Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 20

25 March 2020

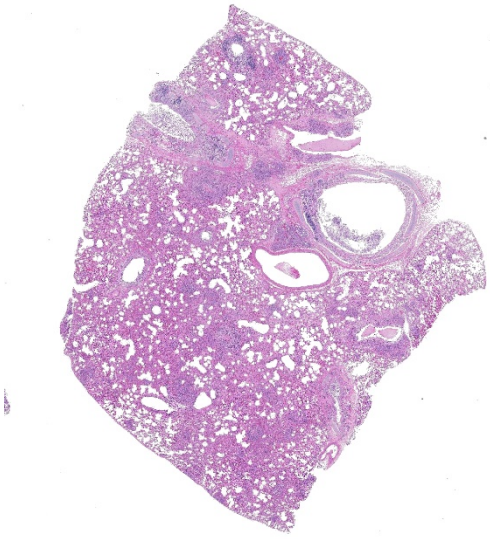
CASE I: T-1708131 (JPC 4117493).

Signalment: Seven-month-old, female, domestic short hair cat, *Felis catus*

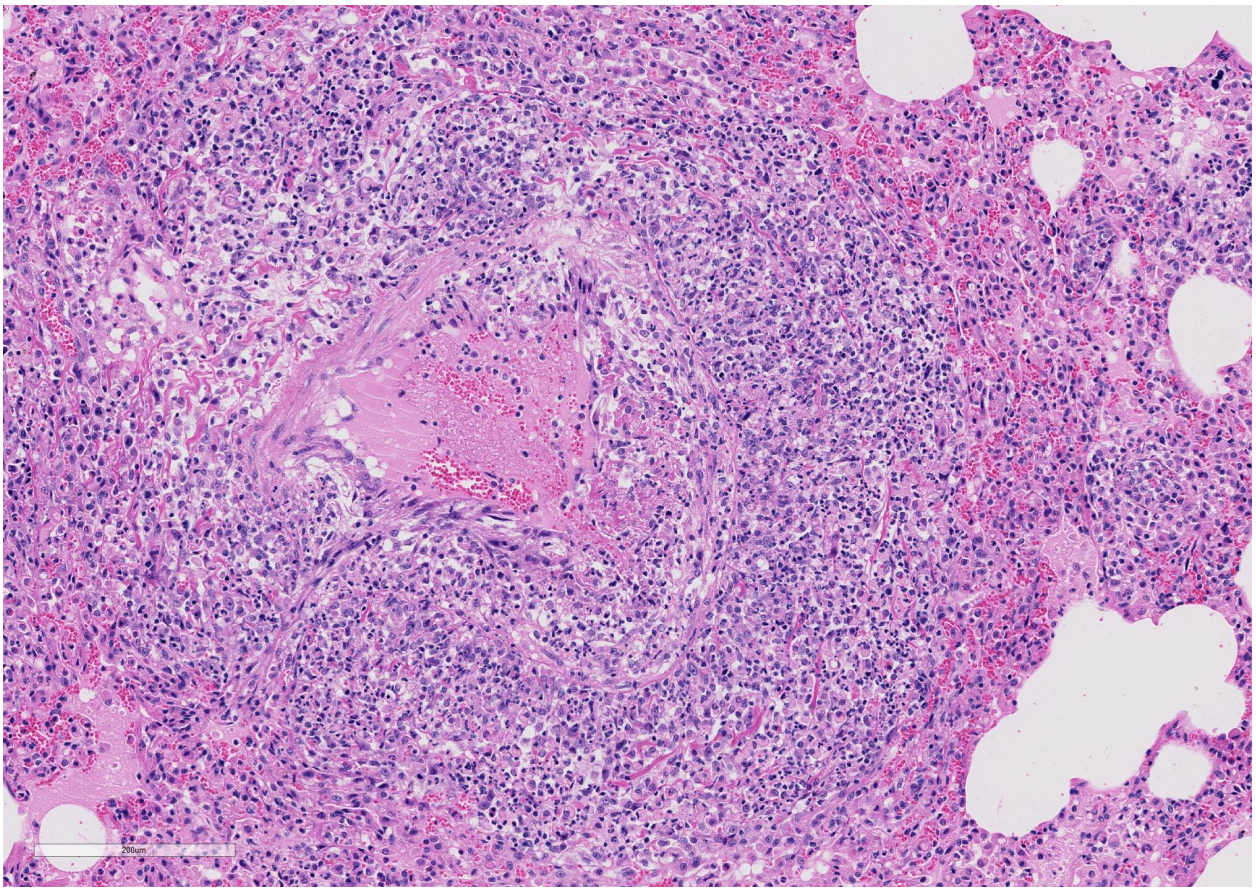
History: The animal presented to the referring veterinarian for general malaise and chronic weight loss. The cat returned home with the owner and was subsequently found dead a few days later. The carcass was submitted to the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) for necropsy.

Gross Pathology: At necropsy, the musculature was mildly atrophied. The oral mucosa was pale beige to grey. Lung lobes were mottled dark red to beige with frequent pinpoint pale foci. Liver had numerous 1 mm to 25 mm in diameter, slightly firm, pale foci. Multifocal to coalescing, irregular, and yellow beige foci, ranging from 2 to 20 mm in diameter were also observed on renal capsule and in renal cortices. Mesenteric lymph nodes were markedly enlarged. There was no other grossly visible lesion at necropsy.

Laboratory results: Bacterial culture on the lung and liver yielded no bacterial growth. Fluorescent antibody tests on lung, spleen, and kidney were negative for both feline infectious peritonitis and feline leukemia virus. PCR for feline coronavirus on pooled



Lung, cat. A single section of lung is presented for examination. At subgross examination, arterioles are surrounded by a cellular infiltrate, and there is exudate within airways. (HE 5X).



Lung, cat. A single section of lung is presented for examination. At subgross examination, arterioles are surrounded by a cellular infiltrate, and there is exudate within airways. (HE 5X).

samples from lung, kidney, liver, and brain was positive.

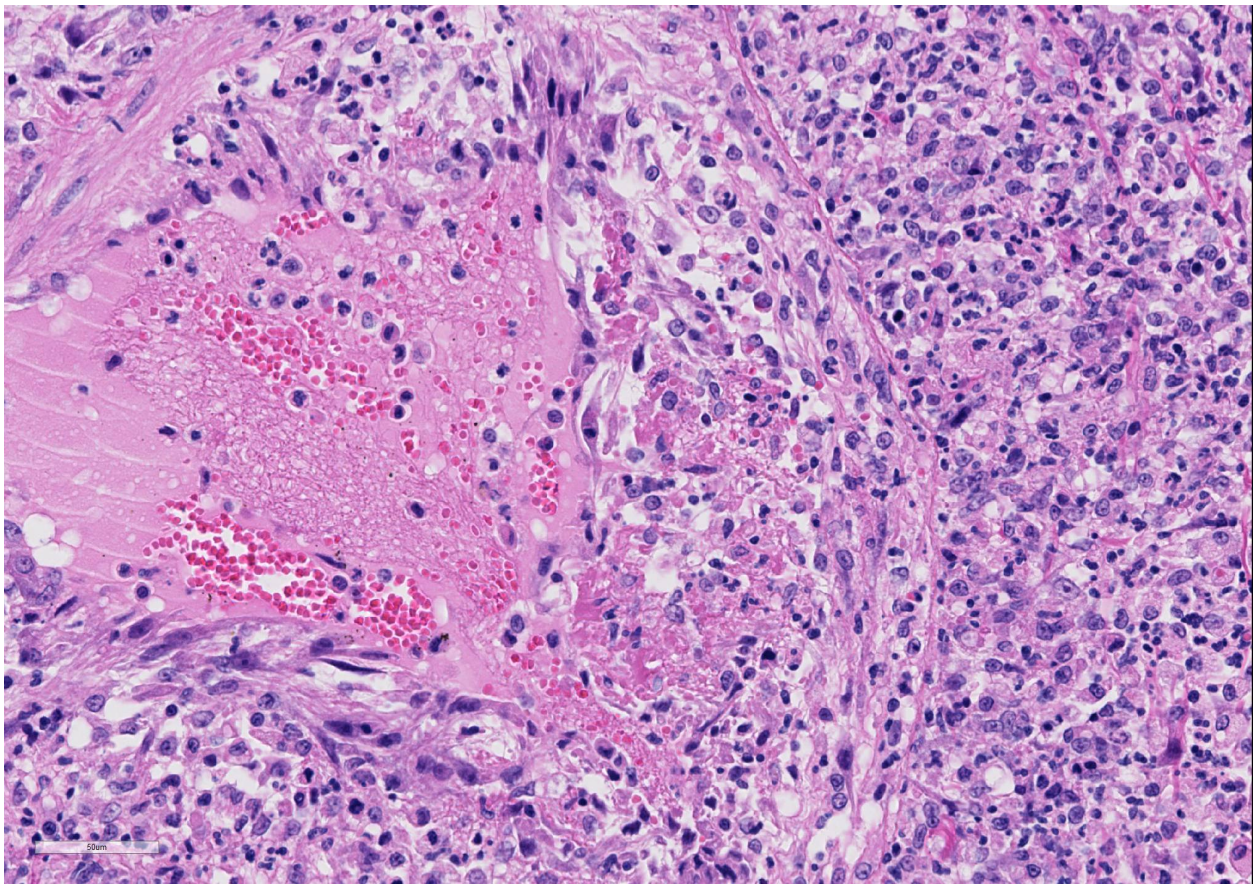
Microscopic Description: Lung sections were congested and disrupted by multifocal to coalescing areas of marked inflammation composed of large numbers of macrophages and neutrophils admixed with fewer plasma cells and lymphocytes. Occasional inflammatory foci contained multinucleated giant cells. Multifocally, tunica media and tunica intima of the pulmonary vessels were disrupted and expanded by an eosinophilic fibrillar material, degenerate neutrophils, and karyorrhectic debris. Scattered vessels were partially occluded by thrombi. Alveoli often contained an eosinophilic material admixed with low to moderate numbers of macrophages. A few bronchioles and bronchi contained an eosinophilic wispy material admixed with low numbers of macrophages and neutrophils. The peribronchiolar lymphoid tissue was hyperplastic. The pleural mesothelium exhibited mild hypertrophy. In a few regions, the pleural surface was overlaid with fibrin mats that contained degenerate neutrophils (not present in all sections).

Contributor's Morphologic Diagnosis:

1. Pneumonia, interstitial, pyogranulomatous, subacute to chronic, multifocal to coalescing, marked with vasculitis, lung.
2. Nephritis, interstitial, pyogranulomatous to plasmacytic, subacute to chronic, multifocal to coalescing, marked with vasculitis, kidney (not present in slide).

3. Hepatitis, pyogranulomatous to plasmacytic, subacute to chronic, multifocal to coalescing, marked, liver (not present in slide).
4. Meningoencephalitis, neutrophilic and histiocytic to plasmacytic, subacute to chronic, multifocal, marked with vasculitis, brain (not present in slide).
5. Lymphadenitis, pyogranulomatous to plasmacytic, subacute to chronic, multifocal to coalescing, marked, mesenteric and splenic lymph nodes (not present in slide).
6. Myocarditis, necrotizing to pyogranulomatous, subacute to chronic, multifocal, moderate to marked, heart (not present in slide).
7. Cystitis, interstitial, pyogranulomatous, subacute to chronic, multifocal, moderate to marked, urinary bladder (not present in slide).

Contributor's Comment: Histopathology confirmed a marked pyogranulomatous interstitial pneumonia with vasculitis. Similar foci of pyogranulomatous inflammation and vasculitis were observed in the kidney, liver, brain, lymph nodes, heart, and urinary bladder. The pattern of multisystemic pyogranulomatous inflammation with vasculitis was consistent with the noneffusive form of feline infectious peritonitis (FIP). PCR on fresh samples of lung, liver, kidney, and brain were positive for feline



Lung, cat: Higher magnification of the affected vessel demonstrating segmental necrosis, mural fibrin and hemorrhage, and effacement of the wall by infiltrating neutrophils and macrophages, admixed with abundant cellular debris. (HE, 400X).

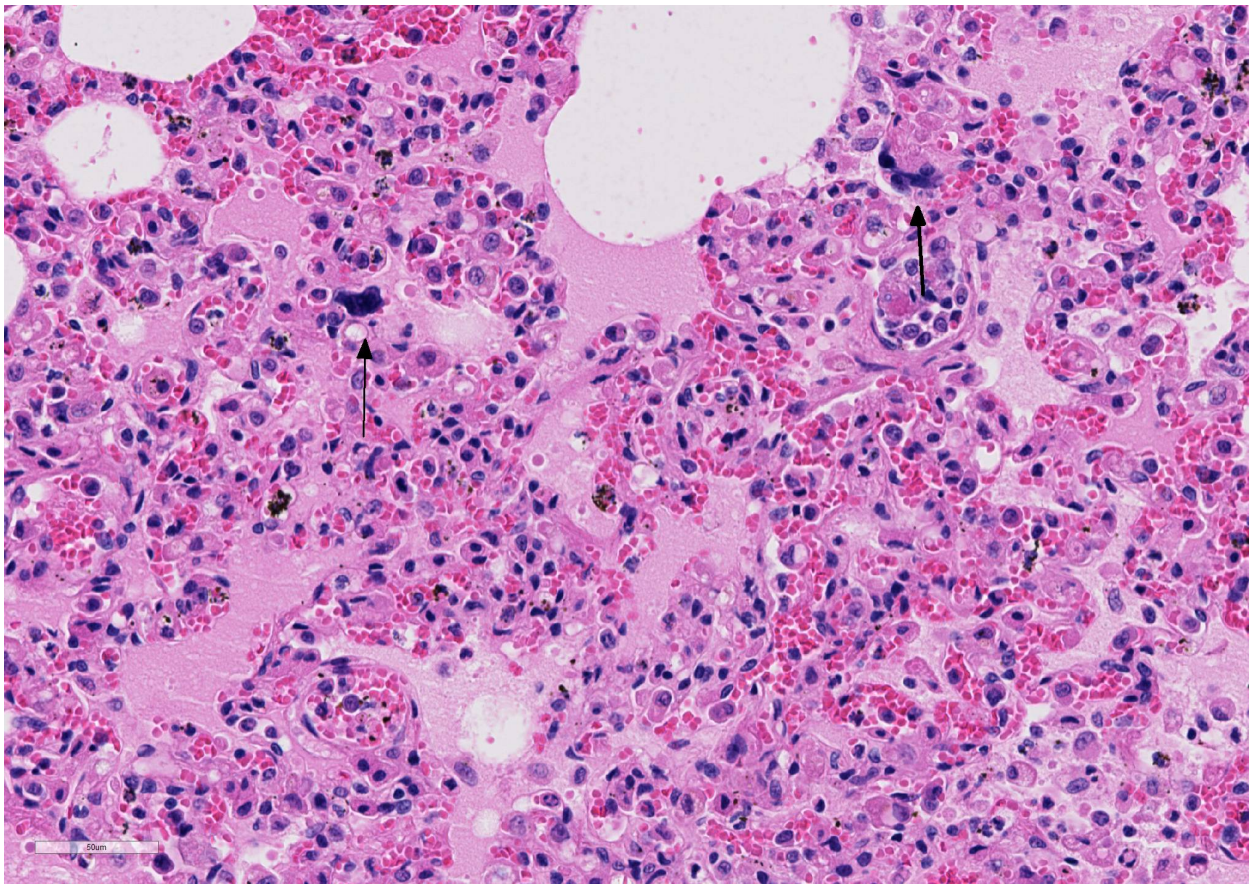
coronavirus. These results in conjunction with the histopathologic lesions confirmed FIP.

FIP is a fairly common infectious disease of cats with a low morbidity, yet high mortality. FIP is a coronaviral disease that can affect cats of any age, but is most prevalent among cats <3 years of age and especially from 4 to 16 months of age.[4] The disease continues to be a major killer of young cats.[3] In general, FIP tends to affect young cats, but can still occur in middle-aged to older cats. Purebred cats and male cats appear to be more susceptible.[4] It has also been well documented in virtually every species of Felidae, and FIP of domestic cats and cheetahs has been historically intertwined.[2,4]

Coronaviruses have adapted themselves over thousands of years to virtually every species of mammals and birds, and are a common cause of transient enteritis and respiratory disease.[3] The causative agent of FIP is feline coronavirus and more specifically, the feline infectious peritonitis virus (FIPV) biological pathotype. The virus is an Alphacoronavirus in the family Coronaviridae.[5] The other pathotype is feline enteric coronavirus (FECV), which is restricted to the intestinal tract and generally considered to be avirulent.[5] The exact pathogenesis of the viral infection and development of clinical disease is not fully elaborated, but the ability of the virus to replicate within macrophages is important in the development of FIP.[5,6] Mutation of FECV to a virulent FIPV has been considered as a possible contributing factor to the development of feline infectious peritonitis.[4,5,6] Other factors contributing to FIP include viral strain, genetic predisposition, and the host's immune response (e.g. ineffective cell mediated immunity of the host will favor an FIP virus infection).[5,6] The role of genetic factors in resistance or susceptibility to FIP is based on both indirect and direct observations. Pedigreed cats are more likely to develop FIP than random-bred cats and certain breeds are also more severely affected than others.[3]

Resistance to FIP is complicated and involves genetic susceptibility, age at the time of exposure and a number of stressors that occur at the same time as infection and have a negative impact on the ability of the infected cat to eliminate the virus. The time period between initial FECV exposure and clinical signs of disease can be as short as 2–3 weeks, as long as several months or, rarely, years, reflecting the time it takes for mutant FIPVs to evolve, or for the infection to progress from a subclinical to clinical disease. After an onset of overt clinical disease, a return to normal health is extremely uncommon and rarely may a cat will make an apparent recovery, but only to have clinical signs recur months and even years later. The disease course is generally shorter in younger cats and cats with effusive disease than in older cats and cats with non-effusive disease.[3]

FIP manifests itself in two forms the non-effusive ("dry") form as displayed in this case and the effusive ("wet") form. Despite the distinct nomenclature, these forms likely represent the two extremes of a spectrum. The effusive form typically presents with multiple acutely developing effusions in body cavities predominantly the abdominal and pleural cavities. Also, serosal surfaces often have fibrin deposits with necrotic foci in the associated parenchyma.[5] The noneffusive form however is chronic and characterized by multisystemic vasculitis and perivascular inflammation. The inflammatory infiltrate is classically histiocytic to pyogranulomatous with variable numbers of neutrophils, plasma cells and lymphocytes.[5] The lesions are commonly observed in the kidney, eye, brain, lung, liver, lymph nodes, and serosal surfaces. Grossly, the noneffusive form of FIP can present



Lung, cat: Throughout the section, alveolar spaces are flooded with edema fluid. Alveolar septa are markedly congested, contain scattered fibrin thrombi, and rare megakaryocytes (arrows). (HE, 125X).

similar to systemic bacterial, fungal or protozoal infections and disseminated neoplasia, especially lymphoma.[5]

Diagnosis of FIP is based first and foremost on consideration of the age of the patient, origin, clinical signs and physical examination. Abdominal distension with ascites, dyspnea with pleural effusion, jaundice, hyperbilirubinuria, discernible masses on the kidneys and/or mesenteric lymph nodes, uveitis and a range of neurological signs associated with brain and/or spinal cord involvement are all common in cats with either the effusive or non-effusive form of FIP. At this point, the diagnosis of FIP can be made with reasonable certainty.[5]

The diagnosis of feline infectious peritonitis is typically based on the gross lesions and histopathology in combination with molecular diagnostics and/or immunohohistochemistry.[5] Other ancillary tests such as serology (e.g. ELISA) or fluorescent antibody testing can help support the diagnosis of FIP,[5] but may be less reliable.

Contributing Institution: The University of Georgia, College of Veterinary Medicine, Department of Pathology, Tifton Veterinary Diagnostic & Investigational Laboratory, Tifton, GA 31793; http://www.vet.uga.edu/dlab/tifton/index.php

JPC Diagnosis: Lung: Phlebitis, lymphohistiocytic, diffuse, severe, with fibrinoid necrosis, diffuse moderate interstitial pneumonia and marked alveolar and interstitial edema.

JPC Comment: The contributor has provided a fine overview of the disease of feline infectious peritonitis. Over the last decade, extensive research has provided insight into the "internal mutation theory" that the mutations transforming FeCV to FIPV occur internally within each individual. Three different genes have been identified have been identified with this conversion.[3]

The ORF 3c gene (whose protein product is unknown) was the first mutated gene to be identified, and approximately 2/3 of FIP viruses have ORF3c truncating mutations resulting in the ability to replicate in macrophages. (Other mutated ORF 3c genes which are not truncated (in this case causing a premature stop codons) do not confer this ability on the virus.[3]

Multiple mutations of the S gene, which encodes the fusion protein, has been identified in mutated forms in FIPVs and in FIP-infected cats, but not in FECVs. Single nucleotide alterations, including at the S1/S2 cleavage have been associated with macrophage tropism. [3]

Macrophage activation is one of the most important drivers of FIP in the cat. The typical vasculitis in FIP is as seen in this case - a vasculitis almost exclusively affecting veins and driven by virus-infected macrophages.[1] This phlebitis has been purported to develop as a result of interaction between the viral-infected monocytes and activated endothelial cells. The preponderance of vascular lesions is limited to veins in a number of organs (kidney, lung, meninges, eyes, liver), suggesting that not all endothelial cells share responsiveness to macrophages-secreted cytokines.[1] The incredible outpouring of fluid from affected vessels in the wet form of FIP (and likely the tremendous amount of fluid in the lungs of FIP cats such as this submission) has been associated with overproduction of vascular endothelial growth factor by virally-infected macrophages. Other cytokines overproduced by macrophages in FIP-infected cats include TNF-alpha, GM-CSF and G-CSF.[1] Macrophages secrete the adhesion molecule CD18 in order to attach to activated endothelium, and matrix malloproteinases to digest the vascular basement membrane at sites of emigration. The relative lack of lymphocytes in these lesions helps distinguish this lesion from a true immune-medicated vasculitis.[1]

References:

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CASE II: 19-2535 DVD (JPC 4135747).

Signalment: One-year old female sheep

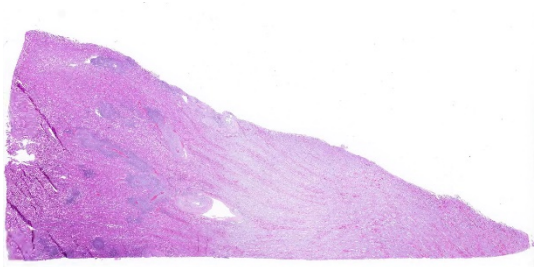
History: Two yearling sheep in a group of fifteen died within the span of two weeks. A field necropsy with limited tissue collection was performed by the primary veterinarian.

Gross Pathology: Clinician reported adequate internal fat, heavy and wet lungs, and several < 1 cm in diameter abscesses in the liver.

Laboratory results: Quantitative PCR for ovine herpesvirus 2 revealed high copy numbers of viral DNA in liver and lung (73,400 and 195,000 copies/5 ng total tissue DNA).

In situ hybridization: Leukocytes demonstrate positive intranuclear staining for ovine herpesvirus 2.

Microscopic Description: Kidney: Multifocal vessels within the renal cortex and the arcuate arteries are transmurally disrupted by moderate numbers of densely packed macrophages, lymphocytes, plasma cells, and a few neutrophils. The endothelial cells are circumferentially plump, and the internal elastic lamina of the tunica intima is multifocally interrupted or obscured by the mixed inflammation admixed with small amounts of hypereosinophilic fibrillar material, shrunken and hypereosinophilic cellular silhouettes that have pyknotic nuclei (necrosis), and eosinophilic granular debris. The tunica media and adventitia are expanded by increased numbers of plump fusiform cells and collagenous connective tissue (hypertrophy). The mixed inflammation fills the periportal connective tissue and multifocally obscures the venous silhouettes. The renal parenchyma adjacent to the affected arteries is also expanded by small to moderate numbers of lymphocytes and plasma cells.



Kidney, sheep. A wedge of kidney is submitted for examination. All arterioles (arcuate, lobar, interlobar) are outlined by hypercellularity. (HE, 8X)

Other tissues:

Liver: Multifocally within the portal triads or around hepatic arteries, the walls of the arteries are similarly disrupted. The mixed inflammation fills the periportal connective tissue and multifocally obscures the venous silhouettes.

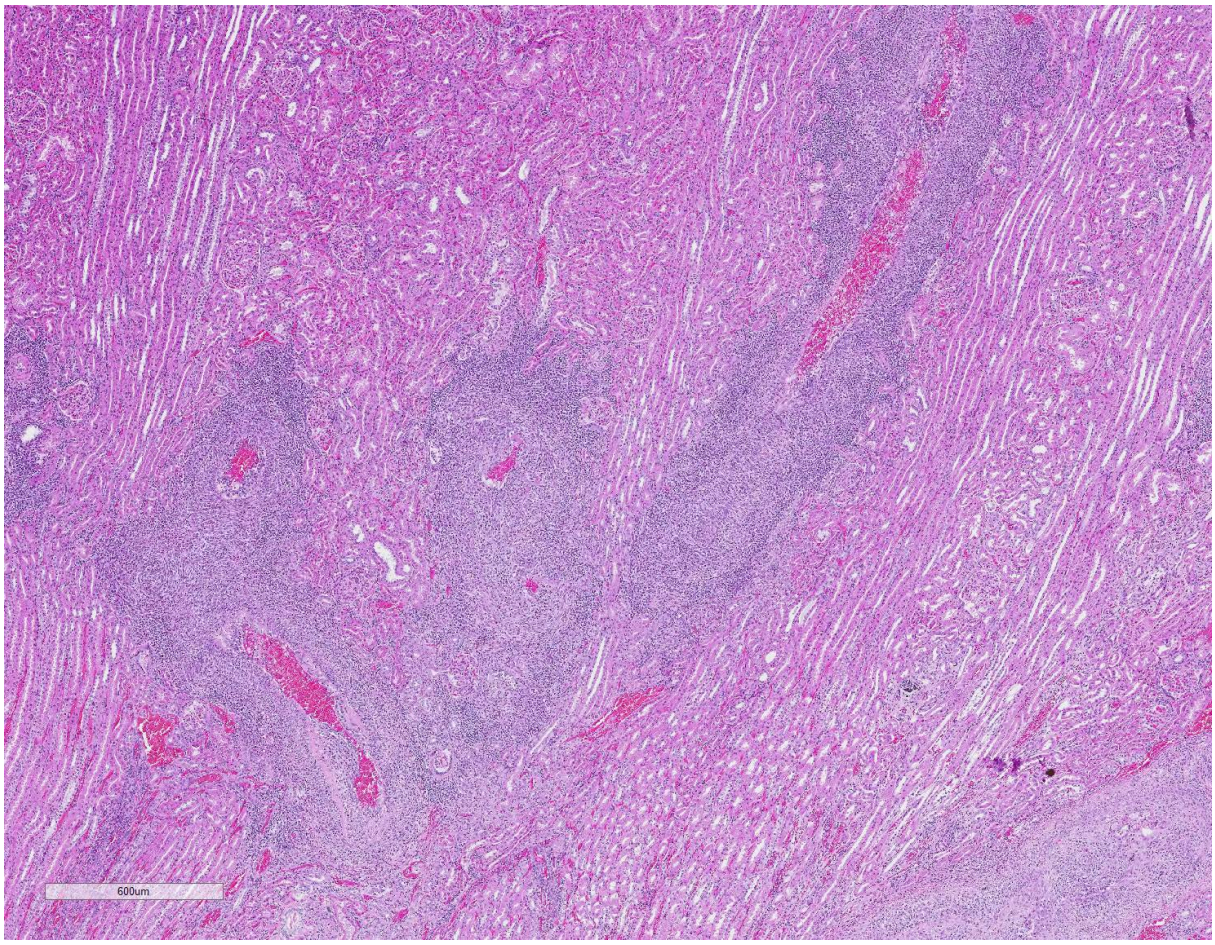
Lung: A group of arteries adjacent to the cartilage of a bronchus is similarly disrupted by mixed inflammation that surrounds and extends into the wall.

Heart: A large caliber artery is disrupted by smaller amounts of mixed inflammation. All arteries have moderate to marked medial hypertrophy and thickening.

Contributor's Morphologic Diagnosis:

Vasculitis, histiocytic and lymphoplasmacytic, multifocal, chronic, marked with vascular hypertrophy; liver, kidney, heart, and spleen

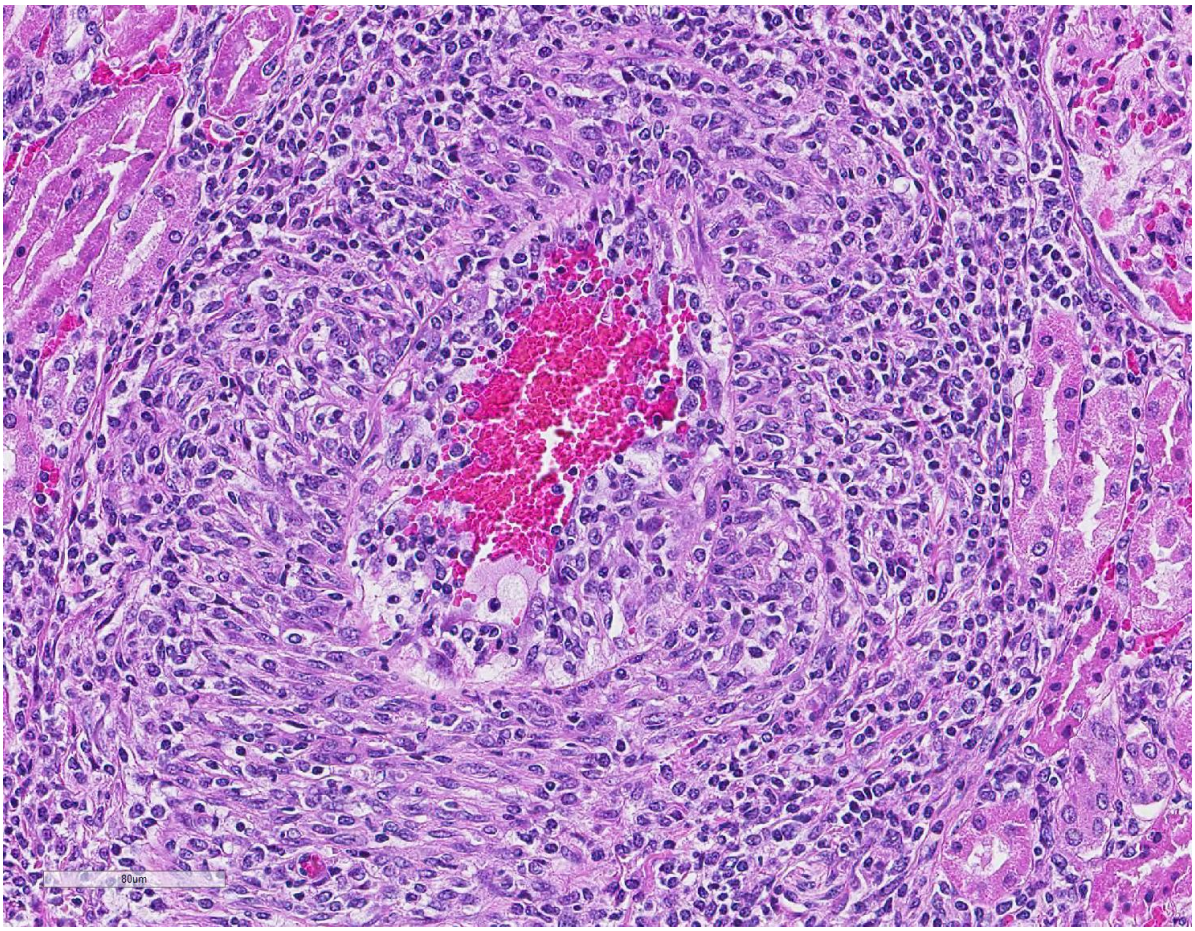
Contributor's Comment: The presented case is consistent with systemic necrotizing vasculitis of sheep, a sporadic disease affecting individuals or clusters of sheep ranging from 5 months to 3 years old in the literature.[2,7,9,10] Clinical signs of this disease may vary, and subcutaneous and joint swelling, diarrhea, chronic weight loss, and hemorrhage into body cavities with a case of aneurysmal dilation and rupture of the



Kidney, sheep. Higher magnification showing effacement of medium-caliber arterioles by a lymphohistiocytic infiltrate which extends into and effaces the surrounding renal parenchyma. (HE, 8X)

gastroduodenal artery have been described. Histologically, the findings are characterized by a primarily circumferential lymphocytic inflammation disrupting small- to medium-sized arteries. The inflammation and local necrosis is often most densely affecting the adventitia and outer muscular wall, but can extend to be transmural in affected vessels. Comparisons between systemic necrotizing vasculitis of sheep and polyarteritis nodosa have been described,[2] and the etiology in individual cases are often speculated to be viral or immune-mediated.[9,10]

Recently, infection with the gammaherpesvirus ovine herpesvirus 2 (OvHV-2) has been demonstrated to be associated with systemic necrotizing vasculitis in sheep.[7] Our presented case recapitulates this association by identifying OvHV-2 nucleic acid within the vascular lesion via *in situ* hybridization. OvHV-2, a causative agent for malignant catarrhal fever (MCF) affecting several ruminant species such as cattle, deer, and confined bison, is generally thought to be a subclinical infection in domestic sheep. Like other herpesviruses, OvHV-2 establishes persistent infection in sheep, which are the natural hosts, and the virus is shed throughout the lifetime of the host. Although complete details of the epidemiology and viral life cycle of OVH-2 infection in domestic sheep remain to be determined, the majority of lambs acquire OvHV-2 at about



Kidney, sheep. The tunica intima is effaced by inflammatory cells, infiltrating smooth muscle cells and collagen. Similar changes affect the tumina media and inflammatory cells, with a predominance of lymphocytes replace the adventitia and extend into the surrounding parenchyma. (HE, 283X)

10 weeks of age, and the highest levels of viral DNA is detected in nasal secretions when individuals are between 6 and 9 months of age.[5] Viral DNA is detected in peripheral blood leukocytes of lambs earlier than in nasal secretions, which suggest that initial infection may occur in lymphocytes.[4] Diagnosis of OVH-2 associated MCF-like syndrome in sheep is a diagnostic challenge given that the virus is readily detectable in clinically healthy animals. Supportive evidence for diagnosis in this case included characteristic vasculitis in multiple organs, quantitative PCR demonstrating high copy numbers of OVH-2 DNA in tissue, and in situ hybridization revealing OHV-2 genomic material within lesion leukocytes.

Differentials for viral-associated vasculitis in sheep are summarized in Table 1, below.

Name	Virus	Associated Disease	Ancillary testing
Bluetongue	Orbivirus	Vascular thrombosis, tissue infarction, and hemorrhage +/- vascular inflammation	PCR of affected tissues

Maedi-Visna / Ovine Progressive Pneumonia	Small Ruminant Lentivirus	Chronic lymphocytic pneumonitis, encephalitis, arthritis, mastitis and vasculitis	Positive lentivirus ELISA with concurrent and characteristic lesions, IHC
Border Disease	Pestivirus	Disseminated nodular periarteritis with a predilection for CNS tissues	Viral isolation and/or rt-PCR

Table 1. Differential diagnoses for viral vasculitides in sheep.[1,3,6,11]

Contributing Institution: Washington State University Department of Veterinary Microbiology and Pathology (https://vmp.vetmed.wsu.edu/)

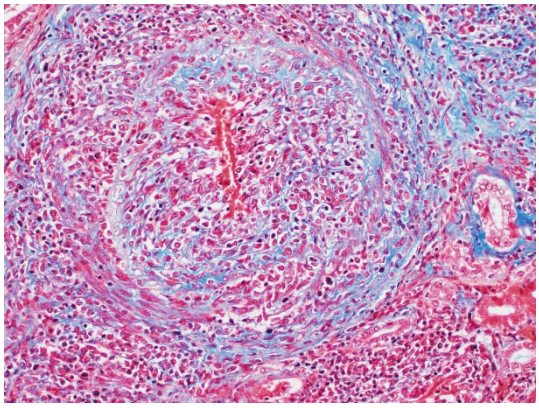
Washington State Animal Disease Diagnostic Laboratory (https://waddl.vetmed.wsu.edu/)

JPC Diagnosis: Kidney, arteries: Arteritis, lymphohistiocytic, multifocal, chronic, severe, with moderate perivascular lymphohistiocytic nephritis.

JPC Comment: The contributor has provided a concise but thorough review of malignant catarrhal fever (MCF) in sheep. MCF has been a frequent contribution to the WSC over the years (Table 2), even before ovine herpesvirus-2 (OvH-2) was identified as a potential cause. In addition to ovine herpesvirus-2, five other MCF-inducing viruses have been identified in ungulates, including alcelaphine herpesviruses 1 and 2, caprine herpesviruses 2 and 3, and ibex malignant catarrhal virus.[8]

The contributor has mentioned the difficulty in establishing the diagnosis of MCF in sheep. Clinically healthy sheep are thought to be widely infected with OvH-2, and would normally be PCR-positive for the present of this virus. A recent paper[7] described a diagnostic protocol utilizing a combination of in-situ hybridization and quantitative PCR to compare viral nucleic acid between tissues of vasculitis and those of clinical healthy normal carriers; it suggested that the presence of ISH positivity in vascular lesions and high levels of OvH-2 DNA may be used to confirm cases of ovine MCF.[7]

Other unique features of MCF in the sheep have impacted the elucidation of its pathogenesis. Unlike other MCF viruses, such as alcelaphine herpesvirus-1, the virus cannot be grown in cell culture, relegating researchers to used pooled secretions from multiple lambs. In AHV-1 infection in susceptible ruminants, lymphoid hyperplasia in which the nodes are populated by large lymphocytes heralds early infection, but these changes are not seen in OVH-2 infection, in which viral distribution is limited, and lesion development is proportional to viral infection and distribution.[8] One particular group has recently published a report of their development of a specific OvH-2 ISH probe with high specificity (it does not react with



Kidney, sheep. A Masson-Trichrome demonstrates the amount of collagen within the wall of the inflamed arteriole. (Masson's trichrome, 400X)

AHV-1, ibex-McFV, and CpHV-2). The particular probe was shown to be effective in both experimental and natural infections, even in cases with low viral copy number, and that ISH signals correlate positively with lesion severity.[8]

References:

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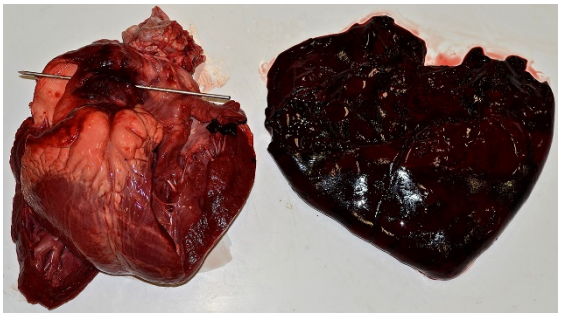
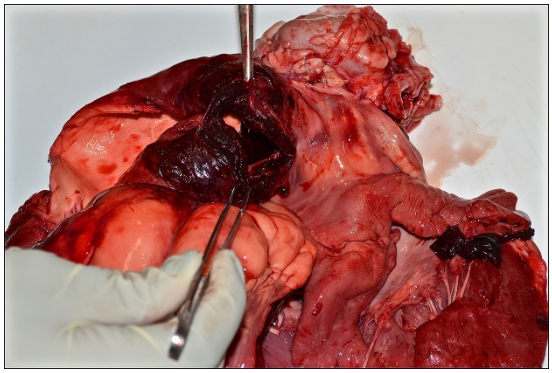
CASE III: Blank label (JPC 4117883).

Signalment: 1-year-old, Aberdeen Angus steer*, Bos taurus*

History: Found dead in pen.



Lung, ox. A ~2L blood clot filled the pericardial sac. There is a full thickenss tear at the base of the pulmonary artery. (Photo courtesy of: Colorado State University Veterinary Diagnostics Laboratories, 200 West Lake Street, Fort Collins, CO 80523-1644, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx)



Lung, ox. Close up of the tear at the base of the pulmonary artery with marked hemorrhage within the arterial wall. (Photo courtesy of: Colorado State University Veterinary Diagnostics Laboratories, 200 West Lake Street, Fort Collins, CO 80523-1644, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx

Gross Pathology: Presented for postmortem examination was a 1-year-old Black Angus steer in adequate body condition (BCS 2.5 out of 5) with minimal postmortem autolysis. A mild amount of edema expanded the subcutaneous fascia of the ventral thorax and mandible. Markedly distending the pericardial sac and enveloping the heart was a large ~2L coagulum of blood (Figure 1). The wall of the right ventricle was markedly thickened and firm, with a 1:1 ratio of right to left ventricular free walls. The pulmonary artery was severely dilated and there was a 4 x 2 cm acute full thickness tear at the base of the pulmonic outflow track (Figure 2). The intimal surface of the pulmonary artery was irregularly roughened and granular and the tear had hemorrhagic borders. The liver was slightly enlarged with rounded borders and was slightly firm in texture on cut section. The remainder of the post mortem examination was within normal limits.

1. Heart/pulmonary artery: Severe right ventricular concentric hypertrophy with pulmonary artery aneurysm, acute rupture, and hemopericardium with cardiac tamponade.
2. Liver: Moderate chronic passive congestion.

Laboratory results:

Bacteriology - Aerobic culture, lung

No significant growth

Molecular diagnostics - lung

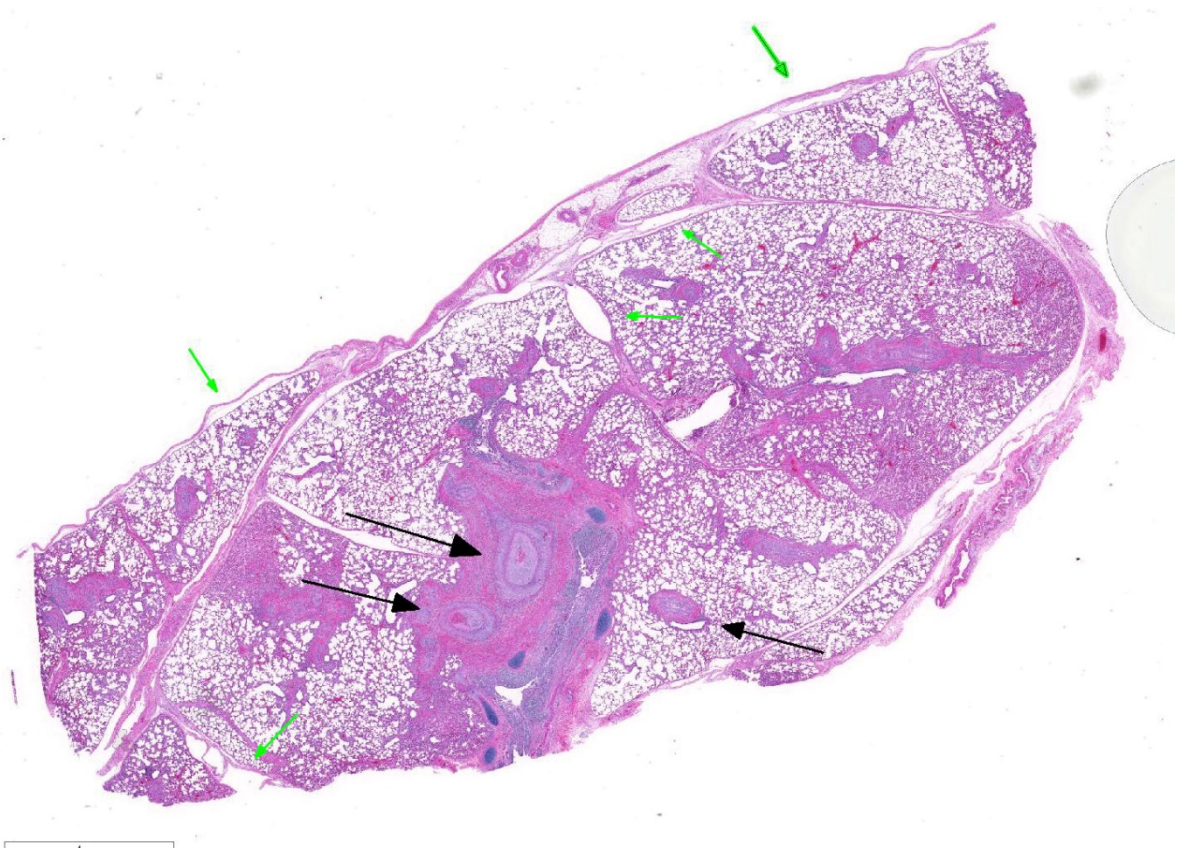
Mycoplasma PCR: Not detected

Bovine respiratory syncytial virus (BRSV) real-time PCR: Not detected

Bovine herpesvirus-1 (IBR) real-time PCR: Not detected

Bovine viral diarrhea virus (BVDV) real-time PCR: Not detected

Microscopic Description: Lung: Diffusely throughout sections examined, the vascular lumina of small to large caliber muscular arteries, and to a lesser extent intraparenchymal pulmonary veins, are diminished due to marked expansion of the tunica intima and media by hypertrophic and hyperplastic smooth muscle and fibroplasia. Severely affected vessels are also segmentally to circumferentially expanded by poorly organized and edematous adventitial fibrosis which supports multiple cross sections of proliferative capillary profiles (complex plexiform arteriopathy/vasa vasorum hypertrophy). Adjacent alveolar spaces are occasionally compressed and collapsed. Vessels are internally lined by endothelial cells which are plump and reactive. Rare vessels are partially to completely occluded by organized thrombi. The tunica intimal and medial hypertrophy of both arteries and (to a lesser extent) veins was confirmed on a Van Gieson stain.



Lung, ox. At subgross magnification, the walls of pulmonary arteries are markedly expanded (black arrows), and there is marked edema and emphysema affecting the interlobular septa and pleura (green arrows). (HE, 6X)

There are multifocal regions of mild alveolar interstitial necrosis. In these areas alveolar septae are lined by hyalinized webs of fibrin. In other locations alveolar septae are lined by plump hyperplastic type II pneumocytes. Alveolar spaces are multifocally expanded by fibrinous and edematous exudates which support scant numbers of neutrophils and plump foamy alveolar macrophages. The pleural surface and interlobular septae are mildly expanded by edema.

Contributor's Morphologic Diagnosis:

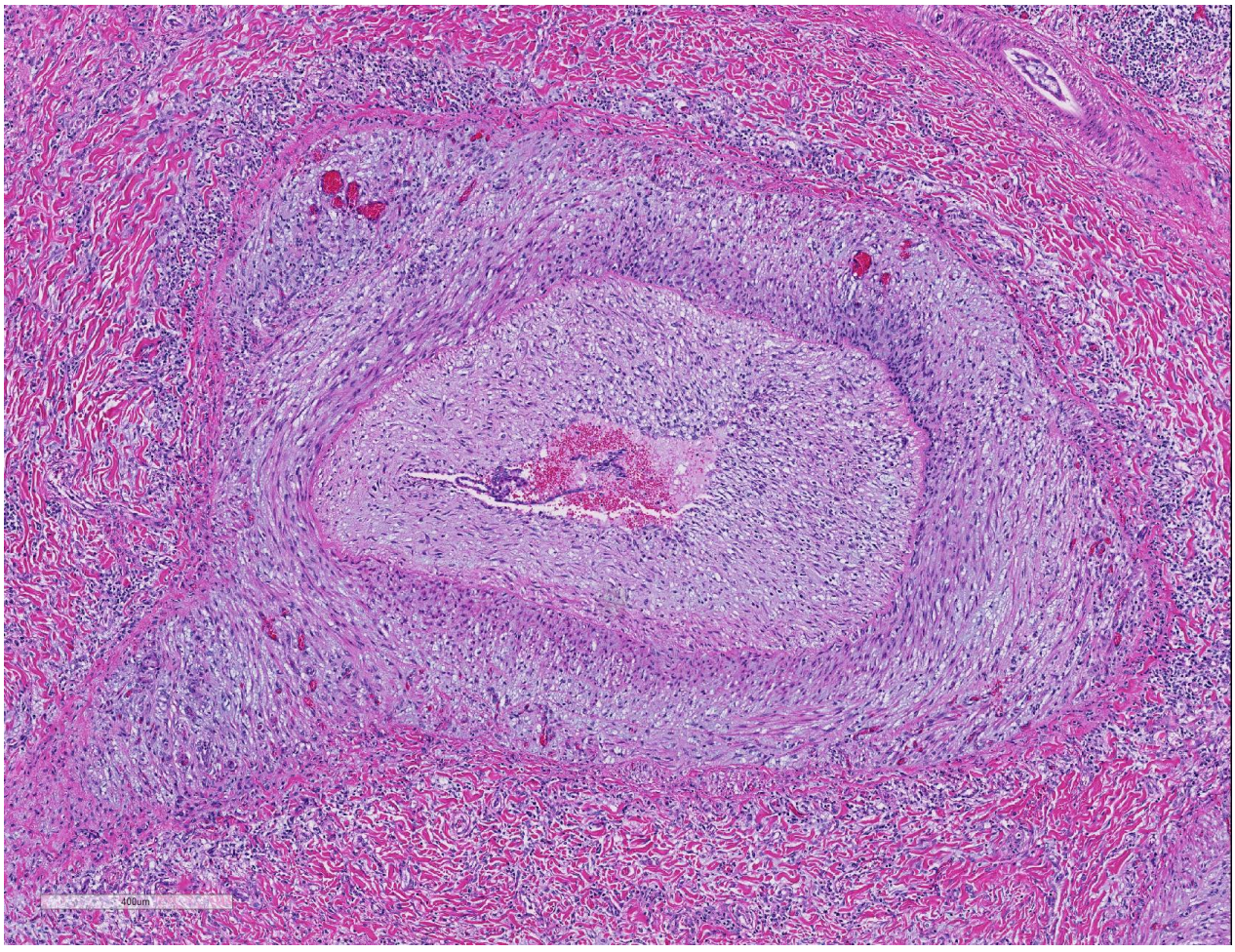
1. Lungs, pulmonary arteries: Severe diffuse chronic arterial mural hypertrophy and hyperplasia with adventitial fibrosis and vasa vasorum hypertrophy (plexiform arteriopathy).
2. Lungs, pulmonary veins: Multifocal mild segmental mural hypertrophy.

Contributor's Comment: The most significant gross lesion detected on post mortem examination was severe right ventricular concentric hypertrophy with pulmonary artery aneurysm, rupture, and hemopericardium. Histologic lesions within the lungs are consistent with high mountain disease, also known as brisket disease, due to typical presentation with submandibular, cervical, and cranioventral thoracic subcutaneous edema.

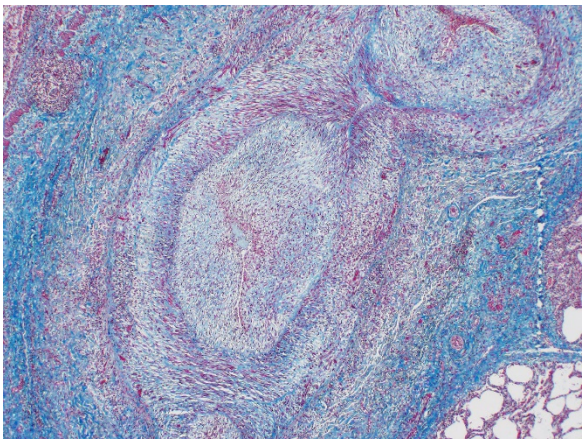
Brisket disease was clinically recognized in Colorado in the early 1900's and was initially reported by Glover and Newsom in the *Colorado Agriculture Experimental Station* bulletin in 1915 and 1917.[2,3] In 1959

Alexander and Jensen reported that this condition is associated with right ventricular hypertrophy in both natural cases as well as in experimental cattle residing at high altitude.[1] Chronic hypoxia and pulmonary hypertension were proposed as an underlying etiology.

In acute hypoxic conditions, such as encountered at high altitude (elevation > 1600m), pulmonary arteriolar vasoconstriction results in both pulmonary hypertension and vascular shunting.[5] Chronicity results in remodeling of pulmonary arteries, including hypertrophy of the tunica intima, media, and adventitia, which further increases pulmonary resistance to flow. Neary et. al speculated that proximal remodeling of large pulmonary arteries (in additional to precapillary arterioles) may play a role in the pathogenesis of bovine pulmonary hypertension.[8] Stenmark et al. introduced an "outside in" hypothesis which postulates that vascular remodeling may be initiated by activated pro-inflammatory adventitial fibroblasts which mediate remodeling in adventitial, medial, and intimal layers and induce proliferation of the vasa vasorum.[6,10] Long-standing pulmonary hypertension may result in cor pulmonale or right ventricular cardiac hypertrophy and eventually right-sided congestive heart failure.[5]



Lung, ox. At subgross magnification, the walls of pulmonary arteries are markedly expanded (black arrows), and there is marked edema and emphysema affecting the interlobular septa and pleura (green arrows). (HE, 6X)



Lung, ox. A Masson's trichrome demonstrates the amount of fibroplasia within the walls of the hypertrophied arterioles. (Masson's trichrome, 40X)

Pulmonary hypertension is detected in live cattle by direct measurement of pulmonary arterial pressure (PAP) via catheterization of the right ventricle. Routine pulmonary arterial pressure testing (PAP) was initiated in the 1960s and early studies concluded that the degree of pulmonary hypertension detected by the PAP measurement was directly related to the degree of histologic pulmonary arterial hypertrophy.[5] Thus a pulmonary arterial pressure above 30 mmHg is considered diagnostic for pulmonary hypertension in cattle and it is recommended bulls with an elevated PAP be removed from breeding stock.

As demonstrated in this case by Verhoff-Van Gieson elastin staining, vascular lesions are not limited solely to pulmonary arteries but also rarely involve pulmonary veins. The degree of this lesion is mild in this case, but may be playing a perpetuating role in the underlying pathogenesis of this disease.

In addition to cattle, cardiac and pulmonary arterial remodeling was described in various zoo mammals housed at high altitudes at African Safari in Puebla, Mexico (elevation 2,100m) (8). Species included in this study were 10 maras (*Dolichotis patagonum*), 2 cotton-top tamarins (*Saguinus oedipus oedipus*), 2 capybaras (*Hydrochaeris hydrochaeris*), a Bennet's wallaby (*Macropus rufogriseus*), a nilgai antelope (*Boselaphus tragocamelus*), and a scimitar-horned oryx (*Oryx dammah*).[9]

It is important to rule out other diseases that may mimic brisket disease such as hardware disease, cardiomyopathy, heart failure secondary to chronic pneumonia, diffuse pulmonary parenchymal disease such as pulmonary fibrosis, or disseminated pulmonary abscesses, parasitic pneumonia, pulmonary intoxications, and/or emphysema.[7] In this case, the histologic changes consistent with a mild interstitial pneumonia retrospectively prompted a comprehensive bovine respiratory disease screen for case completeness; no underlying infectious etiologies were identified.

Given the history of this animal being raised along the Colorado Front Range (elevation ~1700m) combined with the gross and histologic diagnoses we conclude the constellation of these findings are consistent with bovine high altitude pulmonary hypertension with core pulmonale, pulmonary aneurysm and rupture.

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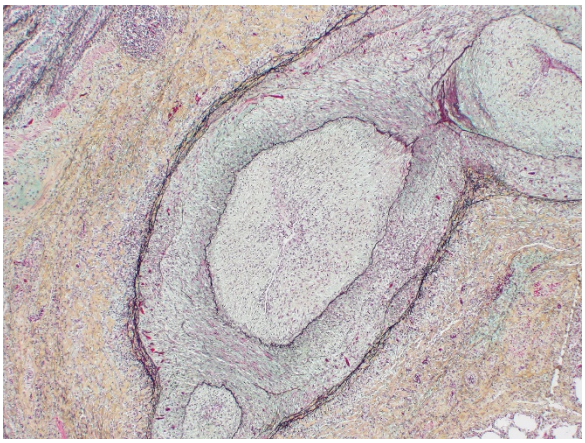
JPC Diagnosis: Lung, pulmonary arterioles: Smooth muscle hypertrophy and hyperplasia, diffuse, severe, with mural fibroplasia, venous fibroplasia, and multifocal alveolar edema.

JPC Comment: The World Health Organization has classified six different types of pulmonary hypertension: pulmonary arterial hypertension, pulmonary veno-occlusive disease, hypertension resulting from left heart disease, hypertension with pulmonary disease or hypoxemia (which includes chronic forms of altitude disease), chronic thromboembolic pulmonary disease, and pulmonary hypertension with unclear multifactorial mechanisms.[11]

In humans, altitude disease ("mountain sickness") takes both an acute and chronic form. Acute exposure to hypoxic conditions at high altitude (2500 ft above sea level) manifests within 6-12 hours, characterized by headache, loss of appetite, vomiting, dizziness, and fatigue. Two theories as to its pathogenesis predominate: a) acute cerebral hyperperfusion resulting from impeded autoregulation of cerebral blood vessels and increased levels of VEG-F and circulating radicals, and b) inhibition of astrocytic Na-K ATPase and resulting edema and irritation of sensory trigeminal nerve fibers. If unattended, especially in additional altitude is attained, life-threatening cerebral or pulmonary edema may result.[4]

Chronic hypoxia resulting from high altitude in humans results in significant remodeling of small- and medium caliber pulmonary arterioles. The remodeling is the result of increased proliferation, decreased apoptosis, and migration of a population of less well-differentiated pulmonary artery smooth muscle cells. In addition, a mural population of fibroblasts may differentiate into a smooth muscle phenotype, while maintaining the ability to secrete matrix proteins. The end result of both proliferations results in effacement of the tunica intima and expansion of all layers of the arterial wall by smooth muscle hyperplasia, disarray, and fibrosis. In

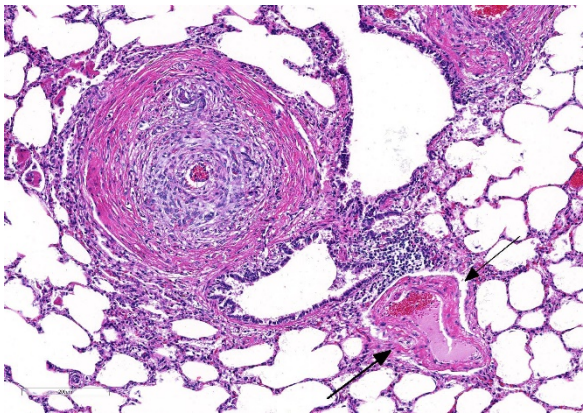


Lung, ox. The internal elastic lamina is largely intact, although the tunica intima is markedly expanded by migrating smooth muscle cells and fibroplasia which markedly compromises the limen. . (Masson's trichrome, 40X).

affected lungs, previously non-muscularized precapillary vessels may develop a muscular wall. However, all of these changes are reversible after prolonged re-exposure to normal oxygen levels.[4]

Plexiform lesions are not seen in remodeling of pulmonary arteries in chronic hypoxia in humans, and are associated with the most severe group of diseases resulting in pulmonary hypertension - those in WHO group 1, pulmonary arterial hypertension. This group includes the idiopathic and congenital forms of pulmonary arterial hypertension, which results in severe, non-reversible remodeling changes of pulmonary arterial remodeling, to include fibrinoid arteritis and plexiform lesion development.[11] An excellent example of these changes may be seen in WSC 25, Case 4 of 2018-2019, in a West Highland White Terrier, a breed in which several cases of idiopathic pulmonary hypertension similar to that seen in humans have been documented.

Several animal models for pulmonary hypertension exist. Chronic exposure of rats and mice to diminished levels of oxygen in normo- and hypobaric settings have been utilized for years and produce predictable



Lung, ox. Multifocally, there is fibrosis of walls of the larger pulmonary veins (arrows). (HE, 100X)

and reproducible results, depending on the strain of rodent. Fawn-hood rats appear to produce the most dramatic tissue changes. Oral administration of monocrotaline, a toxic pyrrolizidine alkaloid derived from *Crotalus spectabilis* also produces arterial changes consistent with pulmonary hypertension in rats dosed orally. While the precise mechanism is unknown, the prevailing hypothesis suggests direct endothelial damage to pulmonary arterioles. In addition, several genetically engineered mouse strains, including a BMPR2 knock out model (a genetic mutation seen in humans with severe forms of pulmonary arterial hypertension) have been developed.[11]

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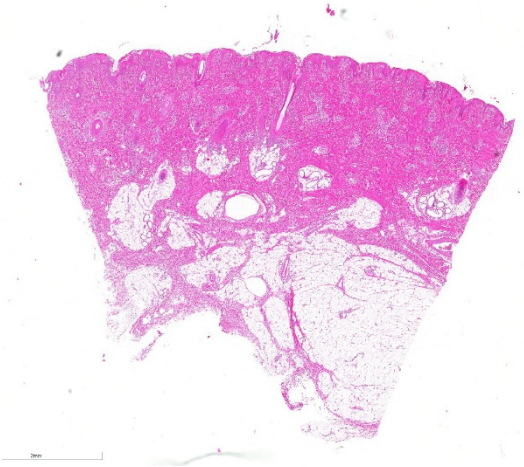


Lung, ox. Multifocally, there is fibrosis of walls of the larger pulmonary veins (arrows). (HE, 100X)

CASE IV: LE-2 (JPC 4049442).

Signalment: Porcine, breed unspecified (*Sus scrofa domestica*), 14-weeks-old, neutered male, body weight 56.6 kg

History: This pig was submitted from a feeder-to-finish pig farm. Due to alterations of the skin a contagious disease was suspected. Therefore, the pig was euthanized and submitted for a complete post mortem examination. A more detailed clinical history was not available.

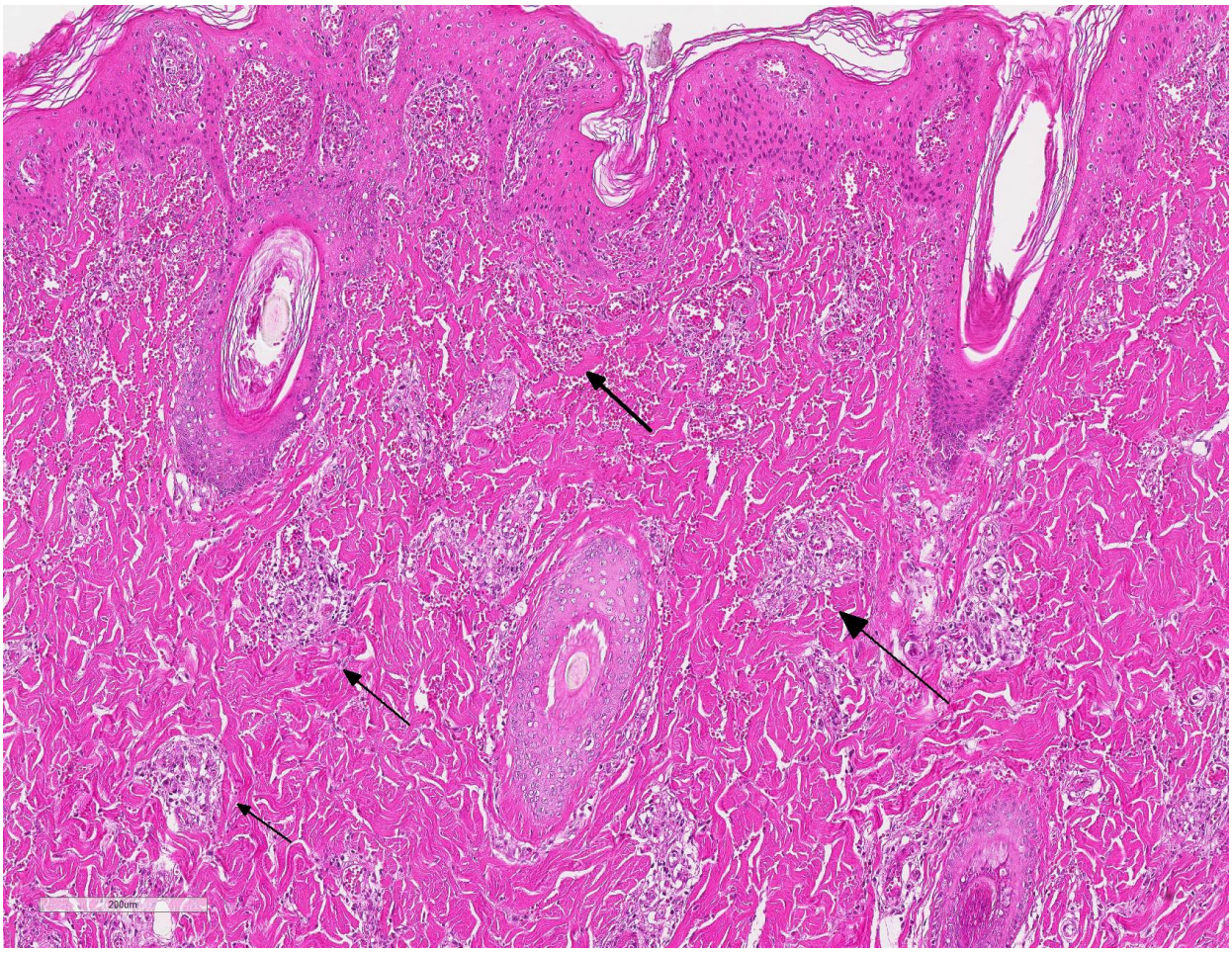
Gross Pathology: The carcass showed multiple cutaneous red macules and patches measuring up to 2 cm in diameter that were predominately located on the head, over the scapulae, as well as at the distal extremities and the anus.

Peripheral and visceral lymph nodes were enlarged measuring up to three times of their normal size. Their cut surfaces contained multiple slightly elevated white foci measuring 0.2 cm in diameter (indicative of a marked follicular hyperplasia) and displayed a red marginal coloration (consistent with a moderate blood resorption). Furthermore, both kidneys had multiple red foci measuring 0.1 cm in diameter that were restricted to the cortex; renal alterations were interpreted as a diffuse moderate acute glomerulonephritis. In addition, lungs showed a mild to moderate suppurative bronchopneumonia in both cranial lobes.

Laboratory results: PCR was positive for the detection of porcine Circovirus type 2 in tissue samples of brain, lungs and kidneys.

PCR was negative for the detection of classical swine fever virus in tissue samples of brain, lungs, lymph nodes, tonsils and spleen.

Microscopic Description: Haired skin: Multifocally, vascular walls of dermal capillaries, arterioles and venules showed deposition of homogenous eosinophilic material associated with loss of normal architecture interpreted as fibrinoid degeneration. Endothelial cells were swollen and bulged into vascular lumens (activated endothelial cells) or showed different stages of degeneration or necrosis (karyopyknosis and karyorrhexis). Some affected vessels were surrounded by a few extravasated erythrocytes and/or were cuffed by moderate numbers of inflammatory cells that extended within the adjacent dermis.Some affected vessels contained intraluminal fibrin thrombi and/or moderate numbers of neutrophils. The inflammatory infiltrates consisted of



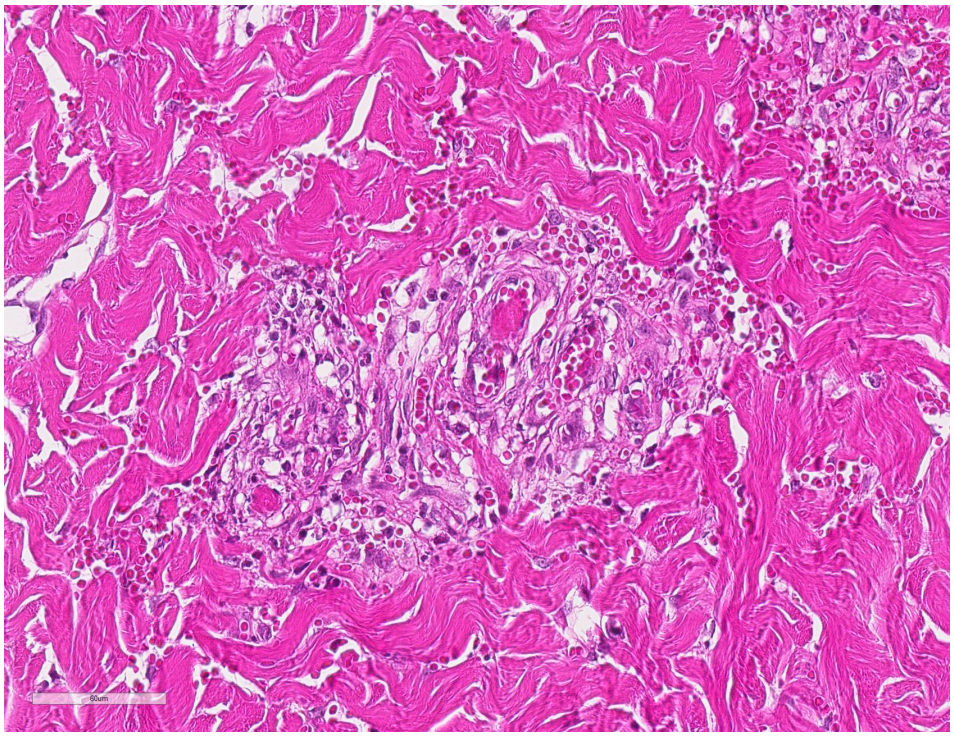
Haired skin, pig: All vessels within the dermis are outlined by edema and a cellular infiltrate (arrows). (HE, 5X)

predominately mononuclear cells (macrophages, lymphocytes, plasma cells) and a few neutrophils. Furthermore, a diffuse mild to moderate perivascular edema existed. The epidermis showed mild irregular epidermal hyperplasia and mild orthokeratotic basket-woven hyperkeratosis. In some slides, the described vascular lesions were also observed within the subcutis.

Contributor's Morphologic Diagnosis:
Haired skin, dermis and subcutis: Perivasculitis and vasculitis, necrotizing and mixed-cellular, acute to subacute, multifocal, moderate, with perivascular hemorrhage and edema

Contributor's Comment: Porcine circovirus type 2 (PCV2) belongs to the family Circoviridae, that encompass the two genera Circovirus and Gyrovirus. Porcine circovirus type 1 (PCV1) and type 2, beak and feather disease virus, canary, goose, pigeon, duck, finch and gull circovirus belong to the genus Circovirus. The genus Gyrovirus contains only the chicken anemia virus (CAV).[13]

The replication of the virions takes place within the nucleus of the host cell during the S phase of the mitosis by using the host cell DNA polymerase. PCV2 is reported as the smallest known pathogenic virus of our domestic animals with a size of approximately 16 to 20 nm in diameter.[13]



Haired skin, pig: Vessels have smudgy indistinct walls with mural inflammation, debris, luminal thrombi, and perivascular hemorrhage (vasculitis). (HE, 400X)

The porcine circovirus was first mentioned in 1974 from Tischer and colleagues.[11] It had been isolated from a pig kidney cell line PK 15 as a contaminant. At this time it was thought to be a picornavirus.[11] In the 1980s the same investigator group classified the virus as a porcine circovirus.[12] It has an isometric shape. The DNA is a covalently closed circular molecule. Furthermore, DNA is single stranded and has a genome size of 1.76 kb.[12]

In 1997, Clark described a new pig disease that occurred in Canada and called it the Post-Weaning Multisystemic Wasting Syndrome (PMWS).[3] Typical pathomorphologic findings are interstitial pneumonia characterized by mononuclear cellular infiltrates including predominately large macrophages and numerous multinucleated syncytial cells as well as enlarged lymph nodes characterized by a lymphoid depletion, an infiltration with large histiocytic cells and multinucleated syncytial cells.[3] In some cases, intralesional histiocytic cells display intracytoplasmic basophilic inclusions (botryoid inclusion bodies).[3,6] At this time the porcine circovirus has been suggested as the infectious agent.[3]

A comparison of the viruses of the pig kidney cell line PK 15 with those isolated from the animals with PMWS showed that they were antigenically distinct. Allan and colleagues designated the original PCV isolate from the pig kidney cell line PK 15 as PCV1 and the disease associated circovirus as PCV2.[1]

Retrospective studies showed the presence of PCV2 back to the 1970s in the United Kingdom pig population and at least since 1962 in Northern Germany.[4,6] Nowadays, a global distribution within the pig population is known.[7] PCV2 can be found in healthy as well as in diseased pigs.[2] It is speculated that a breakout of a PCV2-associated disease is multifactorial in causality, like host susceptibility, immune modulation, coinfection.[7] Viruses spread via fecal-oral route, but vertical transmission has also been demonstrated.[13]

Opriessnig and Langohr summarized the diversity of PCV2-associated diseases in pigs.[9] Those can be divided into subclinical PCV2 infection, PCV2-associated systemic infection, PCV2-associated respiratory disease, PCV2-associated enteritis, PCV2-associated reproductive failure and PCV2-

associated porcine dermatitis and nephropathy syndrome (PDNS).[8]

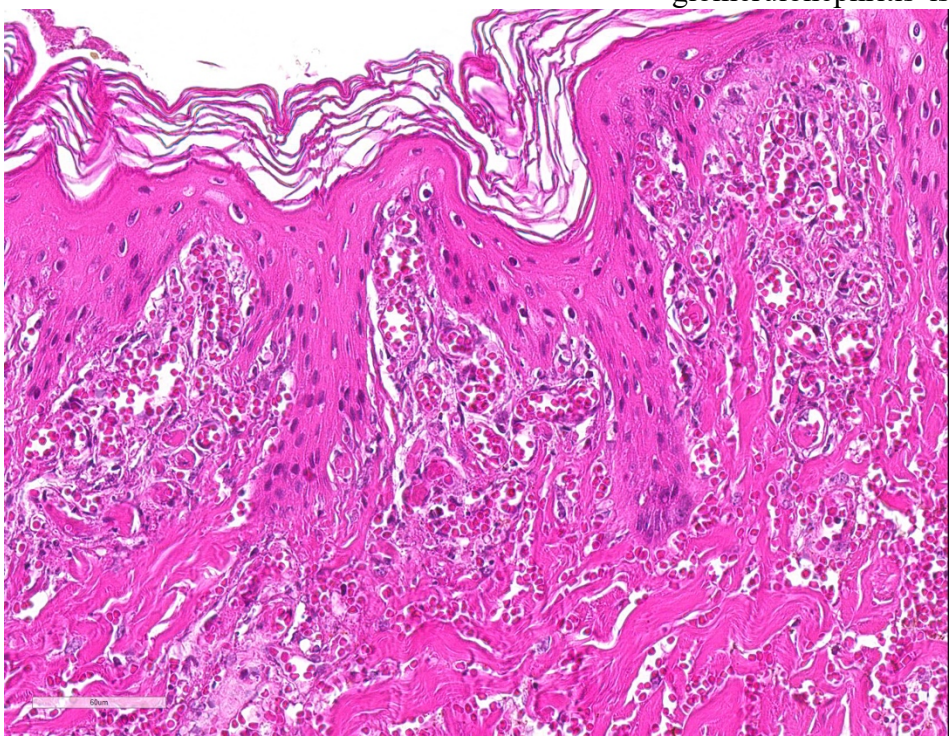
In the present case, microscopic examination of enlarged lymph nodes revealed lymphocytic depletion as well as infiltration by numerous multinucleated giant cells (syncytial cells). Furthermore, lungs showed a marked diffuse interstitial non-suppurative pneumonia and a moderate multifocal acute suppurative bronchopneumonia. The kidneys displayed histologically a multifocal moderate non-suppurative interstitial nephritis, a suppurative glomerulonephritis and a multifocal marked necrotizing vasculitis and perivasculitis.

PCV2-associated PDNS is characterized by typical gross lesions of the skin and the kidneys together with the hallmark microscopic lesions (systemic vasculitis and glomerulonephritis) and detection of PCV2 mRNA or antigen. Higgins mentioned PDNS also in association with drugs, other infectious agents and neoplasia.[5]

Therefore in the present case, PCV2-associated PDNS is most likely due to the gross lesions, histopathological findings and the detection of PCV2 mRNA within multiple tissues of this pig.

The proposed pathogenesis of PDNS is a type III hypersensitivity reaction with deposition of immune complexes in vessel walls and glomerular tuffs. In regards to the PCV2-associated PDNS, Rosell and collegues couldn't always detect porcine circovirus nucleic acid in vascular and glomerular lesions.[10] They supposed a transient deposition and a promptly removal of immune complexes or that the immune complexes lack of viral genome and only contain viral protein.

One of the major differential diagnoses of a disseminated acute vasculitis and glomerulonephritis is classical swine fever, in addition other viremic or septicemic diseases have to be ruled out. Further virological examination is required in such cases, i.e. PCR and/or virus culture, to definitively rule out the presence of a highly contagious and notifiable disease. The present case was negative for classical swine fever virus.



Haired skin, pig: There is marked congestion and hemorrhage within dermal pegs and necrosis of the overlying epidermis. (HE, 400)

In case several animals of this farm may present with similar lesions, management

practices, disinfection, control of coinfections and PCV2 vaccines should be recommended.[8]

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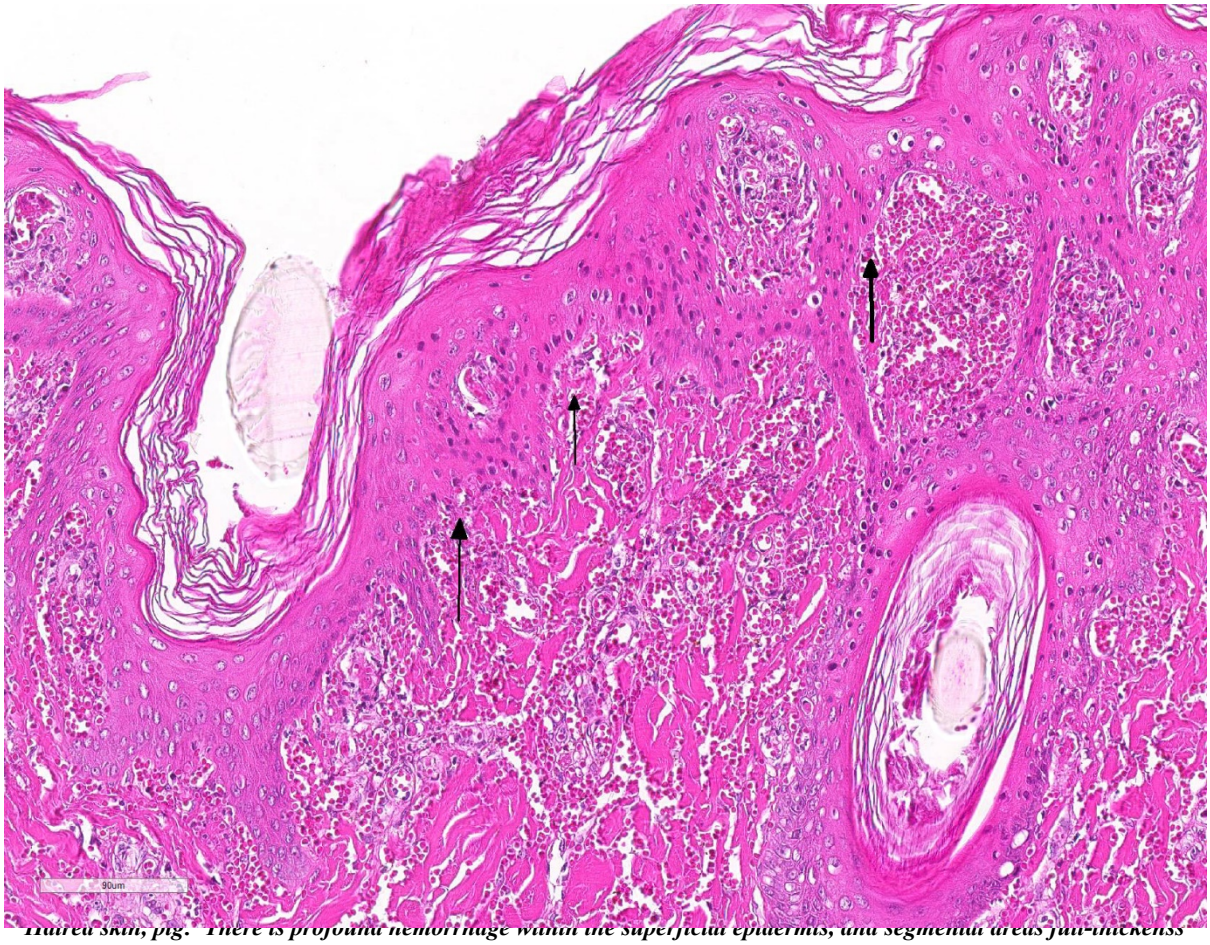
JPC Diagnosis: Haired skin: Vasculitis, necrotizing, diffuse, severe, with multifocal dermal and epidermal necrosis.

JPC Comment: The contributor has provided an excellent review of the history of porcine circovirus-2 investigation. PCV-2 has made a number of appearances in the WSC demonstrating the wide spectrum of disease associated with this virus. Previous WSC submissions falling into this category include two previous cases of PCV-associated myocarditis (WSC 2019, Conference 17 Case 2 and WSC 2016, Conf 8, Case 2), cerebellar vasculitis and necrosis (WSC 2014, Conf 21, Case 4), granulomatous lymphadenitis and hepatitis (WSC 2013 Conf 25 Case1) and



***Haired skin, pig:** There is profound hemorrhage within the superficial epidermis, and segmental areas full-thickness epidermal necrosis (black arrows) characterized by hypereosinophilic and nuclear pyknosis. Note the viable, more vesicular nuclei in the apidermis at left, and then the lower left portion of the hair follicle. (HE, 231X)*

tubulointerstitial nephritis (WSC 2011, Conf 7, Case 3.)

Vasculitis is a common lesion in association with PCV-2 infection. Porcine dermatitis and nephritis syndrome is characterized by a necrotizing and neutrophilic vasculitis of arterioles and capillaries of the skin and kidney (to include glomerular capillaries). Fibrinoid necrosis of septal capillaries within the lung and resultant focal alveolar hemorrhage and edema has been widely reported in the US and Europe.[7] Necrotizing vasculitis within the cerebellum has been documented in porcine multisystemic wasting disease. Finally, cases of myocarditis in piglets are often associated with lymphohistiocytic coronary arteritis and periarteritis.[7,8]

The presumed pathogenesis of vasculitis in various forms of PCVAD is a Type III hypersensitivity reaction. In Type III hypersentivity, systemic disease results from the formation of antibodies to a protein (in this case, viral protein) with deposition of antigen-antibody complexes within the walls of vessels. Vessels in which blood is filtered to form other bodily fluids, such as urine and synovial fluid, are most affected (resulting in glomerulonephritis and polyarthritis respectively). Anitbodies (IgG and IgM) which have the ability to bind complement or bind to Fc receptors on leukocytes are the most likely to result in vascular lesions of fibrinoid necrosis. Analysis of damaged vessels may demonstrate the presence of immune complexes or complement, although levels in vessels outside the kidney may be low. PCV-2 antigen has been demonstrated within the walls of damaged blood vessels.

The effects of PCV-2 on vessels remains to be elucidated, but previous studies have shone light on some of its effects. PCV-2 antigen has been demonstrated in endothelial and inflammatory cells within the walls of vessels in both experimentally- and naturally-infected pigs. PCV-2 has the ability to infect endothelial cells, causing them to increase the expression of surface procoagulant activity. PCV-2 antigen has been identified in endothelial and inflammatory cells within necrotic vessels in infected pigs with granulomatous lymphadinitis as well as a peracute syndrome of acute pulmonary edema syndrome.[8]

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