Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 5

27 September 2017

Corrie Brown, DVM, PhD, DACVP
Josiah Meigs and University Distinguished Professor
Department of Veterinary Pathology
College of Veterinary Medicine
University of Georgia
Athens, GA 30602

CASE I: S17-3588 (JPC 4100988).

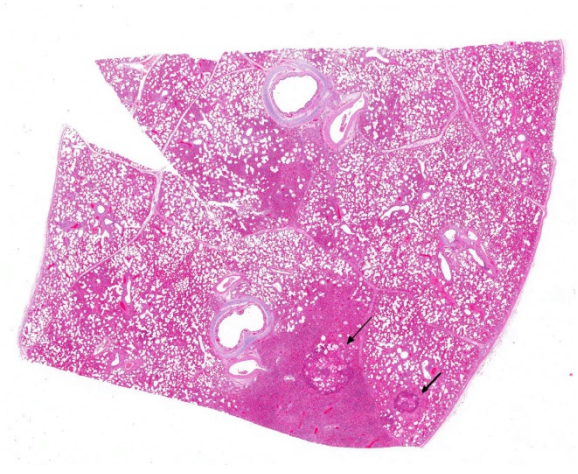
Signalment: 8-week-old, male, breed not specified, *Sus scrofa domesticus*, porcine.

History: A piglet from an intensive pig farm, where a mortality rate increase of approximately 10% was registered in weaned piglets within a short time span, was submitted for necropsy. Affected animals displayed diarrhea that was occasionally bloody. All suckling piglets in this farm were vaccinated against porcine circovirus type 2 (PCV-2) and *Lawsonia intracellularis*.

Gross Pathology: The piglet was severely emaciated and moderately dehydrated and its hind limbs were soiled with dry, dark brown feces. The cranial lobes of the lung were bilaterally dark red and with a firm consistency, whereas the rest of the lungs were bilaterally diffusely firm, heavy and did not collapse after the thorax was opened. The intestine was filled with a small amount of brown, liquid to creamy ingesta. No further gross lesions were detected in other organs.

Laboratory results:

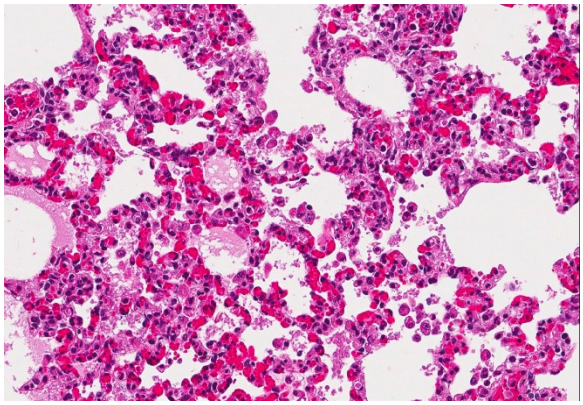
PCV-2 antigen immunohistochemical detection: High amount of PCV-2 antigen visible within the macrophages in the lung and lymphoid organs (spleen, Peyer´s patches, retroperitoneal and mesenteric



Lung, pig. At subgross magnification, there is a diffuse interstitial pneumonia with randomly scattered areas of consolidation. There are two circular areas of necrosis outlined by cellular debris (arrows). (HE, 5X)

lymph nodes, tonsils).
Bacteriology lung: High content *Pasteurella multocida*; moderate content *Streptococcus suis*.
Bacteriology intestinal content: Inconclusive (mixed microbial flora detection).
Serology: No antibodies against porcine respiratory and reproductive syndrome (PRRS) virus and classical swine fever (CSF) virus detected.

Microscopic Description: Lung: In approximately 90% of the parenchyma the alveolar septae are diffusely congested and multifocally severely thickened due to the presence of a high number of macrophages, lymphocytes and plasma cells, as well as fewer multinuclear giant cells of the foreign body type with up to five nuclei, neutrophils and accumulation of eosinophilic, foamy material (edema). The bronchus associated lymphoid tissue (BALT) is severely depleted and infiltrated by macrophages displaying moderate numbers of intra-cytoplasmatic, basophilic botryoid inclusion bodies. The bronchiolar epithelium is multifocally severely disrupted and the bronchiolar lumen is filled with a high amount of degenerated neutrophils intermingled with desquamated respiratory epithelium and occasional multinuclear giant cells. The surrounding alveoli are filled with high numbers of viable neutrophils and increased amounts of alveolar macrophages with a foamy cytoplasm, as well as a moderate number of free erythrocytes (hemorrhage) and edema. In most of the slides there are focal-extensive, hypereosinophilic areas displaying loss of cellular detail, karyorrhexis, karyolysis and pyknosis (lytic necrosis) associated with multiple, non-pigmented, septated fungal hyphae with 5-8 µm thick parallel walls and an acute angle dichotomous branching. Surrounding these necrotic areas there is a prominent granulomatous reaction characterized by the presence of high



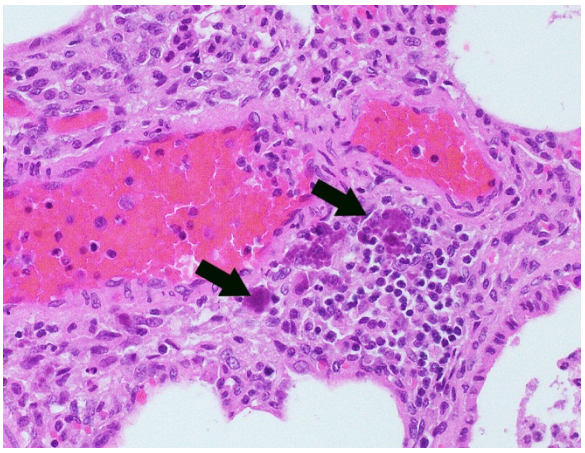
Lung, pig. Alveolar septa are widened and hypercellular with hypertrophic pulmonary intravascular macrophages and abundant granular eosinophilic alveolar contents. (HE, 360X)

numbers of degenerate and viable neutrophils, epithelioid macrophages, lymphocytes and occasionally fibroblasts and sparse collagen proliferation. The interstitial septae and the pleura are diffusely moderately edematous.

Contributor's Morphologic Diagnosis:
Lung: 1. Severe, diffuse, chronic, granulomatous, bronchointerstitial pneumonia with intralesional intra-cytoplasmatic botryoid basophilic inclusion bodies
2. Moderate, multifocal, acute, suppurative bronchopneumonia
3. Mild, focal-extensive to focal, chronic, granulomatous and necrotizing pneumonia with intralesional fungal hyphae (some slides only)

Contributor's Comment: The postmortem and histological findings are compatible with an infection with porcine circovirus type 2 (PCV-2).

PCV are small non-enveloped, single-stranded DNA viruses with a circular

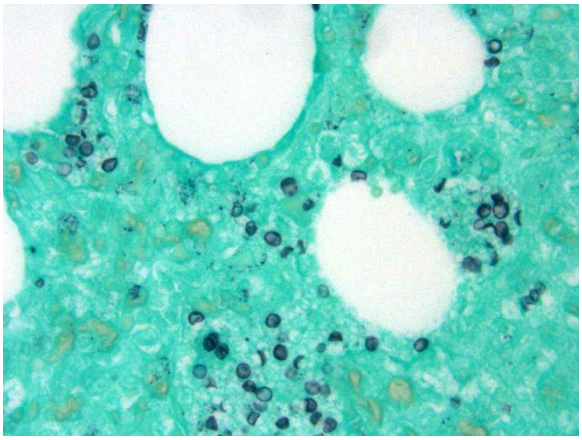


Lung, pig: The BALT is severely depleted and infiltrated by macrophages displaying moderate numbers of intracytoplasmic, basophilic botryoid inclusion bodies (arrow) (HE, 20X). (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland (http://www.itpa.vetsuisse.unibe.ch/index_eng.html)

genome that belong to the genus *Circovirus* from the *Circoviridae* family; two genotypes (PCV-1 and PCV-2) are currently described. While PCV-1 is nonpathogenic, PCV-2 is highly virulent and was isolated for the first time in 1997 in association with postweaning multisystemic wasting syndrome (PMWS), an important, globally present swine disease of great economic impact.[1,6] Since several other often overlapping clinical syndromes were linked to PCV-2 infection in several age groups following its discovery, namely porcine respiratory disease complex (PRDC), porcine dermatitis and nephropathy syndrome (PDNS), enteric disease, reproductive failure and, more recently, PCV-2-associated cerebellar vasculitis, the term PCV-associated diseases (PCVADs) is nowadays used.[1,5,6] The most common clinical presentation is systemic PCV-2 infection (PMWS) associated with PRDC-associated pneumonia.[5] Other swine disease entities such as proliferative and necrotizing pneumonia, sow abortion and mortality syndrome, congenital hypomyelination and abortion with fetal myocarditis may also be associated to PCV-2 infection, but definite proof is still lacking.[1]

PCV-2 infection is usually slow progressing[1] and infection occurs following inhalation or ingestion of viral particles present in contaminated oronasal-pharyngeal body fluids, feces and urine. Establishment of lymphoid tissue infection in the tonsils and Peyer's patches is achieved through infection of mucosal dendritic cells, macrophages and lymphocytes, although the mechanisms that allow the virus to penetrate past the epithelial body barriers have yet to be fully elucidated. While macrophages are non-permissive to virus replication and act primarily as viral "carriers" within the organism, lymphocytes allow PCV-2 replication. Viral replication and release is associated with lymphocyte injury and lysis, which leads to severe lymphoid depletion and subsequent immunosuppression.[10]

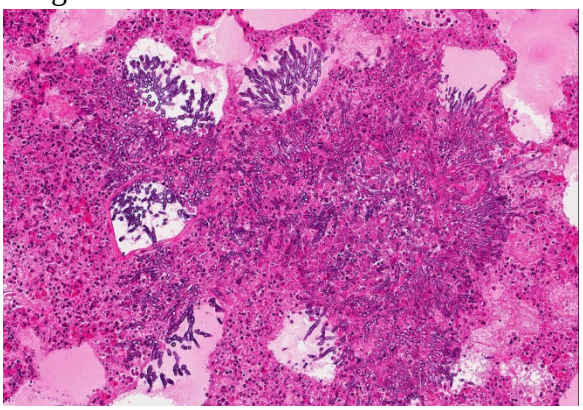
Piglets between 5 and 12 weeks of age are usually most affected and display strong growth impairment or wasting, as well as a wide range of unspecific clinical signs, namely tachypnea, respiratory distress, fever, diarrhea, pallor, jaundice and central nervous signs. Sudden death can also occur.



Lung, pig. A GMS stain demonstrated numerous trophozoites of Pneumocystis within alveoli. (Gomori methenamine silver, 400X)

Morbidity is usually around 5-10% and affected animals usually die or are euthanized due to poor prognosis.[1,6] The most commonly PMWS-associated lesions at necropsy include poor body condition, enlarged or atrophic lymph nodes, and interstitial pneumonia, very often associated with cranioventral bronchopneumonia.[1,5] Histologically, the most commonly found lesions include severe lymphoid depletion associated with granulomatous lymphadenitis, bronchointerstitial pneumonia, granulomatous enteritis, interstitial nephritis, meningoencephalitis, and vasculitis. Sharply demarcated, single or in grapelike clusters (botryoid) arranged, basophilic inclusion bodies can be observed in the cytoplasma of macrophages of the lymphoid system, although their presence in other locations is also described.[1,6]

Besides the above-described lung lesions of the piglet, a severe, diffuse depletion of lymph follicles associated with a severe infiltration of macrophages displaying abundant intracytoplasmatic, botryoid, basophilic inclusion bodies in the tonsils, the spleen, Peyer's patches, and in the retroperitoneal and mesenterial lymph nodes was observed. A high amount of PCV-2 antigen could be detected via immunohistochemistry both in the lymphoid organs and in the lung, thus confirming the PMWS diagnosis. Although PCV-2-associated lymphoid depletion does not always correlate with clinical disease and has also been described in subclinically infected pigs[6], it is most likely that in the present case the PCV-2 infection led to a severe immunosuppression, which allowed the infection with opportunistic bacterial (*Pasteurella multocida* and *Streptococcus suis*) and fungal (most likely *Aspergillus sp.*) pathogens.



Lung, pig: Numerous septate, dichotomously branching fungal hyphae, measuring 4um in diameter, efface a pulmonary arteriole. (HE, 246X)

Interestingly, this piglet came from a farm where vaccination against PCV-2 was performed in suckling piglets. Several highly effective vaccines against PCV-2 are currently available on the market and have been widely used since 2006, which subsequently caused a reduction of the PCVAD disease prevalence;[2,4,6,7,8,9] however, occasional outbreaks in vaccinated animals have been described.[3] Even if PCV-2 infection alone is known to be sufficient to cause PMWS[1], it is quite likely that host, management, co-infections (namely with parvovirus or PRRS virus) or immuno-stimulation play a crucial role in disease progression to PCVAD.[1,7] In the present case the piglet was serologically negative for anti-PRRS virus antibodies and there was no evidence of a co-infection with other major swine pathogens. Therefore, an individual immune impairment or an individual vaccination failure due to an insufficient vaccination dosage may explain the above-described findings in the piglet.

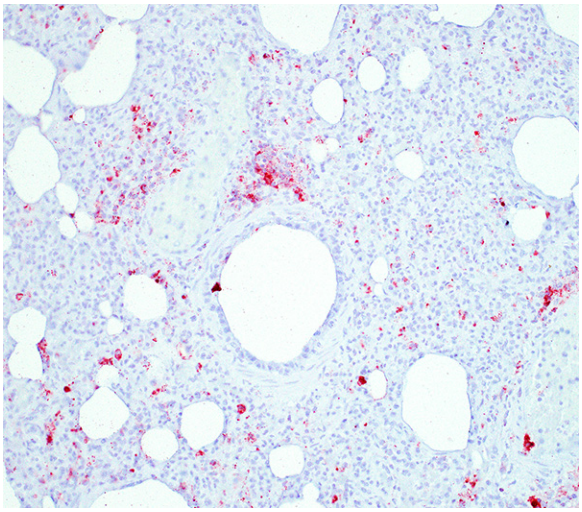
JPC Diagnosis: 1. Lung: Pneumonia, interstitial, necrotizing, diffuse, moderate with diffuse, severe, bronchoalveolar-associated lymphoid tissue (BALT) depletion, rare intrahistiocytic botryoid inclusions, multinucleated macrophages, and intra-alveolar fungal cysts (etiology

consistent with *Pneumocystis* sp.), breed not specified, porcine.
2. Lung: Bronchopneumonia, suppurative and histiocytic, multifocal to coalescing, moderate with intra and extracellular bacilli.
3. Lung: Arteritis, necrotizing, focally extensive, severe with intraluminal fungal hyphae.

Conference Comment: This case provides a slide that is ripe with descriptive features. Participants identified the same key features as the contributor: viral interstitial pneumonia, bacterial bronchopneumonia, and intra-arteriolar fungi.

There were very few examples of botryoid intracytoplasmic viral inclusions in macrophages present within depleted BALT tissues. The moderator noted that inclusions are seen less frequently in more recent cases, which has been described but not explained in the literature. The contributors submitted images were reviewed and discussed, including immunohistochemistry for PCV-2. We thank the contributor for providing additional images, which adds to the teaching/learning value of the case.

Conference participants described the fungal hyphae as angiocentric. Although there is significant tissue damage surrounding the mass of fungal hyphae, it appears to be surrounded by a thin layer of smooth muscle which could represent either the wall of an artery or a bronchiole. Based on the pathogenesis of *Aspergillus* sp., our morphologic diagnosis is arteritis. Although *Aspergillus fumigatus* and *A. flavus* are often associated with chronic destructive bronchitis in German shepherd dogs, in other species they commonly disseminate and spread systemically to other organs. Fungal conidia and hyphae are able to block immune responses and evade killing by phagocytes. Macrophages phagocytize fungi



Lung, pig: Large amount of PCV-2 antigen (stained red) visible within macrophages and most prominent in the BALT (anti-PCV-2 IHC, 20X). (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland (http://www.itpa.vetsuisse.unibe.ch/index_eng.html)

by identification of pathogen associated molecular patterns (PAMPs) using pattern recognition receptors (PRRs) expressed on the surface of macrophages and other phagocytic cells. Specifically, *Aspergillus fumigatus* uses β-glucans, melanin, and other molecules to block lysosomal killing by reactive oxygen species, phagolysosomal acidification, and other destructive mechanisms in macrophages and neutrophils to allow for systemic spread via leukocyte trafficking. In addition, hyphae can travel separately by invading endothelial cells lining capillaries and access the circulatory system, break off in the blood stream, attach to endothelium, and invade tissues at distant sites. It is postulated that ligand-receptor interactions determine where the fungal hyphae decide to stop and spread.[10]

In addition, conference attendees described small, punctate, fungal cysts that partially fill alveoli giving the spaces a honeycombed appearance. These were interpreted as secondary pulmonary pneumocystosis. *Pneumocystis carinii* is a common

secondary disease induced by immune suppression and has been associated with Porcine Respiratory and Reproductive Syndrome (PRRS) and PCV-2 in pigs. Pneumocystis is a fungus with a small uninucleate trophic form that replicates by binary fission and a larger multinucleate cyst form with 8 intracystic bodies formed by sexual replication. These intracystic bodies are released and attach to type I pneumocytes where they mature into trophic forms. Binding to type I pneumocytes, macrophages, surfactant proteins, and fibronectin is mediated by glycoprotein A (cell surface protein). Grossly, Pneumocystis results in a diffusely firm, rubbery, lung. The characteristic microscopic finding is a "honeycomb" material filling alveoli which represents intracellular and extracellular fungal bodies. The wall of the cyst form stains with Gomori methenamine silver (GMS) and Periodic acid-Schiff (PAS).[1] In this case, GMS identified numerous *Pneumocystis* sp. organisms scattered throughout the lung parenchyma often lining alveoli.

Contributing Institution:

Institute of Animal Pathology
Vetsuisse Faculty
University of Bern
Länggassstrasse 122, 3012
Bern, Switzerland
http://www.itpa.vetsuisse.unibe.ch/index_eng.html

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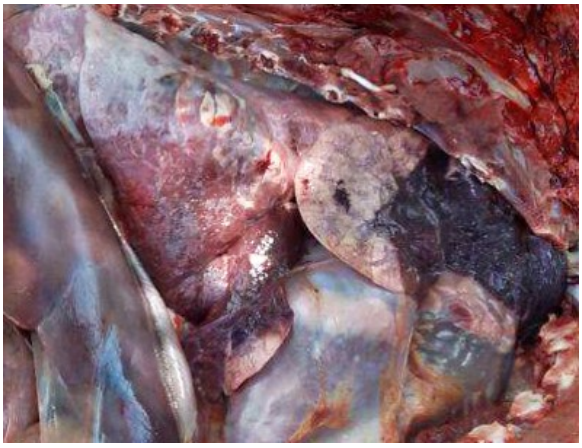
CASE II: 16-180 (JPC 4102148).

Signalment: 6-month-old female Normande, *Bos taurus,* bovine.

History: Six of ten Normande heifers developed acute respiratory disease characterized by coughing and dyspnea. The animals had not been vaccinated against bovine respiratory disease complex and had not received antibiotic treatment recently. The heifers were grazing on a pasture of ryegrass and clover, with concentrate and silage supplementation.

Gross Pathology: Bilaterally, the cranial lung lobes showed homogeneous dark red discoloration and firm consistency (consolidation) involving approximately 30% of the pulmonary parenchyma. The affected regions were well demarcated from the non-affected adjacent parenchyma. There was moderate amount of pink stable froth admixed with mucus in the thoracic portion of the trachea, accompanied by extensive petechiation in the tracheal mucosa.

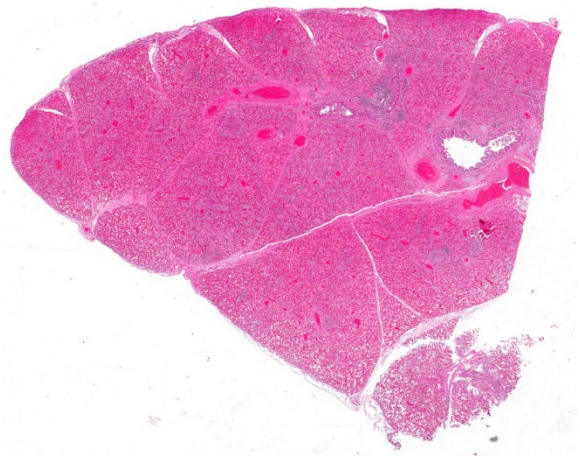


Lung, calf. There is marked collapse and consolidation of cranioventral lobes and moderate interlobular emphysema scattered throughout all lobes. (HE, 5X)(Photo courtesy of: Animal Health Platform, National Institute of Agricultural Research (INIA), Uruguay, www.inia.uy)

Laboratory results: No ancillary laboratory testing was done before the autopsy was performed. Immunohistochemistry was performed and was positive for bovine respiratory syncytial virus (BRSV) antigen within lung lesions. Additionally, PCR detected the viral genome in lung tissue.

Microscopic Description: Lung: Multifocally, bronchioles are mildly ectatic and contain intraluminal necrotic cellular debris admixed with neutrophilic and fibrinous exudate that frequently occludes their lumen. Bronchiolar epithelial cells are either necrotic and sloughed, or markedly attenuated, with loss of apical cilia. In the alveolar spaces there is an amorphous homogeneous light eosinophilic material (edema), fibrin and moderate neutrophilic and multifocal histiocytic infiltrate (alveolar macrophages), with occasional and infrequent bi- or multinucleated syncytial cells, admixed with necrotic epithelial cells with hypereosinophilic cytoplasm and pyknotic nuclei. Multiple, variably sized intracytoplasmic eosinophilic inclusion bodies are observed infrequently in some multinucleated syncytial cells. Some alveolar septae were lined by hypertrophied (type II) pneumocytes.

Contributor's Morphologic Diagnosis:



Lung, calf. There is diffuse consolidation of the alveoli and filling of airways with exudate throughout the section, as well as mild BALT hyperplasia. (HE, 5X)

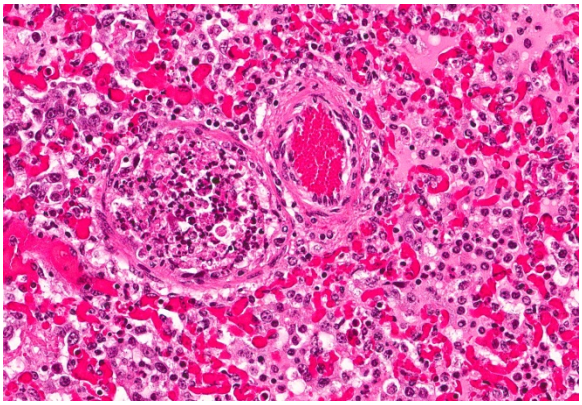
Lung: Bronchointerstitial pneumonia with necrotizing and neutrophilic bronchiolitis, type II pneumocyte hyperplasia, and rare multinucleated syncytial cells with intra-cytoplasmic viral eosinophilic inclusion bodies consistent with bovine respiratory syncytial virus, moderate, acute.

Condition: Bovine respiratory syncytial virus (BRSV) pneumonia.

Contributor's Comment: The bovine respiratory disease (BRD) complex is a multifactorial disease caused by several viruses and bacteria, including BRSV, parainfluenza 3 (PI3), bovine herpesvirus 1 (BHV-1 or infectious bovine rhinotracheitis virus, IBRV), bovine viral diarrhea virus (BVDV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis*,[2,4,6] among other possible causes.

BRSV (genus *Pneumovirus*, family *Paramyxoviridae*) is the most important viral etiological agent of the BRD complex, given its widespread distribution and high pathogenicity, [2] and is responsible for numerous outbreaks of respiratory disease worldwide.[1,2,6] BRSV by itself causes an acute respiratory syndrome including dyspnea, tachypnea, coughing, anorexia, and hyperthermia, occasionally leading to death due to severe respiratory distress.[6] Additionally, BRSV infection predisposes calves to secondary bacterial infections, such as *M. haemolytica*, *P. multocida*, and *H. somni*. It is estimated that the economic losses in cattle with BRD are in the range of US$23.23 and US$151.18 per animal, including deaths, decrease production and prevention and treatment costs.[1] For detailed information on BRSV epidemiology, pathology and pathogenesis, diagnosis, and immunology, please refer to the excellent review by Sacco et al. 2014.

In the case presented herein, the diagnosis of BRSV was made by intralesional detection of BRSV antigen by immunohistochemistry, and by detection of the viral genome by PCR in lung tissue. In cases of respiratory disease, the histologic lesions of broncho-interstitial pneumonia with necrotizing bronchiolitis and syncytial cells are highly suggestive of BRSV and should raise a suspicion for this agent. However, the observation of syncytial cells alone is not conclusive for the diagnosis, particularly if typical inclusion bodies are not observed

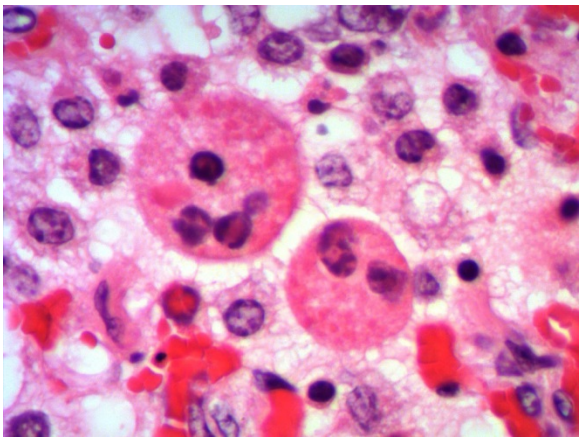


Lung, calf. Diffusely, there is necrosis of airway epithelium primarily of small bronchioles, and the lumen is filled with necrotic cellular debris as well as rare necrotic viral syncytia. (HE, 400X)

upon histologic examination. PI3 infection may also produce syncytial cells and inclusion bodies similar to those produced by BRSV. Furthermore, fibrinous bronchopneumonia caused by several bacteria induces formation of macrophagic multinucleate giant cells morphologically similar to syncytial cells in the lumen of bronchioles and alveoli. Consequently, viral detection by PCR or IHC is necessary for the etiologic diagnosis of BRSV pneumonia.[2] In this case, IBRV, PI3 and bacterial agents were ruled out in lung tissue by IHC, PCR and bacterial cultures, respectively.

In Uruguay, the distribution, frequency and economic impact of BRSV for the cattle industry are unknown. In a serologic study in 100 cattle from different regions of the country, 95% were positive to anti-BRSV antibodies by ELISA,[3] which suggests that the agent has a wide geographic distribution. However, there are few reports of BRSV-associated pneumonia in cattle in this country.[5]

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing and fibrinosuppurative, diffuse, severe, with rare alveolar and bronchiolar multinucleated viral syncytia with intracytoplasmic viral inclusions, Normande, bovine.



Lung, calf. Viral syncytia often contain or one more irregularly round eosinophilic viral inclusions.

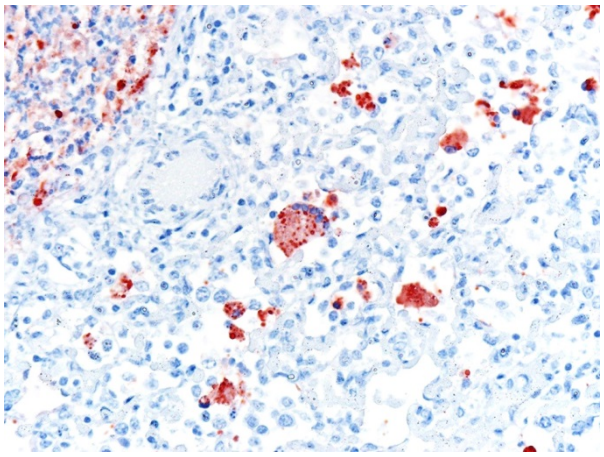
Conference Comment: This is apparently a straightforward case of uncomplicated bovine respiratory syncytial virus (BRSV) in a young heifer. Uncomplicated cases are rare since BRSV impairs the mucociliary apparatus, predisposing affected animals to secondary bacterial infections.

Conference participants identified classic microscopic features of BRSV, centered on the bronchioloalveolar junction, including necrotizing bronchiolitis, alveolar edema and inflammation, and moderate numbers of viral syncytial cells within the bronchiolar and alveolar lumens containing eosinophilic intracytoplasmic viral inclusion bodies.

Attendees also discussed the possibility of bronchiolitis obliterans formation due to chronic bronchiolar damage. Bronchiolitis obliterans is a polypoid proliferation of fibrous connective tissue lined by attenuated epithelial cells that causes obstruction of airways, resulting in hypoxic vaso-constriction of the pulmonary parenchyma, pulmonary hypertension, and right heart failure.[2]

Bovine respiratory syncytial virus is common among beef and dairy herds in Europe and North America and is an important cause of acute outbreaks of respiratory disease and enzootic pneumonia in 2-week-old to 5-month-old calves.[2] BRSV is spread via aerosol and targets the ciliated cells of the conductive system and alveolar type II pneumocytes of the gas exchange component of the respiratory system. The virus (like other *Paramyxoviruses*) attaches via heparin binding domains on envelope glycoprotein G, and enters the cell using envelope

Lung, calf. A variety of cells demonstrate immunopositivity for BRSV antigen including airway epithelium and sloughed cells within the lumen (upper left), alveolar macrophages, and multinucleate syncytia (anti-BRSV, 400X) (HE, 5X)(Photo courtesy of: Animal Health Platform, National Institute of Agricultural Research (INIA), Uruguay, www.inia.uy)



glycoprotein F, its fusion protein, which also induces the formation of syncytial cells. Viral syncytia allow interaction with and spread to adjacent unaffected cells. Once host cells are infected a cascade of pro-inflammatory cytokines is released, recruiting neutrophils, lymphocytes, and macrophages to the lesion.

Experiments in tissue culture have revealed that the virus itself does not cause very much damage to respiratory epithelium. It has been presupposed that most of the damage is due to exuberant host defense mechanisms.[7] Gross lesions associated with BRSV infection range from red, rubbery cranioventral lungs (atelectasis) to firm, heavy, edematous caudodorsal areas of lung that fail to collapse postmortem. However, there are frequent variations in the gross appearance for several reasons: (1) If calves die in respiratory distress, there is often marked subpleural and interlobular emphysema with bullae formation; and (2) Secondary bacterial bronchopneumonia can become so severe as to obscure more subtle viral lesions.[2] The microscopic lesions noted above (necrotizing bronchiolitis with syncytial cells) are highly suggestive of BRSV; however, bovine parainfluenza virus 3 (BPIV-3) must also be considered.

Bovine parainfluenza virus 3 (BPIV-3) is also a member of the *Paramyxoviridae* family. Microscopic lesions can be identical to BRSV although there are usually fewer syncytial cells and the clinical course is typically less severe. Diagnosis requires virus isolation or detection of viral antigen by immunohistochemistry or nucleic acid by RT-PCR testing (as in this case). Unfortunately, definitive diagnosis is often problematic, as viral antigen can be cleared from the body long before the secondary bacterial pneumonia results in death of the animal.[2]

Contributing Institution:

Animal Health Platform
National Institute of Agricultural Research (INIA)
Uruguay
www.inia.uy

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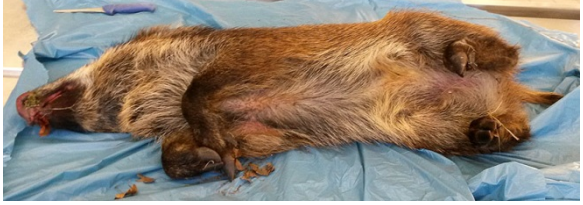
CASE III: 16H0097 (JPC 4101308).

Signalment: 1 to 2-year-old, male, Central European boar (Eurasian wild pig), *Sus scrofa*, porcine.

History: The perished animal was detected on a hunting premise in early December. Several perished wild boars of the same age had been found during the last months.

Gross Pathology: The pig was in a moderate body condition (26 kg body weight). Signs of autolysis were mild (due to the low temperature weather conditions). The pig was moderately infected with lice. The carcass was severely cyanotic (especially snout and ears) and all lymph nodes were severely hyperemic. Multiple abscesses were seen in the mandibular lymph nodes. The lung was not collapsed and heavy. The cranial lobes and multifocally the diaphragmatic lobes of the lung were consolidated and dark red with multiple small abscesses. The trachea was filled with large amounts of pus. The liver was severely hyperemic and had multiple irregularly distributed 1 to 5 mm in diameter foci of necrosis. The spleen was severely enlarged. Embolic suppurative nephritis was present in both kidneys. There were multiple foci of necrosis in the adrenals. The stomach was severely hyperemic. The gastro-



Carcass, boar. The carcass diplays cyanosis of the snout. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institut for Animal Health, Suedufer 10, D-17493, Greifswald-Insel Riems, Germany. www.fli.de)

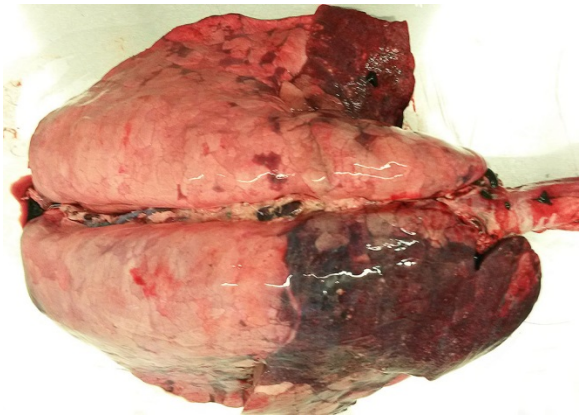
intestinal tract including the colon was almost empty. No signs of gun-shot wounds or injuries caused by an accident (e.g. collision with a car) were detected.

Laboratory results: *Salmonella* Choleraesuis was isolated from multiple tissues.
PCRs were negative for European swine fever virus, African swine fever virus, pseudorabies virus, rabies virus and PCV-2.

Microscopic Description: Lung: There are numerous foci of necrosis which appear to be associated with the airways. They are irregular shaped, of variable size and sometimes confluent. They are surrounded by predominantly neutrophilic infiltrates. Alveoli of the adjacent pulmonary tissue are filled with fibrin, contain few neutrophils and occasionally erythrocytes. The alveolar septae are severely hyperemic. Some contain fibrin thrombi. Numerous bacterial colonies are present throughout the pulmonary tissue (both gram positive and gram negative). Salmonellae are frequently detected in the periphery of the necrotic foci and in bacterial emboli in small blood vessels.

Lymph node (mediastinal): The subcapsular region is dilated by hyperemia, hemorrhages and infiltrates of neutrophils and histiocytes. There are multifocal areas of necrosis and neutrophilic infiltration most likely associated with larger blood vessels. Small blood vessels are obliterated by fibrin thrombi and bacterial emboli. Numerous bacterial emboli are present throughout the section. Lymphoid follicles are severely depleted

Liver: Many small and irregularly distributed foci of necrosis are present throughout the hepatic tissue. Some consist of pure coagulation necrosis; others have foci of hemorrhage or are infiltrated by few neutrophils or histiocytes. The liver is severely hyperemic. In addition to erythrocytes, leucocytes and fibrin are present in hepatic arteries and veins. Sinusoids are dilated by hyperemia and leukocytostasis. Bacterial colonies and emboli occur in a multifocal distribution. They stain positive for salmonella LPS by IHC. There are a few areas with loss of hepatic tissue structure and infiltration of large numbers of long bacilli without inflammatory reaction.



Lung, boar. The cranial ventral lobes and scattered lobules throughout the remainder of the lung are consolidated and hemorrhagic. Multiple abscesses may be seen in these areas. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institut for Animal Health, Suedufer 10, D-17493, Greifswald-Insel Riems, Germany. www.fli.de)

Contributor's Morphologic Diagnosis:
Lung: Severe, diffuse acute fibrinopurulent and necrotizing bronchopneumonia with bacterial colonies, fibrin thrombi and bacterial emboli in small blood vessels.

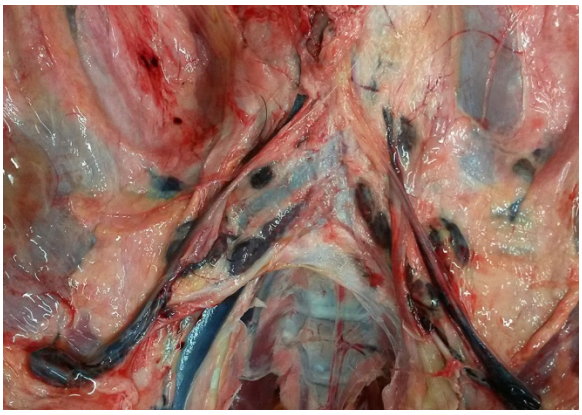
Lymph node (mediastinal): Severe purulent and necrotizing lymphadenitis with severe hyperemia, hemorrhages and lymphatic depletion as well as fibrin thrombi and bacterial emboli in small blood vessels.

Liver: Severe multifocal acute necrotizing hepatitis with bacterial emboli in small blood vessels.

Contributor's Comment: Groups of gram-negative bacteria were seen in all tissues examined. IHC using a primary antibody against salmonella LPS (MCA2832, Bio-Rad) identified them as salmonella. In the lung, gram-positive coccoid bacteria were found in addition. Gram-labile long slender bacilli in the liver were interpreted as post mortem invasion.

Salmonella are gram-negative, aerobic to facultative anaerobic, motile, facultative intracellular bacilli. They represent one genus in the family Enterobacteriaceae. The genus *Salmonella* encompasses more than 2500 species, subspecies and serovars. *Salmonella (S.) enterica* subspecies *enterica* has the largest number of serovars (>1500) including the most important animal and human pathogens, e.g. *S. enterica* subspecies *enterica* Choleraesuis, abbreviated as *S.* Choleraesuis.

Pathogenic salmonella can be differentiated in host adapted serovars (e.g. *S.* Choleraesuis in pigs, *S.* Dublin in cattle, *S.* Typhi in humans) and serovars with a wide host spectrum (e.g. *S.* Typhimurium and many others). Host-adapted salmonella cause severe generalized disease both in young and adult hosts; serovars with a wide host spectrum cause predominantly enterocolitis in young animals.



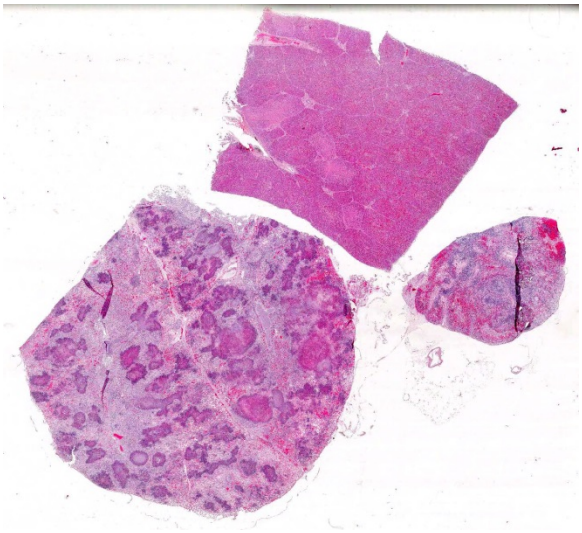
Mandibular lymph nodes, boar. The lymph nodes are enlarged and hyperemic with small abscesses. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institut for Animal Health, Suedufer 10, D-17493, Greifswald-Insel Riems, Germany. www.fli.de)

Three syndromes are associated with salmonella infections of swine[11]: (1) septicemic salmonellosis usually associated with *S.* Choleraesuis, (2) acute or chronic enterocolitis and rectal stricture associated with *S.* Typhimurium and (3) ulcerative enterocolitis and/or caseous tonsillitis and lymphandenitis associated with *S.* Typhisuis. In addition, clinically in apparent infections by numerous other salmonella serovars are possible.[4]

The main reservoir for *S.* Choleraesuis is the intestinal tracts of pigs.[4] Persistent carriers occur and shedding can be activated by stress. During acute disease, up to 10^6 *S.* Choleraesuis/g feces are excreted. Salmonella remain infectious for 3 months in wet and for 6 months in desiccated feces. Therefore contact to other pigs or environment contaminated by pigs are the main sources of infection. *S.* Choleraesuis is rarely associated with contamination of carcasses or pork products. Thus, it is unusual as cause of human disease. If human disease occurs, it is severe.

The main route of infection is the alimentary tract including the tonsils. Irrespective of the route of inoculation, *S.* Choleraesuis was most frequently recovered from the ileocecal junction, ileocolic lymph node, cecal contents, tonsil, lung and colon.[3] After crossing the mucosal barrier, infection of macrophages and dendritic cells occurs. Survival in phagocytes is an important feature of virulence and allows systemic bacterial dissemination.[11]

Lesions in septicemic salmonellosis are mainly associated with endothelial damage and microvascular thrombosis due to endotoxins, cytokines released by endotoxin activity and bacterial emboli.[3,9,11] This results in congestion of many organs, widespread hemorrhages, often as petechiae, and pulmonary edema and causes fatal disease. At necropsy, thrombosis of capillaries and venules in the dermal papillae of the skin, glomerulonephritis, paratyphoid nodules (ranging from coagulative necrosis to granulomas) in the liver, splenomegaly with small lymphoid



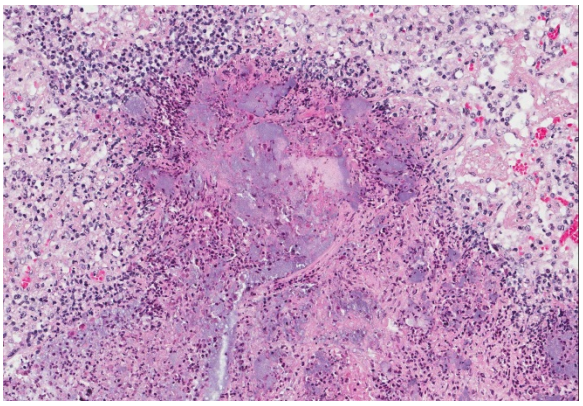
Lung, liver, and lymph node, boar. Multiple abscesses are visible at subgross magnification in the section of lung (lower left). The lymph node (right) exhibits marked lymphoid depletion and hemorrhage, and the liver (top) displays hypocellular areas of necrosis. (HE, 6X)

follicles, but histiocytic infiltrates and

meningoencephalitis are common findings. The injury to the capillaries in pulmonary interalveolar septae may result in a fulminant fibrinous bronchopneumonia.

Differential diagnoses are other causes of generalized disease with high fever (classical swine fever, African swine fever) or septicemia (pasteurellosis, streptococcal infection, erysipelas) or fibrinous pneumonia (e.g. *Actinobacillus pleuro-pneumoniae* infection).

During the 1950s and 1960s, *S.* Choleraesuis was the predominant serovar isolated from pigs worldwide. At the present time, it is still highly prevalent in domestic pigs in North America and Asia, but rare in Australia and Western Europe.[1,7] There are a few case reports of *S.* Choleraesuis in wild boars in Germany.[6,8,10,12] A survey of perished wild boars submitted to the regional diagnostic laboratory of Thuringia, Germany, detected *S.* Choleraesuis in 20% (24 pigs) of the pigs examined.[5] Further characterization of the isolates revealed that distinct isolates were circulating in herds of



Lung, boar. Airways are necrotic and filled with large colonies of small bacilli which extend into the surrounding alveoli. Surrounding alveoli contain variable amounts of degenerate neutrophils admixed with necrotic debris, polymerized fibrin, and hemorrhage. (HE, 168X)

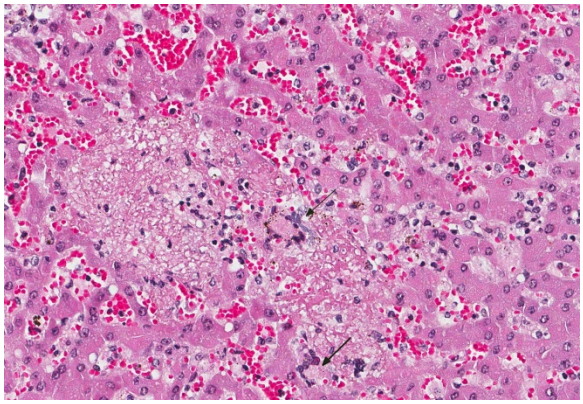
wild boars separated by natural barriers or arterial roads. There appears to be a higher susceptibility of young animals, because predominantly shoats and juvenile pigs were affected. The percentage of carriers and shedders without clinical signs remained unresolved. *S.* Choleraesuis infection of wild boar may serve as reservoir for domestic pigs especially if kept outdoors. Attention should be paid to game meet inspection to reduce health risks for hunters and persons preparing and consuming pork from wild boars.

JPC Diagnosis: 1. Lung: Bronchopneumonia, necrotizing multifocal to coalescing, severe with numerous large bacterial (do we want to specify / i.e.; coccobacilli?) colonies, Central European boar, porcine.
2. Liver: Hepatitis, necrotizing, multifocal, mild to moderate with occasional large bacterial colonies.
3. Lymph node: Lymphadenitis, necrotizing, acute, multifocal, marked with hemorrhage and occasional bacterial colonies.

Conference Comment: This case provided a comprehensive presentation of *Salmonella choleraesuis* in a wild boar. There were a few atypical microscopic findings noted during the conference. First, there were rare multinucleated giant cells in the liver and lymph node which is not a typical finding salmonellosis. Second, the pattern of pneumonia in the lung appears to be bronchocentric, which is unusual for S. Choleraesuis, which often presents as an embolic interstitial pneumonia.[11] Affected animals may develop bronchopneumonia as a result of secondary bacterial opportunists but in this case IHC using a primary antibody against salmonella LPS (MCA2832, Bio-Rad) demonstrated the presence of bacilli in large numbers within multiple airways. An alternate expalantion was proposed by the moderator, who

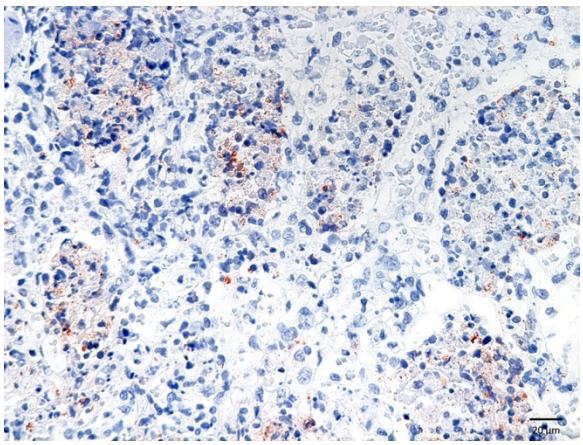
suggested the bronchogenic appearance may have resulted from an outgrowth of inflammation within the adjacent bronchial vascular tree. Finally, conference participants noted that it is unusual to see such large colonies of *Salmonella* sp. within lesions. However, after much discussion, attendees were resigned to the fact that under favorable circumstances excessively large burdens of any bacteria could form colonies in affected tissues.

The contributors comments abovesuperbly reviews the serovars of *Salmonella* sp. affecting domestic animals, porcine specific serovars and their clinical syndromes, pathogenesis, and gross findings, and the history and distribution of salmonellosis in wild boars in Europe. Salmonellae are often found in the intestinal lamina propria and regional lymph nodes of poultry and cattle, which are important reservoirs for the organism. . *Salmonella choleraesuis* is a common co-infection with hog cholera (classical swine fever), and was originally thought to be the causative agent of classical swine fever, with which it shares many gross lesions.[11]



Liver, boar: There are randomly scattered foci of hepatocellular necrosis and loss throughout the section. Additional, several multinucleated cells are present in this image. (HE, 400X)

During the discussion on the pathogenesis of salmonellosis, the conference moderator pointed out that the cause of all of the microscopic changes associated with salmonellosis is due to endothelial damage resulting from endotoxin release. Endotoxin (lipopolysaccharide or LPS) is a virulence factor encoded on Salmonella pathogenicity islands (SPI-2), which are clusters of plasmid genes that can be shared among bacterial colonies. In addition to LPS, SPIs also code for other virulence factors such as fimbriae, motility, and other secreted proteins. Endotoxins induce the release ofcytokines which leads to thrombosis of capillaries and venules (Shwartzman reaction), hemorrhage, and eventual



Lung, boar: Intra- and extracellular bacilli are immunopositive for antibodies against Salmonella lipopolysaccharide (anti-S. Cholerasuis MCA2832, 400X) (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institut for Animal Health, Suedufer 10, D-17493, Greifswald-Insel Riems, Germany. www.fli.de)

ischemic necrosis of visceral organs. Additionally, endotoxins help bacteria evade phagocytosis, decreases lysis within the phagolysosome, and renders complement useless, thus perpetuating survival and replication of the organism.[2]

Contributing Institution:
Friedrich-Loeffler-Institut
Federal Research Institut for Animal Health
Suedufer 10, D-17493
Greifswald-Insel Riems, Germany

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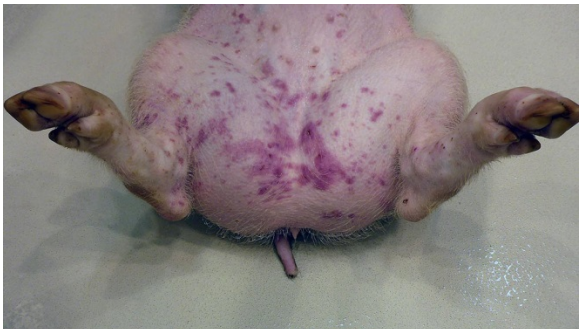
CASE IV: P16-555 (JPC 4101307).

Signalment: Juvenile, male, domestic pig, *Sus scrofa domesticus*, porcine.

History: This case is one out of four pigs from an unvaccinated control group of a vaccine development study. The pigs were experimentally infected i.m. with 10^6 $TCID_{50}$ of classical swine fever virus wild type strain Alfort/Tuebingen. They were humanely killed after developing clinical disease 15 days post infection including fever up to 41°C, apathy, changed defecation and dermal macula.



Skin, pig. There are confluent dermal hemorrhages within the skin of the caudal abdomen. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de)

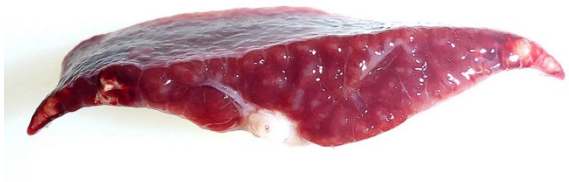
Gross Pathology: The splenic surface showed multifocal, distinct, flame-shaped white streaks with a variably distinct peripheral plum-colored border extending 2-3 mm from and perpendicular to the acute angle. Cut sections revealed roughly wedge-shaped white areas with a variably distinct peripheral plum-colored border at the acute angle, interpreted as mild, multifocal, (ischemic) infarcts.

Other macroscopic changes in this pig were mild hydrothorax and ascites, mild, multifocal, pleural fibrosis with synechiae between parietal and visceral pleura, and moderate hyperplasia of the lymphocentrum bronchiale.

Macroscopic changes in the other pigs included mild to moderate ascites which was the only constant change observed in all four animals. Further important macroscopic changes included moderate, multifocal, irregular cherry red dermal maculae in the inguinal area in two pigs, and peripherally cherry red and white marbled hepatic and gastric lymph nodesinterpreted as blood-filled sinusoids in one pig.

Laboratory results: Classical swine fever genomic RNA was detected in multiple samples of this pig beginning 5 days post infection and up to day 15 using diagnostic real-time RT-PCR.[5] The results were clearly positive with threshold cycle values of 25.12 on the day of killing.

Microscopic Description: Spleen: Located at the acute angle and affecting 10-20% of the splenic parenchyma is a wedge-shaped area displaying diffuse, pale, eosinophilic, shadow-like histoarchitecture and a peripheral zone of moderate extravascular erythrocyte accumulation (acute hemorrhage) which extends into the adjacent normal tissue. The stromal and parenchymal cells display ill-defined cellular borders, cytoplasmic hypereosinophilic condensation, nuclear loss, pyknosis and karyorrhexis (coagulation necrosis). There is minor multifocal basophilic granular change of the necrotic debris (dystrophic mineralization). At the border to the normal appearing tissue are oligofocal medium sized arteries with pyknotic, karyorrhectic and lost endothelia and smooth muscular cells, intramural deposition of an hypereosinophilic amorphous mass (fibrin)



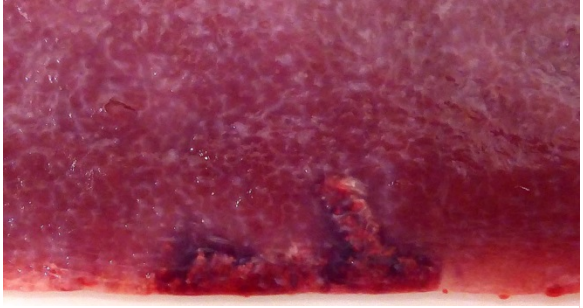
Spleen, pig. There are irregular white marginal infarcts with hemorrhagic seams. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de)

and minor and variable infiltration by degenerating neutrophils, macrophages and foreign body-type and Langhans-type

multinucleated giant cells (fibrinonecrotizing vasculitis). The lumen of multiple affected arteries is occluded by an eosinophilic fibrillary to amorphous mass with scant encased degenerating neutrophils and macrophages (fibrin-rich thrombus). The white pulp in the adjacent parenchyma is of mildly reduced cellularity and displays a mild loss of small differentiated lymphocytes with heterochromatic nuclei (lymphoid depletion) combined with a relative increase in moderately sized lymphoblastic cells with round, euchromatic nucleus, multiple mitotic figures and a mildly increased number of tingible body macrophages. The serosal mesothelial cells are multifocally enlarged and display round euchromatic nuclei (reactive change).

Contributor's Morphologic Diagnosis:
Spleen: Vasculitis, fibrinonecrotizing, oligofocal, acute, moderate, with multinucleated giant cells, arterial thrombosis, necrosis and hemorrhage (infarct).

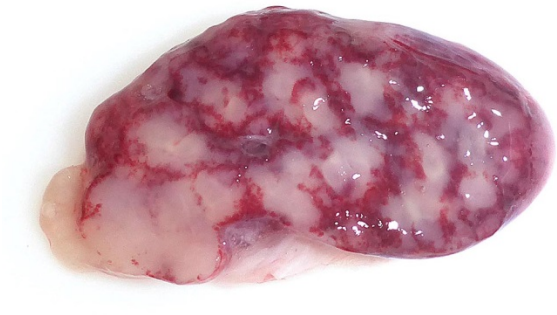
Contributor's Comment: This case of moderate, acute, oligofocal, fibrino-necrotizing vasculitis with multinucleated giant cells, arterial thrombosis, necrosis and hemorrhage is the histopathological correlate of the grossly detected anemic infarcts at the acute angle of the spleen.



Spleen, pig. There are irregular white marginal infarcts with hemorrhagic seams. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de)

These infarcts at the acute angle of the spleen (there is a special German term for that: "Milzrandinfarkte") are considered as one of the most characteristic if not pathognomonic lesions present in 1 – 87% of cases of classical swine fever (CSF; synonym: hog cholera).[13]
This case from an experimental study was selected for the Wednesday Slide Conference because in contrast to most classic textbooks of veterinary pathology including Jubb, Kennedy and Palmer's Pathology of Domestic Animals and Pathologic Basis of Veterinary Disease which describe hemorrhagic infarcts in CSF,[13,14] the current case demonstrates ischemic core infarct areas, suggestive of a very early stage of an infarct pathogenesis. Furthermore, the multinucleated giant cells observed in this case are a rare feature, possibly due to a marked activation of monocytes / macrophages, which is a key element of CSF pathogenesis.[4,8]

Etiology:



Lymph node, pig. Peripheral sinuses of the gastric and hepatic nodes contain abundant hemorrhage. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de)

The etiologic agent of CSF is the classical swine fever virus (CSFV), a pestivirus of the flaviviridae family. It is related to the other well-known species of the genus pestivirus bovine virus diarrhea virus-1 and -2, border disease virus as well as novel pestiviruses including HoBi-like viruses (atypical pestivirus) and Bungowannah virus.[12] Notably, some of the related ruminant pestiviruses including bovine virus diarrhea virus can occasionally infect pigs,[11] leading to cross-reactive antibodies and false positive serological results.[5] In contrast, evidence of naturally occurring CSFV-infection in ruminants or other species is lacking. The CSFV is a spherical, enveloped, single-stranded, positive-sense RNA virus with a diameter of ~ 50 nm. The ~12.3 kb RNA consists of a single open reading frame encoding the four structural proteins C, E^{rns}, E1, and E2, as well as the non-structural proteins N^{pro}, p7, (NS2-3), NS2, NS3, NS4A, NS4B, NS5A, and NS5B.[12]

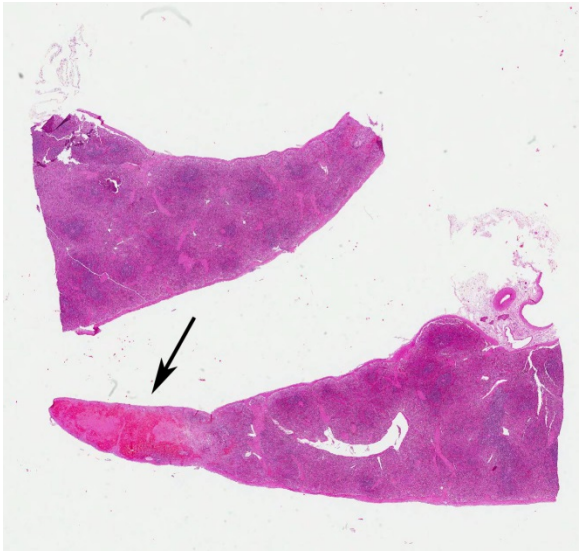
Epidemiology:

CSF is one of the most important diseases of domestic pigs and notifiable to the World Organization for Animal Health. The main hosts for CSFV are domestic pigs (*Sus scrofa domesticus*) and European wild boars (*Sus scrofa scrofa*). Furthermore, it has been proven experimentally that warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus*) are also susceptible to CSFV.[3] Currently CSF is endemic in South and Central America, parts of Eastern Europe, and Asia and represents a constant threat for all other pig producing countries.[1]

CSFV can be transmitted horizontally, mainly by the oronasal route. Furthermore, vertical *in utero* transmission is possible. The normal incubation time is four to seven days. Diseased pigs are highly viremic and shed virus at least from the beginning of clinical disease until death or the occurrence of neutralizing antibodies. Virus is shed in saliva, lacrimal secretions, urine, feces and semen.[1]

Clinical Course:

Depending on the virulence of the CSFV strain and the age and constitution of the host the clinical course of CSF can be



Spleen, pig. There are irregular white marginal infarcts with hemorrhagic seams. At subgross, the acute angle of the section at bottom shows an arterial infarct with peripheral hemorrhage (arrow). (HE, 5X)

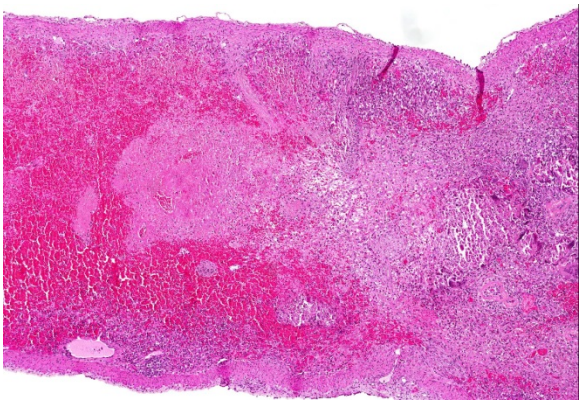
peracute, acute or chronic. In the classical acute form, initial atypical clinical signs such as high fever, anorexia, gastrointestinal symptoms, weakness and conjunctivitis progress to the characteristic hemorrhagic fever and neurological symptoms with a mortality of up to 100% after 2 to 4 weeks. However, a variable proportion of pigs may not progress and recover from the disease. In the chronic form clinical symptoms such as remitting fever, depression, and wasting are usually non-specific and inevitably lead to death after a disease duration of 1 - 3 months. Furthermore, *in utero* infection is a special situation which leads to fetal death, resorption, abortion, mummification, stillbirth, malformations or birth of persistently infected piglets which show runting, late onset disease and inevitably death after 2 – 11 months.[1]

Macroscopic Changes:

Pigs may succumb to peracute CSF without any characteristic gross lesions. Characteristic macroscopic findings in acute CSF are dermal erythema and cyanosis at the margins of the ears, limbs and ventral abdomen. Petechial and ecchymotic hemorrhages can be observed in skin, larynx, epiglottis, urinary bladder, gastric mucosa, and the serosal surfaces of kidneys, lungs, and heart. The lymph nodes can present a typical marbled appearance due to hemorrhages with blood in the peripheral sinuses. Furthermore, there is necrotizing to suppurative tonsillitis, infarcts at the margin of the spleen, as well as hydropericardium, hydrothorax, and hydroperitoneum. In chronic CSF lesions include atrophic lymphatic organs, splenic infarcts, necrosis of gut associated lymphoid tissues including colonic button ulcers. Furthermore, due to the immunosuppression a magnitude of secondary and non-specific lesions can occur. Gross lesions in late onset disease in persistently infected pigs resemble those of the chronic form. Typical malformations in piglets include cerebellar hypoplasia, thymic atrophy, and deformities of the head and limbs.[1,13,14]

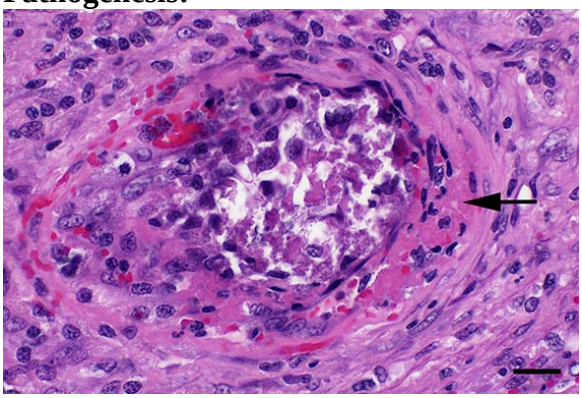
Pathohistologic Changes:

The most important pathohistological findings in acute CSF are multifocal necrotizing vasculitis and lymphoid necrosis and depletion in the lymphatic organs. Vasculitis is often followed by thrombosis, infarction and hemorrhages. In addition to the characteristic gross splenic infarcts, infarcts can also develop in lymph nodes, skin, tonsil, gallbladder, and large intestine. No matter if neurological clinical symptoms have been reported or not, the brain is among the best tissues to check for histological changes. They present as lymphocytic panencephalitis with marked perivascular cuffing and extravasation of blood plasma protein. Chronic cases may also present with mesangioproliferative glomerulonephritis. *In utero* infected piglets can be affected by hypomyelination inducing congenital tremors. Furthermore, the physeal growth plates may exhibit zones of persistent primary spongiosa (growth arrest lines) presumably reflecting viral destruction of osteoclasts.[13]



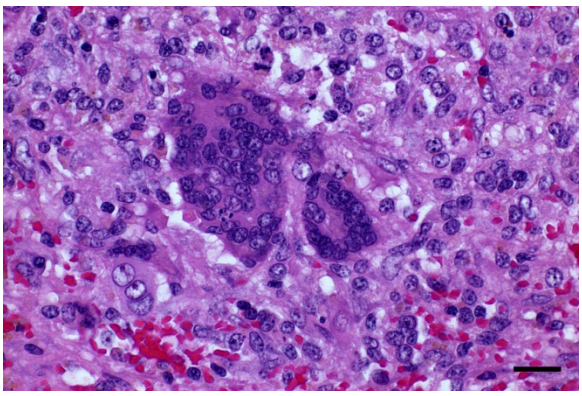
Spleen, pig. Higher magnification of the splenic infarct. An aggregate of multinucleated cells is present at right. (HE, 80X)

Pathogenesis:



Spleen, pig. There is necrosis of the endothelium and extrusion of protein (arrow) within the wall of a medium-sized splenic arteriole. (HE, 400X)

Infection usually occurs via the oronasal route and the CSFV enters the body mainly via the epithelial cells or M-cells of the tonsillar crypts. Primary replication takes place within macrophages in the tonsils and local oropharyngeal lymph nodes, followed by mononuclear cell-associated viremia mainly to other lymphoid organs including spleen, thymus, lymph nodes and mucosa associated lymphoid tissues.[14] Furthermore, CSFV can also be found in variable amounts in all other organs of the body including skeletal muscles, heart, lung, liver, pancreas, stomach, duodenum, jejunum, ileum, kidney, urinary bladder, brain, spinal cord.[6] The CSFV envelope glycoproteins E^{rns} and E2 are responsible for attachment to the putative virus receptor CD46 together with heparan sulfates.[2] The most important host cells are macrophages and dendritic cells, but CSF can also be found in endothelial cells and various epithelial cells, lymphocytes and megakaryocytes. Although activation of macrophages is a well-known feature of CSF, multinucleated giant macrophages as observed in this case are not described.[4,8] Especially the infection of plasmacytoid dendritic cells induces secretion of large quantities of interferon α, which is a key element in the pathogenesis of CSF. The high serum level of interferon α is suggested to be the central inducer of immune cell dysregulation, which manifests as lymphocyte apoptosis, depletion and immunosuppression as well as bone marrow suppression.[10] Furthermore, a marked increase in macrophage / monocyte-derived pro-inflammatory cytokines such as TNF α, IL-1, and IL-6 is suggested to be the main mediator of systemic endotheliotoxicity. Cytokine induced activation of endothelial cells represents the basis for light microscopically visible endothelial swelling. This is followed by degeneration and necrosis of endothelial cells inducing breakdown of the endothelial barrier, fibrinonecrotizing vasculitis, activation of the clotting system, edema and hemorrhages.[14] The formation of arterial infarcts at the multifocal sites of vasculitis within the spleen leads to vascular occlusion followed by ischemic necrotic cell death of the dependent parenchyma. The anatomy of the spleen with single blood supply and minimal anastomoses is an important predisposing factor for infarction.[7] Although it is also most possibly related to some microanatomical features, it is still enigmatic why the infarcts are aligned along the acute angle of the spleen in CSF but not in other diseases with splenic infarcts such



Spleen, pig. Occasional foreign body-type (left) and Langerhans-type (right) multinucleated giant macrophages can be observed within and adjacent to the foci of vasculitis. (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. https://www.fli.de) (HE, 400X)

as African swine fever, highly-pathogenic porcine reproductive and respiratory syndrome and chronic erysipelas.[9] As can be seen in the current case, the infarcted areas initially can be pale due to the lack of blood inflow following complete arterial occlusion. This is rapidly followed by hemorrhage from damaged vessels and inflow of blood from the surrounding parenchyma with intact perfusion leading to the commonly illustrated hemorrhagic infarcts. Secondary pallor due to cell swelling, hemoglobin degradation and diffusion within the affected area usually does not take place in the spleen due to its spongy consistency.[7]

Differential Diagnosis:
A good overview concerning the differential diagnosis of porcine hemorrhagic fevers is presented in Sánchez-Vizcaíno et al. (2015).[9] Concerning splenic infarction in pigs, the most important etiologic differential diagnoses are CSF, African swine fever, highly-pathogenic porcine reproductive and respiratory syndrome, and septic and embolic infarction from other primary lesion sites such as endocarditis valvularis thromboticans in chronic erysipelas.

JPC Diagnosis: 1. Spleen, red pulp: Vasculitis, necrotizing, multifocal to coalescing with multifocal infarcts, *Sus scrofa domesticus*, porcine.
2. Spleen, white pulp: Lymphoid depletion, diffuse, severe.

Conference Comment: The contributor provides a complete review of classical swine fever. The multinucleated giant cells noted by the contributor were seen by conference participants as well. It is possible, that as the moderator suggests, these cells form because monocytes and macrophages are markedly activated as part of the CSF pathogenesis. Attendees debated this concept and pointed out that pestiviruses do not have a fusion protein and should not inherently cause fusion of affected cells.[13] Although the process of multinucleated giant cell formation in inflammation is not well understood it does require that macrophages be bathed in cytokines like IFN-γ, IL-3, IL-4, IL-13 and GM-CSF. Subsequently, membranes of adjacent macrophages express fusogenic proteins such as: DC-STAMP, β1 and β2 integrins, CD44, CD47, macrophage fusion receptor, fusion regulator protein (FRP-1; CD98), and P2X7 (ligand gated ion channel activated by ATP that forms a pore).[1]

Contributing Institution:
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Department of Experimental Animal Facilities and Biorisk Management
Südufer 10, 17493 Greifswald – Insel Riems, Germany.
https://www.fli.de

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