnification, airway are filled with exudate and the pleura and interlobular connective tissue are mildly expanded.***

debris in these alveolar spaces as well as proteinaceous fluid. Bronchioles are occasionally variably filled with neutrophils, and many regions of BALT are mildly hyperplastic.

**Contributor's Morphologic Diagnosis:**

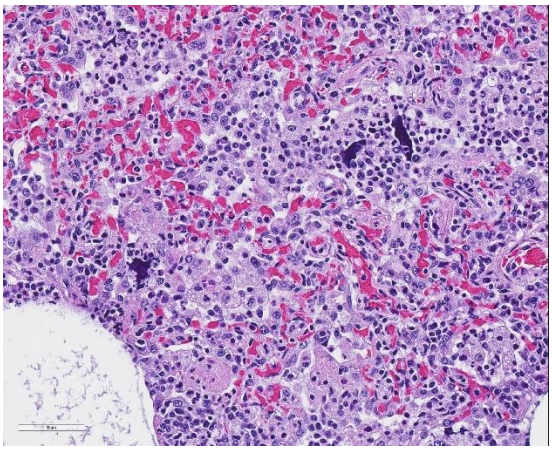
1. Lung: severe, acute, regionally extensive to patchy, interstitial pneumonia with marked type II pneumocyte hyperplasia and intra-alveolar macrophage necrosis
2. Lung: mild, acute, multifocal, cranioventral bronchopneumonia with mild BALT hyperplasia



***Lung, pig. Alveolar septa (arrows) are markedly expanded by a combination of macrophages (infiltrating and activated intravascular), congestion and edema. (arrows). Alveoli are filled with viable and degenerate neutrophils,***

**Contributor's Comment:** Gross and histologic lesions were consistent with a patchy, severe interstitial pneumonia in this case. The most predominant features of the pneumonia were abundant type II pneumocyte hyperplasia as well as the large number of necrotic macrophages admixed with basophilic nuclear debris within the alveolar spaces. These histologic features are highly suggestive of porcine reproductive and respiratory syndrome (PRRS) virus induced interstitial pneumonia; however these features are not always present depending on the chronicity of the pneumonia, among other variables.[2] PRRS virus was detected in the affected lung with PCR and large numbers of macrophages had strong cytoplasmic immunoreactivity with PRRS IHC.

PRRS is caused by PRRS virus, an arterivirus, and is characterized by two overlapping disease presentations, reproductive impairment or failure, and respiratory disease in pigs of any age.[4] It causes estimated yearly economic losses of 660 million dollars in the USA and similar losses in most other countries.[1] The respiratory syndrome is seen more often in young growing pigs but also occurs in naïve finishing pigs and breeding stock.[4] In the respiratory syndrome, the virus is transmitted from an infected pig to the tonsil



***Lung, pig. Deeply basophilic aggregates of degenerated chromatin are often reported in in cases of PRRS. (HE, 354X)***

or upper respiratory system of another pig where primary replication occurs in lymphoid tissues.[4] Viremia follows and may persist for several weeks.[4] The virus infects and compromises the function of pulmonary alveolar and intravascular macrophages resulting in interstitial pneumonia and appears to increase susceptibility of the lungs to other pathogens.[2] Secondary bronchopneumonia is common as was present in the current case.

Systemic infection commonly results in lymphocytic infiltrates in multiple organs, including lymphoplasmacytic rhinitis, myocarditis, endometritis and myometritis, and lymphohistiocytic meningoencephalitis and choroiditis, characterized by perivascular cuffs, vasculitis, gliosis and glial nodules.[2] Vasculitis, fibrin thrombi and rarely fibrinoid necrosis may occur in any organ.[2] A mild mononuclear vasculitis within the brain was also present in the current case, presumably due to PRRS.

Some pigs survive infection and become carriers, which is epidemiologically the most significant aspect of the infection. Commonly, once in a herd, this virus will persist indefinitely as the virus causes immune dysregulation during a critical time in immunological development, allowing the virus to persist.[1] One recently proposed method for this persistence is altered thymocyte development due to the viral infection leading to "holes" in the T cell repertoire resulting in poor recognition of PRRSV and other neonatal pathogens.[1] Currently, there are no effective treatment protocols for acute PRRS infections, and vaccination has not been as efficacious as hoped.[1] Prevention is the primary means of control.[3]

**Contributing Institution:**

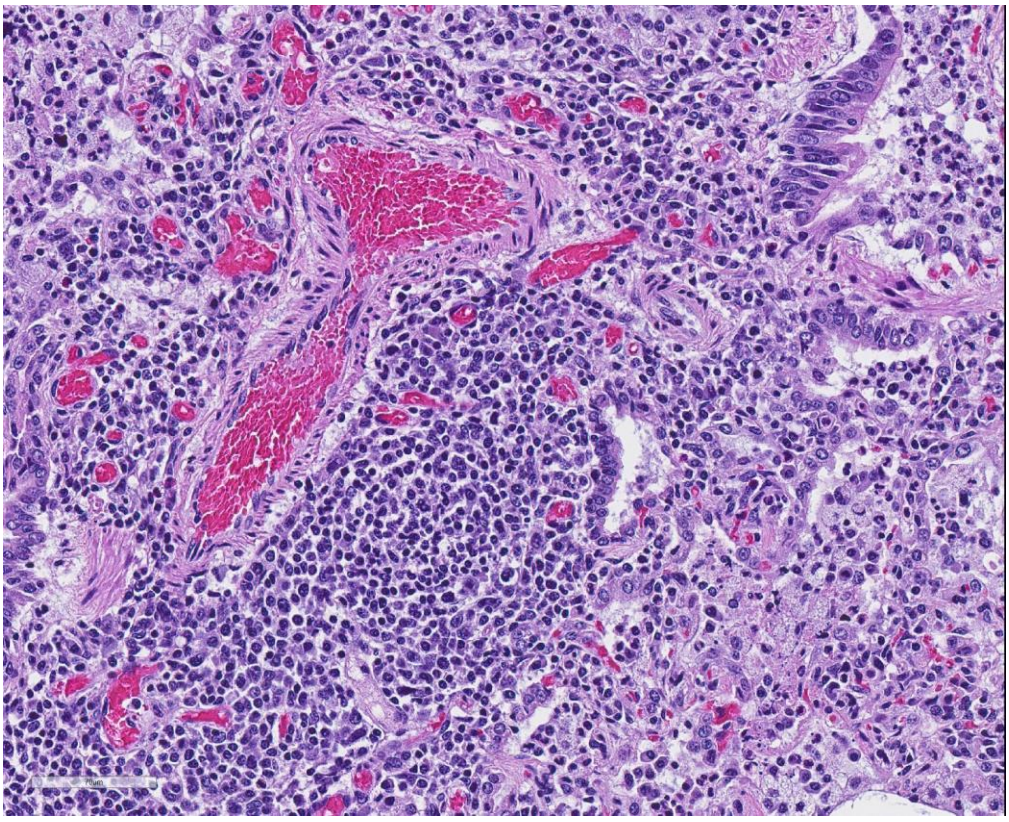
California Animal Health & Food Safety Laboratory, San Bernardino Branch

https://cahfs.vetmed.ucdavis.edu/locations/san-bernardino-lab

**JPC Diagnosis: JPC Diagnosis:** 1. Lung: Pneumonia, interstitial, lymphohistiocytic, diffuse, severe, with type II pneumocyte hyperplasia, intra-alveolar macrophage necrosis and marked peribronchiolar and perivascular lymphoid hyperplasia.
2. Lung: Bronchopneumonia, necrotizing and suppurative, diffuse, mild

**JPC Comment**: The contributor provided a concise but informative review of porcine respiratory and reproductive syndrome.

This particular virus is a global problem within the swine industry around the globe and one of its most costly and continuing problem. Two genotypes exist, type 1 (Europe) and type 2 (North America). A number of difficulties are associated with PRRS infection, including a high virus mutation rate which may result in outbreaks in previously vaccinated herds, prolonged intection, and shedding via a number of routes, to include ingestion, inhalation, venereal and transplacental routes.[3] Fortunately, the virus is relatively fragile in



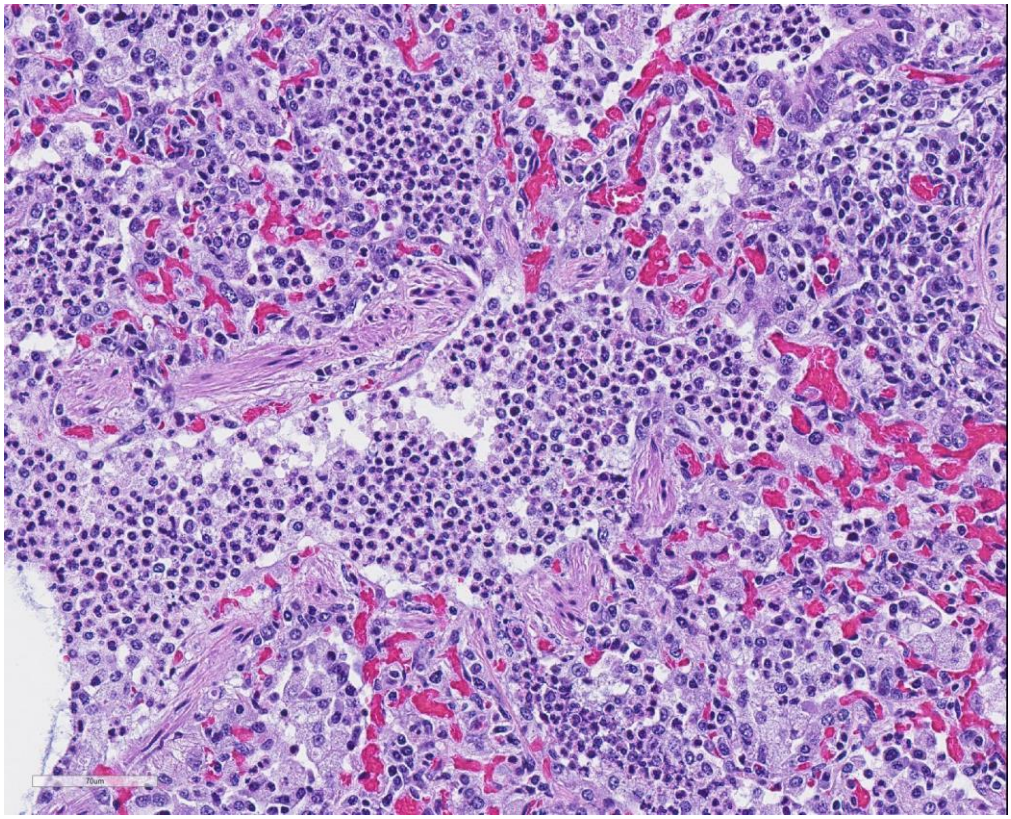
***Lung, pig. Large cuffs of lymphocytes and plasma cells surround vessels of all sizes. (HE, 315X)***

the environment.

PRRS infection generally revolves around macrophage infection, initially in tonsillar, nasal, or sites of pulmonary infection with infection of alveolar, interstitial, and intravascular macrophages, bronchiolar epithelium, and endothelium. Systemic viremia may occur in less than 12 hours with infection of macrophages, monocytes, and dendritic cells in many tissues. Within the lung, infection of macrophages results in markedly decreased macrophage phagocytosis, oxidative burst and cytokine release, predisposing the lungs to secondary bacterial infection. Additionally, the virus induces the release of the immunsuppressive cytokine IL-10 from infected macrophages, further diminishing both the innate and adaptive immune response.[3]Apoptosis of alveolar and intravascular macrophages are also common in infected animals but may not represent a consequence of viral infection.[2]

A number of reproductive abnormalities are seen in infected herds. In naïve herds, upt to 50% of sows may abort and 10% may die. It is a recognized cause of SMEDI (stillbirth, mummification, embryonal death and infertility). Hemorrhage of the umbilical cord due to fibrinoid necrosis and suppurative inflammation of the umbilical artery has been considered to be a characteristic gross lesion.[2] The persistence of one or more strains in endemic herds may result in continued losses from waves of abortions from successive generations of gilts.[2]

The presence of numerous necrotic macrophages and aggregates of bluish chromatin in the alveoli was considered diagnostic for PRRS by attendees. The moderator mentioned that while the presence of intra-alveolar necrotic macrophages are an excellent diagnostic feature for PRRS, they are not present in all cases. Some participants considered the presence of viable and degenerative neutrophils, especially within alveoli and smaller airways, evidence of a secondary



***Lung, pig. Airways and surrounding alveoli denuded of epithelium and filled with viable and degenerate neutrophils suggest the strong possibility of a concomitant bacterial pneumonia. (HE, 315X)***

bacterial infection further complicating the pneumonia in this pig. Attendees differed in their attribution of the BALT hyperplasia to either the interstitial pneumonia or a secondary bacterial infection.

While discussing this case, the moderator walked the participants through a number of differential diagnosis for lung diseases which should be tested for in cases such of this. Epitheliotrophic viruses such as swine influenza and swine coronavirus were discussed as potential viral agents, and bacteria such as *Streptococcus suis*, *Bordetella bronchiseptica*, and *Pasteurella multocida*. The moderator cautioned that some viruses, such as swine influenza, are present for only a short time in the epithelium, and that immunohistochemistry for viral antigen may be negative in more long-standing lesions. The moderator also raised the possibility of this case being a case of "proliferative and necrotizing pneumonia", an acute disease of weaner and grower pigs, in which severe acute disease results in dyspnea. In this condition the entire lobe, or only cranial and middle lobes may be affected, and lymphadenopathy is a consistent feature. The etiology of this condition is yet unknwon and may be the result of infection of certain strains of the PRRS virus, PCV, HeN2, alone or in combination.[2]

The presence of numerous necrotic macrophages and aggregates of bluish chromatin in the alveoli was considered diagnostic for PRRS, and the perivascular cuffs of lymphocytes and plasma cells were considered significant corroborative evidence for this diagnosisSome participants considered the presence of viable and degenerative neutrophils, especially within alveoli and smaller airways, evidence of a secondary bacterial infection further complicating the pneumonia in this pig.

**References:**

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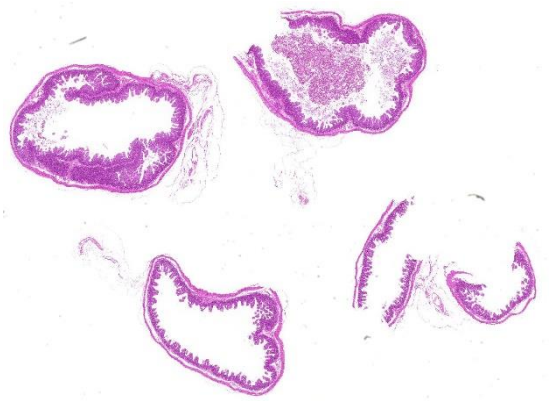
**CASE II:** 2015A (JPC 4167277).

**Signalment:** Four-day-old crossbreed piglet (*Sus scrofa domesticus*)

**History:** This pig had no previous signs of illness, and was found dead.

**Gross Pathology**: At necropsy, the stomach was filled with undigested curdled milk. The small and large intestines were distended by watery yellow-to-greenish undigested milk, and intestinal walls were thin. No gross lesions were detected in other organs.

**Laboratory results:** RT-PCR for porcine epidemic diarrhea (PED) virus (PEDV) and transmissible gastroenteritis (TGE) virus (TGEV) was performed, the expected size of amplification products for PEDV gene, but not TGEV gene, were obtained. The RT-PCR products were directly sequenced and confirmed as PEDV gene.



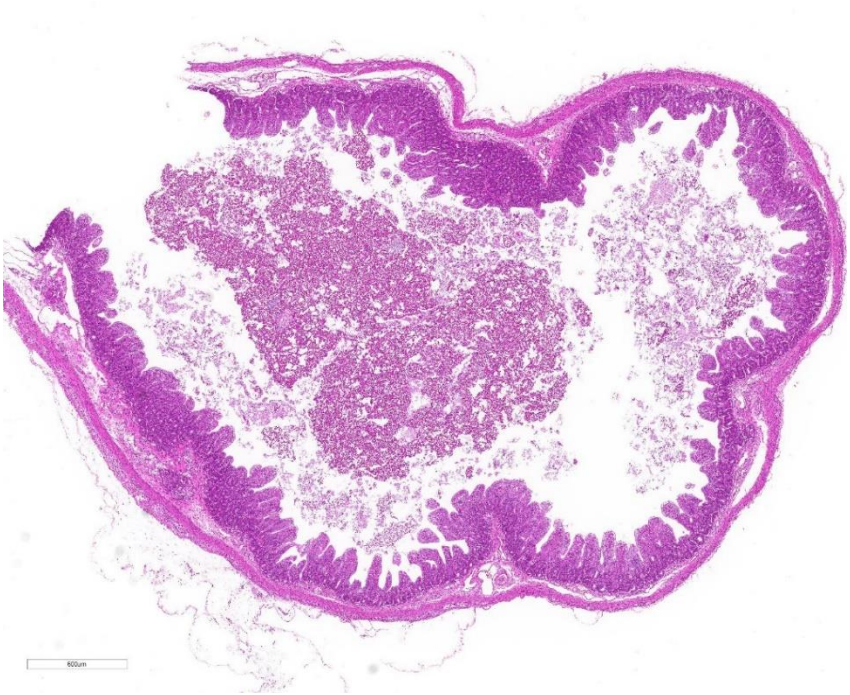
***Jejunum, 4-day-old piglet. Four cross-sections of jejunum are submitted for examination. (HE, 5X)***

**Microscopic Description:** Severe atrophy of villi was detected in all segments of the small intestine. There were vacuolated epithelial cells throughout the small intestine. Immunohistochemical analysis revealed presence of PED viral antigen in the cytoplasm of epithelial cells lining the jejunum. No histopathologic lesions were detected in other organs.

**Contributor Morphologic Diagnosis:**

Jejunum: Enteritis, villous atrophy, acute, diffuse, severe.

**Contributor Comment:** PEDV is an enveloped, positive-sense, single-stranded RNA virus that belongs to the order *Nidovirales*, family *Coronaviridae*, genus *Alphacoronavirus*. PED was first observed among English feeding and fattening pigs in 1971, and then emerged in many European, Asian and North American countries.[9] PED is a highly contagious disease of swine of all ages, and has become a devastating issue in many pig-raising countries in Asia and North America.[1,4,5,7] In October 2013, a PED outbreak was recurred and confirmed in



***Jejunum, 4-day-old piglet. Low magnification of a of cross section of jejunum shows marked villar blunting and fusion, as well as hemorrhage within the***

on the jejunum and ileum. The diffuse villus atrophy of the small intestine is characteristic of the two disorders.[9] The presence of PED virus is confirmed by immunohistochemistry and RT-PCR.[9]

**Contributing Institution:**

National Institute of Animal Health, Japan.
http://www.naro.affrc.go.jp

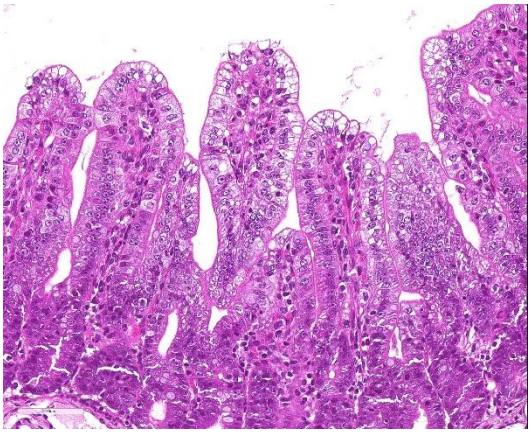
/org/niah/

**JPC Diagnosis:** Intestine: Villar blunting, diffuse, severe, with villar enterocyte vacuolar degeneration, villar fusion, and mild crypt hyperplasia.

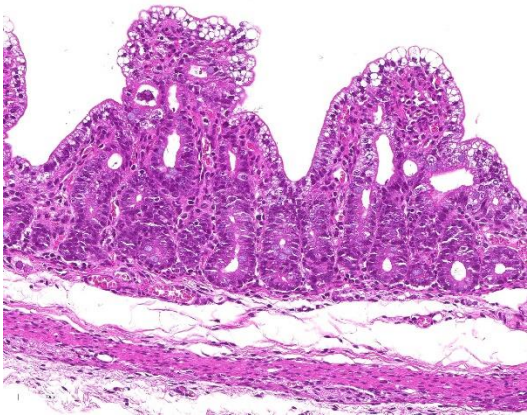
Japan after an absence of seven years in the country.[3] 38 out of 47 prefectures are affected until August 2014, and 817 have been affected among 5,570 farms.[3] PEDV isolates from this outbreak are genetically related to the PEDV isolates recovered from China and the USA in 2013.[3] Several new variants of PEDV emerged in the global pig population.[1,4,5,7] The spike protein of PEDV plays pivotal roles in viral entry and inducing the neutralizing antibodies in natural hosts.[6] Attenuated live vaccines using cell-culture-adapted PEDVs have long been used in Asia for the control of PEDV.[6,8] Recently, it was reported that an inactivated vaccine made from a U.S. field isolate is immunogenic in pigs.[2]

PED is characterized by vomiting and watery diarrhea, followed by dehydration, and a high mortality among suckling pigs.[5,9] Differential diagnoses for vomiting and diarrhea in pigs include PED and TGE, both of which have indistinct gross and histologic lesions centered mainly

**JPC Comment**: Porcine epidemic virus (PEDV) outbreaks were common in Europe in the 1970s and 1980s, and in Asia in the 1980s to 2000s. The first outbreak of PEDV in the United States occurred in April 2013 with explosive epidemics of diarrhea and vomiting affecting all ages, and resulting in 90-95% mortality in suckling pigs.[9]



***Jejunum, 4-day-old piglet. There is degeneration of villar tip enterocytes characterized by the presence of large vacuoles. (HE, 337X)***



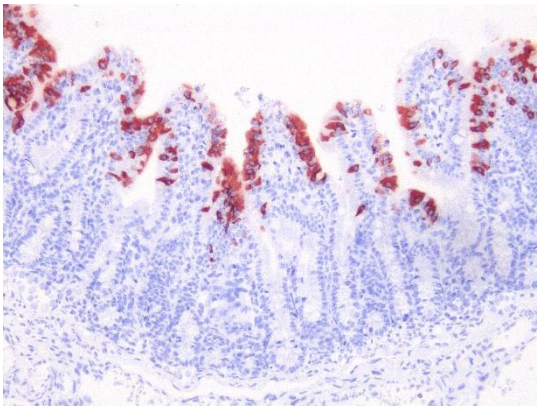
***Jejunum, 4-day-old piglet. Multifocally, remaining villi demonstrate fusion. (HE, 347X)***

In affected pigs, the main and often only sign of disease is watery diarrhea affecting up to 100% of pigs in all age groups, although piglets are more commonly affected. Experimental studies have demonstrated a 22- to 36-hour incubation period with viral replication in villous epithelium in all segments of the small intestine (viral replication can also been seen in colonic epithelium, but without obvious cellular degeneration).[9] Villus:crypt ratios in affected pigs are decreased from 7:1 to 3:1 or less. Characteristic vacuolar change of the villous epithelium resemble that seen with the coronavirus that causes transmissible gastroenteritis (TGE) in pigs, but are considered somewhat less pronounced.[9]

Clinical signs of disease on farms with naïve animals mimic those of epidemic transmissible gastroenteritis virus (TEGV) infection. Mortality is highest in piglets of 1 week of age or less, which often die of dehydration after 3-4 days of illness. Older pigs recover after about a week although recurrent diarrhea may occur in times of stress during intestinal repair, and sows may exhibit depression but no gastrointestinal signs.[10] The disease in the breeding facility is self-limiting, and tends to disappear once sows develop immunity and can produce colostral antibodies.[10]

Other coronaviruses of interest, but of less repute can also cause clinical disease in swine. A betacoronavirus known as hemagglutinating encephalomyelitis virus results in a disease known as vomiting and wasting disease. Infection is consided widespread among swine, although clinical disease is incommon. The virus, as the name suggests, possesses the ability to spontaneous agglutinate the erythrocytes of a number of species, including laboratory rodents.[10] The virus infects the respiratory epithelium of pigs less than 4 weeks of age, which lack protective antibodies. The virus ascends to the CNS via trigeminal, vagal, or spinal nerves. Vomiting in affected pigs is triggered by replication in the vagal sensory ganglion or in nerves that terminate in the vomiting center. Prolonged vomiting results in wasting; young piglets may die of dehydration, older pigs become emaciated.[10]

Another lesser known member of the Coronaviridae family is porcine torovirus, one of three species of totTorovirus, all of which rarely cause clinical disease. but they rarely cause clinical disease. In diarrheic pigs, seroprevalence for porcine torovirus



***Jejunum, 4-day-old piglet. PED antigen is restricted to villar enterocytes. (anti-PED, 400X) (Photo courtesy of: National Institute of Animal Health, Japan. http://www.naro.affrc.go.jp/org/niah/)***

may be high in many countries; however, concomitant enteric pathogens are simultaneously identifed in approximately 75% of cases so a direct link between porcine torovirus and true enteric disease is difficult to prove.[10]

The moderator mentioned the normal crypt ratio of 7:1 for a normal piglet, which demonstrates the marked villar blunting in this individual. A pathologist in the audience comment on the thin muscular tunics; the moderator noted that the muscular tunics of the intestine are thickest in proximity of the gastroduodenal junction and diminish caudally.

The moderator mentioned a number of other coronaviruses causing enteric disease in pigs including the porcine deltacoronavirus, and a bat-origin alphacoronavirus which resulting in almost 25,000 dead piglets on 4 farms in China in October 2017.

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Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, eds. *Diseases of Swine*. 10th ed. Ames, IA: Blackwell Publishing; 2012: 514-521.

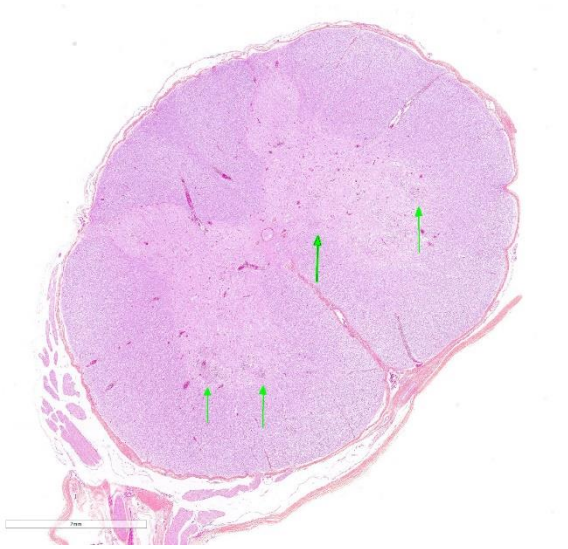
**CASE III**: P590-19 (JPC 4136500).

**Signalment:** 5½ week-old, neutered male, crossbred Landrace, Sus scrofa domesticus, domestic pig.

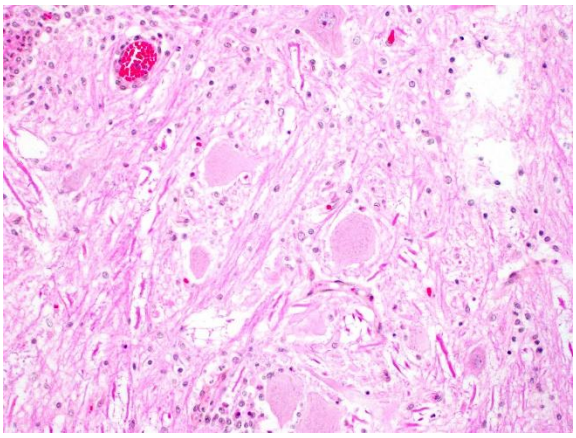
**History:** Two out of 950 weanling piglets showed clinical signs described as paresis progressing to paralysis affecting mostly the forelimbs; they were otherwise alert. The piglets were euthanized and rapidly brought to our diagnostic laboratory for a complete autopsy.

**Gross Pathology**: No gross lesions were seen.

**Laboratory results:** PCR for PRRSV at our institution was negative on samples of brainstem and spinal cord. Similar samples were sent to the Iowa State University's Veterinary Diagnostic Laboratory for PCR and were positive for porcine sapelovirus and negative for porcine teschovirus.



***Spinal cord, piglet. At low magnification, focal areas of inflammation are visible within the ventral horns (arrows). (HE, 5X)***



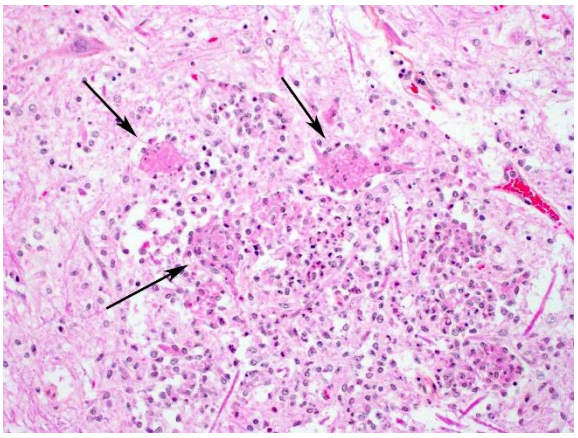
***Spinal cord, piglet. Throughout the grey matter, neurons in early stages of degeneration are multifocally swollen, with a loss of Nissl substance (chromatoysis) (HE, 400X)***

**Microscopic Description:** In this section of spinal cord, lesions involve the grey matter, predominantly the ventral horns. There is marked neuronal degeneration, necrosis and loss. Necrotic neurons are surrounded, infiltrated and eventually replaced by activated microgliocytes (neuronophagia and microglial nodules) with a few admixed neutrophils (Fig. 1). Necrotic neurons are shrunken with a hypereosinophilic, sometimes vacuolated cytoplasm, and the nucleus is often not visible. There is mild to moderate, multifocal perivascular cuffing by lymphocytes with fewer plasma cells also involving, but to a lesser degree, the leptomeninges.

**Contributor Morphologic Diagnosis:**

Mild to moderate lymphoplasmacytic poliomyelitis with marked neuronal degeneration/necrosis (nonsuppurative necrotizing poliomyelitis).

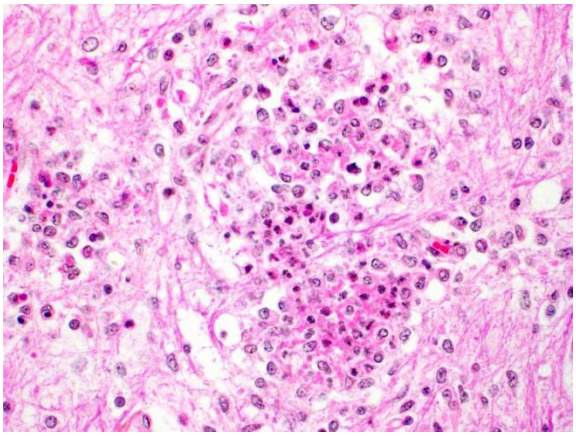
**Contributor Comment:** These lesions were diffuse in the spinal cord, but more severe in the cervical and cranial thoracic segments, consistent with the reported



***Spinal cord, piglet. There are numerous neuronophagic nodules within the gray matter, containing fragmentd and disintegrating neurons admixed with numerous glial cells, macrophages, and fewer neutrophils and eosinophils. (HE, 400X)***

clinical signs. Lesions similar in nature and intensity were present in the brainstem, mainly in the medulla and pons, but also involved the white matter, albeit more mildly. Significant lesions were not present in the cerebellum or cerebrum (minimal lymphocytic meningitis) nor in other organs. The nature of the lesions, i.e. nonsuppurative inflammation involving preferentially the grey matter with neuronal necrosis and microglial nodules, was highly suggestive of a neuronotropic viral infection. Selenium toxicosis causes bilateral symmetrical poliomyelomalacia in the ventral horns, but it was not considered here as it is malacic (necrosis of all CNS components, not only neurons) in nature, and does not cause neuronophagia/microglial nodules or inflammation (although primarily malacic conditions will eventually incite inflammation with microglia, macrophages, and gitter cells). The viral etiologies considered in our geographical area (northeastern North America) were porcine teschovirus A (PTV), porcine sapelovirus A (PSV), porcine hemagglutinating encephalomyelitis virus (PHEV) and, to a lesser degree, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2). These viruses are all specific to the porcine species (in natural disease). The final diagnosis, based on histopathology and PCR results, was encephalomyelitis due to PSV infection.

Porcine teschovirus A (PTV), formerly known as porcine enteroviruses (PEV) 1-7 and 11-13, is a member of the *Picornaviridae* family, which also includes porcine sapelovirus A (PSV) and porcine enteroviruses (PEV).[1] PTV causes a polioencephalomyelitis in pigs known as Teschen and/or Talfan disease; it has also been associated with reproductive failure. Teschen disease, the first one reported, is clinically severe (high morbidity and mortality) and limited mostly to Europe while Talfan disease, reported 20 years later, is a milder form (infection is usually asymptomatic) and is cosmopolitan.[1,2,7] Porcine sapelovirus A (PSV), formerly known as porcine enterovirus (PEV) 8, also causes a polioencephalomyelitis that is essentially similar to PTV, and has only been relatively recently reported in North America in 11 week-old pigs (20% morbidity; 30% mortality); it has also been associated with enteritis, pneumonia and reproductive failure. In contrast to PTV, the

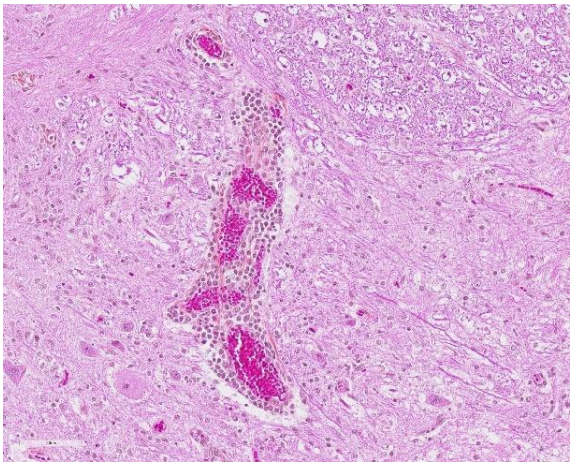


***Spinal cord, piglet. Glial nodules mark areas of neuronal demise. (HE, 400X)***

pathogenesis of PSV polioencephalomyelitis is still largely unknown and, to our knowledge, the disease has not been experimentally reproduced; PSV, but not PTV, is known to be cytopathic in porcine kidney cells.[1] Both PSV and PTV, like PEV, can be found in feces of normal pigs; thus, CNS sampling must be done as aseptically as possible.[1] Microscopic lesions in PTV and PSV are characterized by nonsuppurative polioencephalomyelitis with neuronal degeneration/necrosis and gliosis. Lesions are usually present throughout the neuraxis with some minor differences in the severity and distribution of lesions.[1,10]

PHEV, the only neurotropic porcine coronavirus, causes "vomiting and wasting disease" and/or encephalomyelitis in piglets less than 4 weeks of age, generally in 1-3 week-old piglets. It causes a nonsuppurative encephalomyelitis with neuronal degeneration involving predominantly the grey matter of the brainstem and proximal spinal cord.[2,6]

Although it is a systemic infection, PRRS has rarely been reported to cause predominantly central nervous system (CNS) lesions and clinical signs (neuroinvasive/ neurovirulent).[3,8] Lesions described in the CNS are lymphohistiocytic encephalitis (grey and white matter), with or without vasculitis, and/or meningitis;[3,9] in published reports we found, the spinal cord has apparently not been examined. Neuronal degeneration/ necrosis is not however a feature even in these cases and PRRSV is not considered a neuronotropic virus, although it was detected in neurons in one case.[3,7] PRRS is a common disease in Quebec and a generally mild lymphohistiocytic meningitis and/or encephalitis is often present along with the classical interstitial pneumonia and other lesions (e.g. lymphohistiocytic interstitial myocarditis).



***Spinal cord, piglet. Vessels within the grey matter are cuffed by several layers of lymphocytes and few macrophages, and the surrounding gray multifocal is multifocally gliotic and infiltrated by some of these cells. (HE, 400X)***

PCV-2 also causes a systemic infection that results in a spectrum of diseases known as "porcine circovirus disease" (PCVD) or "porcine circovirus-associated disease" (PCVAD), the best known being postweaning multisystemic wasting syndrome (PMWS). CNS lesions are reported in PCVAD, but the role of PCV-2 is often not clear; they are generally mild and non-specific, e.g. nonsuppurative encephalitis and/or meningitis.[2,4,8] Neurological disease has rarely been reported with PCV-2 infection; in these cases, reported CNS lesions include cerebellar vasculitis and granulomatous meningoencephalomyelitis with multinucleated cells. These lesions were seen concurrently with systemic lesions typical of PMWS (e.g. widespread lymphoid depletion with granulomatous inflammation).[4,8]

Other possible causes of nonsuppurative polioencephalitis ± myelitis in pigs include

rabies (Lyssavirus), pseudorabies (suid herpes 1/varicellovirus; not present in Canada), West Nile virus (flavivirus) infection and "blue eye disease" (rubelavirus; Mexico). Although it is called encephalomyocarditis (cardiovirus), this disease is mainly a necrotizing myocarditis with generally minimal CNS lesions.[2,10]

**Contributing Institution:**

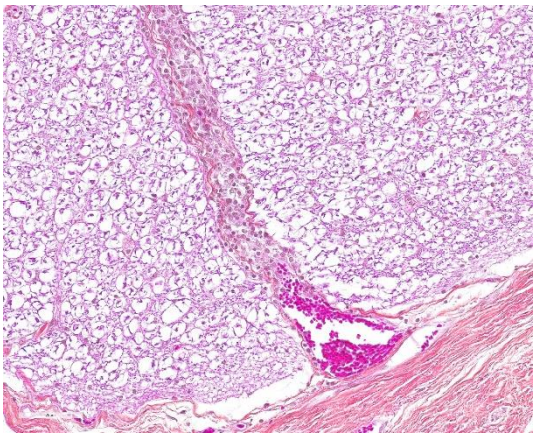
Faculty of veterinary medicine, Université de Montréal: https://fmv.umontreal.ca/faculte/departements/pathologie-et-microbiologie

**JPC Diagnosis:** Spinal cord, grey matter: Poliomyelitis, lymphocytic, diffuse, marked with neuronal necrosis, neuronophagia, glial nodule formation, and meningitis.

**JPC Comment**: The contributor provided an excellent review of neuronotropic viruses that target the spinal cord in swine.

Porcine sapelovirus is a member of the family Picornaviridae and was previously known as porcine enterovirus-8. There are historically two other species within the sapeloviruses, an avian species with one serotype and a simian species with three, hence the name "sapelovirus" for **s**imian, **a**vian, and **p**orcine **e**ntero-**l**ike viruses. More recently, the avian virus was moved to the genus *Anativirus*, and sapeloviruses have been discovered in the fecal flora of the sea lion and of a house mouse.[11]

Porcine sapelovirus has been identified in the feces of both healthy and diseased swine around the world.[5] It may result in SMEDI-like fetal mortality if inoculated into gilts on day 30 of gestation or earlier. In addition to neurologic disease, it may result in pneumonia and diarrhea as well. Histologic lesions in the intestine are those of villous atrophy.[5]



***Spinal cord, piglet. Perivascular cuffs of lymphocytes extend along Virchow Robins spaces at the periphery of the spinal cord. (HE, 100X)***

While most commonly transmitted by fecal-oral contact, this virus is environmentally resistant, and may also be transmitted through fomites. Diarrheic disease is seen intially in experimentally inoculated 50- to 60-day-old pigs, which is followed by ataxia and paraparesis and ultimately paralysis approximately five days later. Death occurs in an additional 2-3 days due to encephalitis. Humoral immunity is important in protection against sapelovirus infection, with maternal colostrum and early IgA production being considered protective against infection in weanlings.[5]

The moderator reviewed a number of viral and nutritional/toxic possibilities for lesions in the gray matter of the spinal cord.

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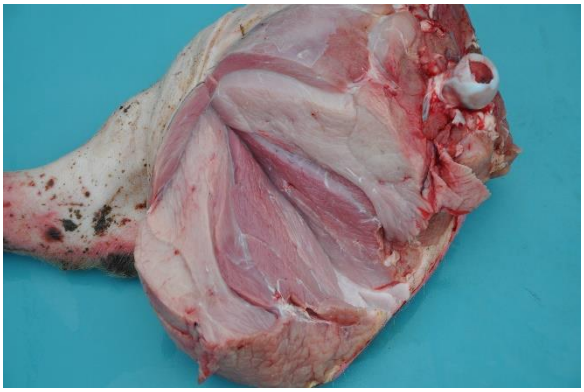
**CASE IV:** N633-16 (JPC 4119040).

**Signalment:** 5½ week-old, neutered male, crossbred Landrace, *Sus scrofa domesticus*, domestic pig.

**History:** This animal was raised in a swine farm located in the state of Rio Grande do Sul, Brazil, and was part of an outbreak which occurred in three distinct swine growing-finishing sites. This outbreak affected pigs with age ranging from 80 to 120 days, and it lasted 60 days. The three sites had a total of 2152 pigs, of which 92 died during the outbreaks. All these pigs, including the one submitted, showed clinical signs of apathy, weight loss, diarrhea, cyanosis, and reddening of the ears, abdomen, and distal parts of the thoracic and hind limbs over a clinical course of 7 to 10 days. In addition, approximately 30 animals from the affected group, including the case referred, displayed stiff gait, muscle weakness, hind limb paresis, and recumbency. Euthanasia was elected due to the poor prognosis and progression of clinical signs.

**Gross Pathology**: Grossly, the muscular lesions consisted of multifocal to coalescing pale discoloration of the skeletal muscles,

***Presentation, 5.5-week-old pig. Affected pigs demonstrated hind limb paresis. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia/)***



***Hind limb, 5.5-week-old pig. Muscles of the hindlimbs demonstrate severe pallor. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia/)***

which was severe for the hind limbs, moderate for the thoracic limbs, and mild for the dorsal lumbar skeletal muscles. Other lesions included generalized lymphadenomegaly with whitish and reddish inter-mixed areas on the cut surface; enlarged kidneys with multifocal to coalescing pinpoint to round whitish areas; and splenomegaly with infarcts and multifocal white pulp hyperplasia. The liver was enlarged with diffuse orange-reddish discoloration; the skin had ulcers and irregular reddened lesions on the limbs and ear cyanosis.

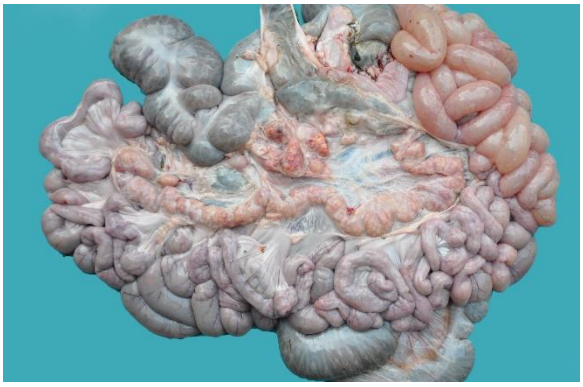
**Laboratory results:**

Immunohistochemistry (IHC) was performed on lymphoid tissue and was characteristic of PCVD, with intense immunostaining in the cytoplasm of macrophages and multinucleated giant cells and moderate immunostaining of endothelial cells. In addition to that, IHC was performed on skeletal muscle tissue, and it showed intense multifocal immunostaining mainly in the cytoplasm and nuclei of inflammatory cells, while mild immunostaining was evidenced in the cytoplasm of endothelial cells, necrotic fibers, and skeletal muscle satellite cells.

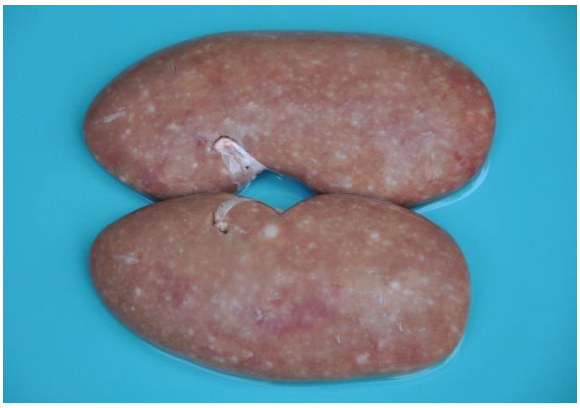
DNA was obtained from the lymph nodes, spleen, and skeletal muscle of this pig, from which a polymerase chain reaction (PCR)

product of 481 bp was amplified. DNA extraction and PCR for PCV2 were performed as described previously.[4] The PCR products were sequenced, which indicated 99% identity with sequences in GenBank from the PCV2b genotype.

**Microscopic Description:** Some submitted slides have two to three sections of skeletal muscle, one of which is from the affected animal and presents prominent histological lesions, and the other ones is a section of normal skeletal muscle from an unaffected control pig. The affected tissue section is characterized by multifocal to coalescing, intense hyaline and floccular necrosis of myofibers, which have swollen and hypereosinophilic sarcoplasm with loss of striations, pyknotic nuclei, in addition to myofiber fragmentation with an intense inflammatory infiltrate of macrophages and multinucleated giant cells, sometimes occupying the whole myofiber sarcoplasm.



***Mesenteric lymph nodes, 5.5-week-old pig. There is marked mesenteric lymphadenomegaly. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia/)***



***Kidney, 5.5-week-old pig. The kidneys are enlarged with white foci. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia/)***

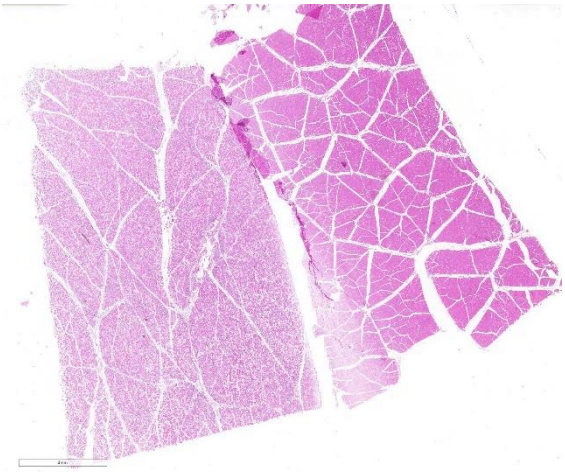
This pig had also in the interstitial and perivascular spaces a moderate inflammatory infiltrate of macrophages, multinucleated giant cells, lymphocytes, and plasma cells, as well as multifocal mild hemorrhage, endothelial cell hypertrophy, mild multifocal thrombosis, as well as occasional regenerating myofibers.

**Contributor Morphologic Diagnosis:**

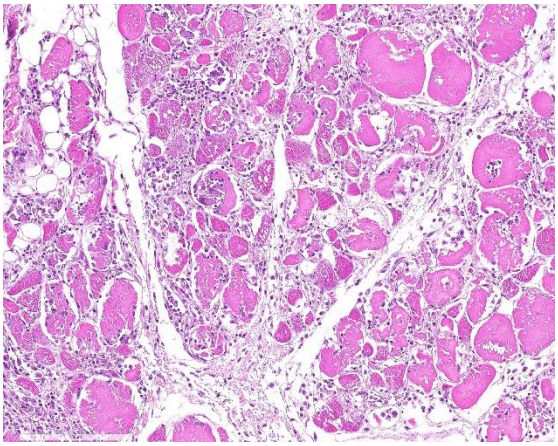
Multifocal to coalescing, severe, subacute, granulomatous necrotizing myositis.

**Contributor Comment:** In the present case, final diagnosis of granulomatous necrotizing myositis due to porcine circovirus infection was reached through the association of clinical, gross, histopathological, immunohistochemical, and molecular findings. Porcine circovirus type 2 (PCV2) is a nonenveloped, circular, single-stranded DNA virus,[17] which presents four genotypes: PCV2a, PCV2b, PCV2c,[3,9,15] and PCV2d.[5,18] The virus is associated with multiple clinical syndromes in pigs, collectively known as porcine circovirus diseases (PCVD).[11] Clinically, PCVD may vary, and affected pigs usually display progressive weight loss, dyspnea, diarrhea, and lymphadenopathy. Histologically, lymphoid depletion and infiltration of histiocytes and multinucleated giant cells are observed in lymphoid organs.[6] In addition to these organs, PCV2 lesions have been described in the kidneys, small and large intestines, stomach, liver, lungs, central nervous system (CNS), and heart.[2,10,11,14]

Even though systemic disease is frequently observed associated with such viral infection, skeletal muscle lesions caused by PCV2 had not been described in the scientific literature prior to the present case, which has been recently published.[7] The etiology of such particular pathological feature is not completely elucidated. Occasionally, PCV2 infection may induce vascular lesions,,[8,11,13] which could induce necrotic changes; however, only mild vascular alterations within skeletal muscle of affected pigs were observed. Thus, we propose that the skeletal muscle lesions observed are most likely a direct effect of the viral infection, since positive immunostaining was noted in the sarcoplasm of myocytes and in macrophages within the muscle.



***Skeletal muscle, 5.5 week old pig. Two sections of skeletal muscle are presented for examination. The section at left demonstrates marked pallor and diffuse cellular interstitial infiltrate visible at low magnification (HE, 7X)***



***Skeletal muscle, 5.5 week old pig. Through the section of muscle, there is myofiber degeneration and necrosis with variation in fiber size, fragmentation, myofibrillolysis, and infiltration of necrotic myofiber by macrophages. The endomysium and perimysium is edematous and infiltrated by moderate numbers of macrophages and giant cells. (HE, 179X)***

Based on the gross findings observed in the present case, ionophore toxicity and vitamin E/selenium deficiency should be included as the main differential diagnosis.[1,16] Vitamin E/selenium deficiency most commonly affects weaned pigs and, microscopically, causes mineralization of degenerate fibers, whereas ionophore poisoning causes a monophasic and multifocal degeneration of myofibers.[1] However, the affected pigs in this outbreak did not consume ionophore antibiotics, and the skeletal muscle microscopic lesions were primarily characterized by granulomatous necrotizing myositis, which causes distinct lesions from the above mentioned toxic and metabolic myopathies.[1,16]

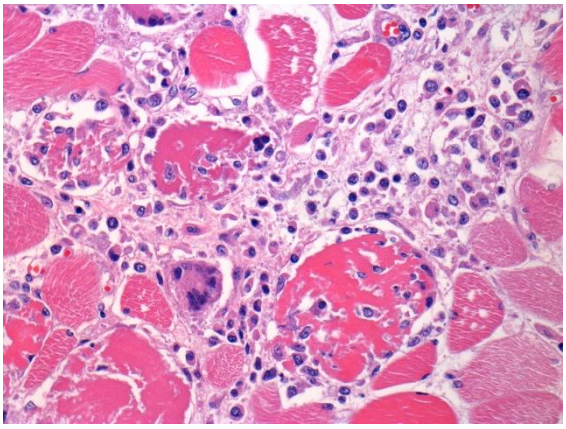
**Contributing Institution:**

Faculdade de Veterinária
Universidade Federal do Rio Grande do Sul
Setor de Patologia Veterinária
http://www.ufrgs.br/patologia/

**JPC Diagnosis:** Skeletal muscle: Myositis**,** necrotizing and granulomatous, diffuse, severe, with multifocal vasculitis.

**JPC Comment**: The contributor summarizes work that he and a team of investigators published in the journal *Veterinary Pathology* in 2018, which was the first published report of skeletal muscle lesions associated with porcine circovirus-2. The potential infection of skeletal muscle (in addition to a long list of other tissues, many of which have appeared in the WSC, as listed below) by porcine circovirus has been considered for a number of years, as it was demonstrated that naïve pigs could be infected by feeding skeletal muscle from PCV-2 infected pigs.

In the report by Konradt et al.,[7] the granulomatous and necrotizing myositis seen in these animals was not an isolated manifestation of porcine circovirus disease, but one of a number of lesions of a group of pigs afflicted by PCD. In these animals , lymphoid tissue, including the spleen,



***Skeletal muscle, 5.5 week old pig. Higher magnification of degenerating and necrotic myofibers. Some myotubes are devoid of a myofiber and contain numerous macrophages. A multinucleated giant cell macrophage is present at center. (HE, 400X) (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária,***

tonsils and lymph nodes, showed marked depletion of lymphocytes and granulomatous inflammation with giant cells. (Multinucleated giant cells macrophages appear to be a relatively constant feature in areas of inflammation in PCV-2 infected pigs, and also appeared in this slide, scattered through the interstitial infiltrate.) Affected pigs in this study also exhibited lymphohistiocytic vasculitis and fibrinoid necrosis in a number of organs including the skeletal muscle, lyphoidtissue, kidney liver, skin, and leptomeninges. Several pigs additionally exhibited granulomatous enteritis, predominantly in proximity to Peyer's patches.[7]

The moderator mentioned a very important point about granulomatous inflammation in this particular case. While widespread damage to muscle often incites a histiocytic response as part of cleanup, the number of epithelioid macrophages within the interstitium exceeds that which would be expected as a result of muscular necrosis and should be interpreted as granulomatous inflammation.

The moderator closed discussion of this case with a comprehensive review of PCV-2, PCV-2b, and PCV-3.

Since 2007, porcine circovirus and the many and varied manifestations of porcine circovirus-associated disease has been an oft-used viral disease and swine disease in the WSC. While space and time prohibit the recapitulation of myriad presentations of PCVD within this comment, the reader is encouraged to visit the various cases listed below (used as exemplars for common manifestations of PCVD) for a wealth of information on this important swine disease.

| | |
|---|---|
| Conference 17, Case 2, 2018-2019<br>Conference 8, Case 2, 2016-2017 | Granulomatous myocarditis |
| Conference 5, Case 1, 2017-2018 | Granulomatous interstitial pneumonia with arteritis |
| Conference 21, Case 4, 2014-2015 | Cerebellar hemorrhage |
| Conference 25, Case 1, 2013-2014<br>Conference 1, case 4, 2007-2008 | Granulomatous hepatitis with marked hepatocellular degeneration and loss |
| Conference 7, Case 3, 2011-2012 | Granulomatous interstitial nephritis |
| Conference 8, Case 4, 2009-2010 | Porcine dermatopathy and nephropathy syndrome |
| Conference 17, Case 4, 1997-1998 | Granulomatous lymphadenitis |

***Table 1 – Previous PCV submissions to the WSC.***

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