Joint Pathology Center
Veterinary Pathology Services
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CASE I: 3121206023 (JPC 4035610).

Signalment: 5-week-old mixed breed piglet, (*Sus domesticus*).

History: Two piglets from the faculty farm were found dead, and another piglet was weak and ataxic and, therefore, euthanized.

Gross Pathology: The submitted piglet was in good body condition. It was icteric and had a diffusely pale liver. Additionally, petechial hemorrhages were found on the kidneys, and some fibrin was present covering the abdominal organs.

Laboratory Results: The intestine was PCR positive for porcine circovirus (>9170000).

Histopathologic Description: Mesenteric lymph node: Diffusely, there is severe lymphoid depletion with scattered karyorrhectic debris (necrosis). Also scattered throughout the section are large numbers of macrophages and eosinophils. The macrophages often contain botryoid basophilic glassy intracytoplasmic inclusion bodies. In fewer macrophages, intranuclear basophilic inclusions can be found.

Liver: There is massive loss of hepatocytes, leaving disrupted liver lobules and dilated sinusoids engorged with erythrocytes. The remaining hepatocytes show severe swelling, with micro- and macrovesiculation of the cytoplasm and karyomegaly. Some swollen hepatocytes have basophilic intranuclear, irregular inclusions (degeneration). Throughout all parts of the liver there are scattered moderate to large numbers of macrophages (without inclusions). Within portal areas there is multifocally mild to moderate fibrosis and bile duct hyperplasia. Some bile duct epithelial cells show degeneration and necrosis, and there is infiltration of neutrophils within the lumen. The limiting plate is often obscured mainly by infiltrating macrophages and eosinophils, and fewer neutrophils, extending into the adjacent parenchyma. Scattered are small areas with extra medullary hematopoiesis.

Contributor's Morphologic Diagnosis: 1. Mesenteric lymph node: Severe lymphoid depletion with moderate diffuse chronic granulomatous lymphadenitis, with intralesional botryoid basophilic intracytoplasmic and intranuclear inclusion bodies.
2. Liver: Severe hepatic degeneration and hepatocellular loss with severe diffuse chronic granulomatous hepatitis and mild neutrophilic cholangitis.

Contributor's Comment: Porcine circovirus type 2 (PCV-2) was first isolated from pigs with postweaning multisystemic wasting disease (PWMD) in 1997. Subsequent retrospective investigations traced PCV-2 DNA antigen back to 1962, and it is likely that this virus has been present in the swine population for much longer. Besides PWMD, PCV-2 has been associated with enteric disease, respiratory disease, porcine dermatitis and nephropathy syndrome and reproductive failure. Vaccination since 2006 has proved effective in preventing PCV2–associated disease, and the prevalence of disease has been reduced. Many detection methods such as immunohistochemistry are no longer used or have been replaced by molecular methods, making the recognition of lesions even more important.

As suggested by the different syndromes associated with PCV-2 infection, clinical signs can be variable. The typical clinical picture includes enlarged lymph nodes, decreased weight gain or wasting, combined with dyspnea, diarrhea, pallor or jaundice. Other signs that have been described include coughing, fever, central nervous system signs, and sudden death.[7]

In the case presented here, the liver lesions were the most striking feature. Although not as widely known as the respiratory, intestinal and cardiovascular lesions,[2] several reports have described lesions similar to those seen in this case. In a field study investigating 100 livers from pigs with clinical PCV2-associated disease, in 70% of the livers, the virus was associated with hepatocytes, Kupffer cells, and inflammatory infiltrates.[9] Hepatic lesions were reproduced experimentally in cesarean-derived colostrum-deprived and gnotobiotic pigs that were infected with PCV-2. Both moderate-to-severe necrotizing and granulomatous hepatitis, acute hepatitis with centrilobular necrosis of hepatocytes, and hepatic atrophy associated with nonsuppurative cholangiohepatitis were observed. In fetuses, the hepatic lesions were described as congestion with hepatocellular loss and nonsuppurative hepatitis with periacinar necrosis.[1,7]

The current case is a representation of the previously described end-stage hepatic disease[1] with extensive swelling and vacuolation of remaining hepatocytes with karyomegaly and progressive replacement of hepatocytes by histiocytic cells. Although immunohistochemistry was not available for this case, PCV-2 antigen is detectable early on in the disease process within the nuclei of hepatocytes and, as the disease progresses, within the cytoplasm of Kupffer cells and infiltrating mononuclear phagocytes.

The lymph node submitted together with the liver sample is a classical histologic presentation of the lymphadenopathy seen in the disease caused by PCV-2: severe depletion of lymphocytes and histiocytic infiltration of lymphoid tissues. It is thought that PCV-2 virus replicates within the histiocytes of lymph nodes. A study regarding the subcellular localization of PCV-2 virus found that in affected lymph nodes, viral particles were exclusively found in histiocytes. The ultrastructural changes found associated with the presence of viral particles were dilatation of the rough endoplasmic reticulum and swelling of mitochondria. With colocalization studies, a close relationship was found between the viral particles and the mitochondria, suggesting that these organelles play a role in replication of the virus.[8]

JPC Diagnosis: 1. Liver: Hepatitis, granulomatous and eosinophilic, diffuse, severe, with portal fibrosis, biliary ductal reaction, hepatocyte karyo/cytomegaly, vacuolar degeneration, chronic-active cholangitis, and rare intracytoplasmic viral inclusion bodies.
2. Lymph node: Lymphadenitis, granulomatous and eosinophilic, diffuse, chronic, severe, with lymphoid depletion and intrahistiocytic intracytoplasmic botryoid viral inclusion bodies.

Conference Comment: Circovirus is a small, non-enveloped DNA virus with a single-stranded circular genome. In addition to porcine circovirus (PCV), members of this genus include psittacine beak and feather disease virus, columbid circovirus, goose circovirus, canary circovirus, and duck circovirus.[6] Two genotypes of PCV have been identified in swine. PCV type 1 (PCV1) does not typically induce disease in pigs, while PCV type 2 (PCV2) is virulent for pigs. Infection produces a wide array of clinical manifestations, including lymphoid depletion and, less commonly, hepatic lesions, as demonstrated in this case. Examples of disease syndromes attributed (at least in part) to PCV2 include post-weaning multisystemic wasting syndrome (PMWS, now known as PCV2-associated diseases or PCVAD), porcine respiratory disease complex (PRDC), porcine dermatopathy and nephropathy syndrome (PDNS), exudative epidermitis, and reproductive failure.[1,7,10]

PCVAD (formerly PMWS) involves multiple organ systems with a highly variable spectrum of gross lesions, the most common of which are emaciation, lymphadenopathy and mild interstitial pneumonia. Activation of the immune system followed by circovirus infection is required for the development of PMWS. Severely affected pigs may develop immunosuppression, which increases susceptibility to opportunistic infections and may result in a poor immune response to vaccines. Reported clinical signs include wasting, dyspnea, coughing, diarrhea, pallor, fever, central nervous signs, and sudden death. Although its name implies that clinical disease develops exclusively in recently-weaned pigs, PMWS can also affect mature pigs.[4,7,10]

PRDC is a multifactorial condition involving several coexisting etiologic agents, especially swine influenza, porcine respiratory and reproductive syndrome (PRRS) virus, PCV2, and porcine respiratory coronavirus. In addition to causing direct damage to the airway and lungs, these viruses predispose swine to secondary infection with pathogens such as *Mycoplasma hyopneumoniae*, *Pneumocystis carinii, Pasteurella multocida, Streptococcus suis, Bordetella bronchiseptica* and *Haemophilus parasuis*.[4] Typically, affected pigs are around 12 to 24 weeks of age and present with fever and varying degrees of sneezing, coughing, nasal discharge, and respiratory distress as well as reduced weight gain.[2,4,7]

PDNS is characterized by a systemic necrotizing vasculitis with tropism for kidney and skin, likely secondary to immune complex deposition. Although the development of PDNS has been attributed to PCV2, this condition has also been reproduced with pathogens other than PCV2, including PRRSV, Torque teno virus (arterivirus), *Staphylococcus hyicus*, *Pasteurella multocida* and *Streptococcus suis*. Grossly, affected swine exhibit irregular red to purple, often crusted papules over hindquarters, perineal area, and ears, in combination with bilaterally enlarged, pale kidneys with petechial hemorrhages.[3,5,7] PCV2 is not a primary cause of skin lesions but, in addition to the vasculitis and necrotizing skin lesions described in connection with PDNS, the virus has been demonstrated in combination with *Staphylococcus hyicus* in several cases of severe exudative epidermitis.[7]

Reproductive failure due to PCV2 is characterized by stillbirths, mummification, embryonic death, and infertility (SMEDI). Reproductive failure is probably the least important manifestation of PCV2, as it is usually only seen in individual dams and is thus not of great economic importance.[7]

Regardless of the tissue or organ affected, PCV2 tends to produce similar histological lesions. Specifically, intracytoplasmic botryoid viral inclusions, granulomatous inflammation and necrotizing vasculitis are common microscopic findings associated with PCV2. Interestingly, there are typically no PCV2-induced microscopic lesions in the musculoskeletal, endocrine and reproductive (although as noted above, sporadic abortions are reported) systems.[2,4,7]

Although the specific mechanisms of PCV2 infection are not completely understood, recent studies have clarified several aspects of its pathogenesis. Viral antigen and/or nucleic acid may be found in multiple cell types; however, histiocytes are the main site of virus localization. Viral attachment to host cell surface receptors is mediated by heparan sulfate and chondroitin sulfate B on the viral surface.[6] Subsequently, histiocytic internalization of PCV2 occurs via clathrin-mediated endocytosis. Recent studies suggest that PCV2 replication also occurs within lymph node histiocytes and that the mitochondria likely play an important role.[8] Once internalized, PCV2 can transiently induce the PI3K/Akt pathway which inhibits apoptosis, thus promoting cell survival and viral replication. PI3K activates its downstream effector, the serine/threonine kinase Akt (also known as PKB), which phosphorylates various substrates, such as caspase-9, BAD, glycogen synthase kinase 3, and FKHR. Overall, this series of reactions leads to induction of NF-κβ and, ultimately, cell survival and growth, as well as the prevention of apoptosis via activation of antiapoptotic factors and inactivation of proapoptotic factors. Akt also results in activation of mTORC1 (which controls cell growth) and mTORC2 (which regulates the actin cytoskeleton), as well as JNK and p38 (which are involved in PCV2-induced apoptosis).[10]

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CASE II: 12 0132-42 (JPC 4019843).

Signalment: 1-year-old neutered male domestic shorthair cat, (*Felis silvestris catus*).

History: A 1-year-old neutered male domestic shorthair cat was presented to our institution for a 7-day history of weakness, anorexia and intermittent dyspnea that evolved over the last hours to persistent lateral recumbency and tremor. Clinical examination revealed a right anterior uveitis with fibrin deposition cranioventrally to the lens. Neurologic examination revealed a non-ambulatory tetraparesis with proprioceptive defect; a C1-C5 myelopathy or brainstem injury was suspected. The cat died two days after its admission and the owner requested a necropsy examination.

Gross Pathology: Apart from the aforementioned right fibrinous uveitis, gross findings included: discrete icterus; a 2-mL serohemorrhagic pleural effusion (consistent with modified transudate); numerous variable-sized and coalescing yellow foci and nodules on the renal capsule and renal cortices (consistent with pyogranulomas), some of which appeared to follow venous tracts (consistent with pyogranulomatous vasculitis (phlebitis)); diffuse, moderate, fibrinous perihepatitis; severe yellow thickening of the meninges, particularly in the ventral brainstem region (consistent with severe pyogranulomatous meningitis). Discrete yellow thickening of the spinal cord meninges was also detected. These findings led to a presumptive diagnosis of feline infectious peritonitis with nervous, ocular, renal and hepatic involvement.

Laboratory Results: Cerebrospinal fluid (CSF) aspiration and examination were performed:

	Present case	Reference values
Red blood cell count (/mm^3)	160	0
Nucleated cell count (/mm^3)	720	2-8
Protein concentration (g/L)	10.6	0-0.3

On microscopic examination of the CSF, there was a marked neutrophilic pleocytosis (90%). Some neutrophils were degenerated. Few large and small mononuclear cells were also present (10%). No infectious agents or neoplastic cells were observed.

RT-PCR (reverse transcriptase polymerase chain reaction) analyses for Feline infectious peritonitis Virus (FIPV) were performed on the cerebrospinal fluid and aqueous humor and were strongly positive. RT-PCR analyses on blood and feces were negative.

Histopathologic Description: Spinal cord with meninges: Meninges (particularly leptomeninges) are severely thickened due to massive infiltration by degenerated neutrophils admixed with macrophages and few lymphocytes and plasma cells (pyogranulomatous meningitis). Infiltration is centered on vessels, particularly veins, and is associated with prominent fibrinoid necrosis and fibrin exudation (pyogranulomatous vasculitis/phlebitis).

Inflammation extends along and inside nerves (neuritis). In nerves, secondary changes include myelin sheath destruction and axonal dilation.

In the spinal cord, there are multiple foci of myelin sheath distension and severe axonal dilation (spheroids), particularly in the ventral and/or lateral funiculi of some sections. Occasionally, inflammation from meninges extends into Virchow-Robin spaces.

Contributor's Morphologic Diagnosis: 1. Meninges: Vasculitis (mainly phlebitis), pyogranulomatous, chronic, diffuse, severe with fibrinoid necrosis and secondary meningitis (due to extension).
2. Spinal cord: Degenerative myelopathy, multifocal, chronic, moderate, with spheroids and dilation of myelin sheaths.
3. Peripheral nerves: Neuritis, pyogranulomatous, chronic, multifocal, severe.

Name of the disease: Feline infectious peritonitis (FIP)
Etiology: Feline infectious peritonitis virus

Contributor's Comment: This case is an example of Feline infectious peritonitis (FIP) with nervous and ocular involvement.

FIP is a fatal immune-mediated disease of cats caused by a member of feline coronaviruses (FCoV), the feline infectious peritonitis Virus (FIPV). Two major forms of FIP have been described: the effusive (wet) form and the non-effusive (dry) form. The distinction between these forms is sometimes arbitrary and they should be regarded as the two extremes of a continuum. Although both forms have been associated with neurological signs, the non-effusive form appears to more commonly produce nervous system lesions.[3,4]

The most widely accepted pathogenesis of FIP considers infection and replication of the virus within macrophages as a key feature. Macrophages can subsequently disseminate the virus through the body (e.g., to liver, visceral peritoneum or pleura, uvea, meninges and ependyma of the brain and spinal cord). In tissues, further replication of the virus results in attraction of neutrophils and macrophages leading to pyogranulomatous inflammation.[10] Both type III and type IV hypersensitivity reactions are believed to occur. The form of FIP is likely determined by the type of immune reaction of the host. Cats that respond with predominantly humoral immunity develop the wet form, whereas those with stronger cell-mediated immunity develop the dry form.[3,13]

The origin of FIPV is still subject to many debates. The most widely accepted hypothesis is that FIPV could be a mutant strain of the feline enteric coronavirus (FECV), another member of the FCoV that causes enteric infection. This mutant strain would be able to infect monocytes and macrophages, causing FIP. FECV is antigenically and morphologically indistinguishable from FIPV. Whether or not FIP develops would also depend on host factors (immune response, cytokine response) in conjunction with viral factors.[5,13]

Feline neurological disease accounts for approximately 10% of total referrals in some feline medicine clinics.[2,8] A specific clinical diagnosis is made in only 30-40% of cases and 30-45% of cases are believed to be infectious in origin. The most common infectious causes of encephalitis in cats are FIP and toxoplasmosis.[8]

Because FIP is a fatal transmissible disease without effective treatment, it is essential for clinicians to make a rapid diagnosis. Histopathology remains the only conclusive means of diagnosis of FIP, particularly in cases of atypical presentation. Surprisingly, FIP has been diagnosed histopathologically in the brains of cats without neurological clinical signs.[4] Occasionally, histopathology can be inconclusive and immunohistochemistry can be useful to confirm or exclude the disease.[7]

In practice, diagnosis is often based on the combination of signalment, history and clinical signs.[8] Although the nervous form of FIP can affect cats at any age, it is considered to be the most common cause of neurological disease in cats less than 4 years of age. Purebred cats are at increased risk and male cats are more frequently affected than females.[4]

Abnormal laboratory findings frequently observed in FIP are hyperproteinemia with a low albumin/globulin ratio (<0.7), anemia, elevated hepatic enzyme levels and hyperbilirubinemia.[17]

CSF analysis is useful in the diagnosis of cats with FIP. Marked elevation of protein concentration (greater than 2 g/L), severe pleocytosis (> 100 cells/µL) with neutrophils (more than 70%) and systemic signs increase the index of suspicion for FIP.[14,16]

Infection by FIPV can be demonstrated by serology and by RT-PCR. Currently available serological tests have low specificity and sensitivity for detection of active infection and cross-react with FECV.[15] RT-PCR is rapid and sensitive but results must be interpreted in the context of clinical findings. RT-PCR on CSF detected only 31% of cats with neurologic FIP. This low sensitivity could be explained by the low CSF cellularity on most cats and the paucity of virus detected immunohistochemically.[6]

JPC Diagnosis: Spinal cord: Phlebitis, meningomyelitis and polyradiculoneuritis, pyogranulomatous, multifocal, moderate, with axonal degeneration, vascular fibrinoid change and thrombosis.

Conference Comment: The viral order *Nidovirales* is composed of the families *Coronaviridae*, *Arteriviridae* and *Roniviridae*. *Coronaviridae* contains two genera: *Coronavirus* and *Torovirus*. Coronaviruses are enveloped, ssRNA viruses that are important in a wide variety of animal

diseases (see table 1).[12,18] Feline coronavirus (FCoV) is a group 1 coronavirus with two serologically and morphologically indistinguishable subtypes: feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV). FECV (i.e., non-mutated FCoV) replicates within enterocytes of affected cats, which may shed virus but are typically asymptomatic or exhibit mild diarrhea. The development of virulent FCoV (i.e., FIPV) appears to be due to a spontaneous viral genetic mutation that occurs during replication in the infected host, although the specific nature and location of this mutation has yet to be determined. FECV carriers are thought to play an important role in the epidemiology of FIP.[11] Transmission is typically oronasal via feces, saliva/mutual grooming, and fomites (e.g. food bowls or grooming tools); transplacental transmission is reported although it is fairly uncommon.[1,3,11]

At present, three key features have been identified as prerequisites for the development of FIP lesions: 1) systemic infection with mutated, virulent FCoV (FIPV), 2) effective and sustainable FIPV replication in monocytes, and 3) activation of FIPV-infected monocytes, although the trigger remains unknown. Activated monocytes/macrophages upregulate their expression of adhesion molecules (e.g. CD18), and produce cytokines (such as TNF-α, IL-1b, IL6, G-CSF and GM-CSF), matrix metalloproteinases (e.g., MMP-9), and vascular endothelial growth factor (VEGF). IL-6 stimulates hepatocytes to produce acute phase proteins (such as alpha-1 acid glycoprotein) and drives differentiation of B-lymphocytes into plasma cells. TNF-α, G-CSF and GM-CSF are neutrophil survival factors. IL-1 is a pyrogenic cytokine that activates both B- and T-lymphocytes. MMP-9 is an endopeptidase that breaks down extracellular matrix proteins. VEGF facilitates interaction with activated endothelial cells; it has been proposed that the limited distribution of vascular lesions (only veins in select organs are affected) is a consequence of selective responsiveness of the endothelium.[1,11]

As noted by the contributor, mutated FCoV-infected circulating monocytes are thought to be responsible for viral dissemination, while the nature of the adaptive immune response determines the clinical manifestations of FIP. Cats with a strong cell-mediated immune (CMI) response do not develop FIP, while a weak to nonexistent CMI and strong humoral response results in effusive, or "wet," FIP, characterized by vasculitis, peritonitis and intracavitary effusions. In contrast, noneffusive, or "dry" FIP with widespread pyrogranulomatous inflammation predominates in cats with a moderate CMI response. As the contributor noted, the "wet" and "dry" forms of FIP represent a continuum, rather than two specific disease processes; mixed effusive and noneffusive forms are not uncommon.[1,11] Additionally, antibody-dependent enhancement (ADE) can affect the severity of clinical disease. ADE is a phenomenon in which cats with preexisting antibodies (e.g. those that were vaccinated with some trial vaccines or received blood from FCoV positive donors), develop disease more quickly, and perhaps more severely than seronegative cats; however, ADE does not appear to affect seropositive cats infected naturally, who are relatively resistant to reinfection.[1]

FIP has historically been regarded as an immune complex-mediated type III hypersensitivity disease (see WSC 2013-2014, conference 22, case 4), based upon 1) the presence of cell-free fibrinogen, C3, viral antigen, IgG, and complement within leukocytes in vascular and focal granulomatous to necrotizing lesions, 2) FCoV-specific immune complexes in blood and glomeruli and 3) elevated serum γ-globulin and C3 levels. Nonetheless, circulating FCoV-specific immune complexes can also be detected in clinically healthy seropositive cats, and most

cases of FIP do not exhibit typical features of immune complex vasculitis, such as the involvement of arteries and the prevalence of neutrophils. So, while type III hypersensitivity may contribute to FIP, it has not yet been confirmed as a crucial pathogenic mechanism. Other authors have proposed that type IV hypersensitivity reactions serve as a driving force behind the granulomatous and necrotizing lesions associated with FIP. As discussed above, activation of viral infected macrophages results in the release of various cytokines and growth factors, specifically VEGF, which is a potent mediator of vascular permeability that may produce vascular leakage and acute phlebitis via alterations in endothelial junctional complexes.[11]

The clinical pathology findings associated with FIP can be somewhat variable and non-specific. When present, effusions are usually classified as a modified transudate, with a high protein content (>3.5 g/dL) and a low nucleated cell count (<5,000 nucleated cells/mL), which parallels a serum hypergammaglobulinemia and decreased albumin:globulin ratio. Serum protein electrophoresis often reveals a polyclonal gammopathy (due to immunoglobulins). Normocytic, normochromic, non-regenerative anemia (or anemia of chronic disease) may also be observed.[1] The release of inflammatory mediators (such as IL-1, IL-6 and TNF-α) results in hepatic production of the acute phase protein hepcidin. Hepcidin induces the degradation of the iron export protein ferroportin, effectively sequestering iron within macrophages and enterocytes and ultimately resulting in decreased iron availability with subsequent anemia.[9] As noted by the contributor, CSF evaluation can also be useful in reaching a diagnosis of FIP; however, obtaining a specimen is often quite difficult due to the high viscosity of fluid from increased protein levels.[1] Serum levels of the acute phase protein, α1-acid glycoprotein (AGP) are often significantly elevated (>3 mg/ml), but this is not specific for FIP and may occur with other inflammatory conditions, neoplastic diseases (lymphoma) or in asymptomatic FCoV carriers, especially from households with endemic infection.[11]

Table 1. Select coronaviruses of veterinary importance.[12,18]

Group 1a		**Disease/Symptoms**
	• Feline enteric coronavirus • Mutated feline enteric coronavirus (FIP)	• None/mild gastroenteritis • Peritonitis, vasculitis, granulomatous inflammation
	Canine coronavirus	Mild gastroenteritis
	Transmissible gastroenteritis virus of swine (TGE)	Gastroenteritis, watery diarrhea, dehydration
	Porcine respiratory coronavirus (PRCV)	Mild respiratory disease/interstitial pneumonia
	Ferret (FRECV) & mink enteric coronaviruses	Epizootic catarrhal enteritis
	Ferret systemic coronavirus (FRSCV)	FIP-like (dry/granulomatous form) systemic dz
Group 1b		
	Porcine epidemic diarrhea virus	Gastroenteritis, watery diarrhea, dehydration
Group 2a		

	Porcine hemagglutinating encephalomyelitis virus	Encephalomyelitis, wasting, muscle tremors
	Mouse hepatitis virus	Hepatitis, enteritis, nephritis, demyelinating encephalomyelitis
	Sialodacryoadenitis virus of rats	Salivary/lacrimal gland necrosis/inflammation, chromodacryorrhea
	Bovine coronavirus	Gastroenteritis (winter dysentery), respiratory disease
Group 2b		
	Severe acute respiratory syndrome (SARS) coronavirus	Humans
	SARS coronavirus	Civets, cats, bats; subclinical
Group 3		
	Avian infectious bronchitis virus	Tracheobronchitis, nephritis, rales
	Turkey coronavirus/Bluecomb virus	Enteritis, cyanosis

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CASE III: PV118/13 (JPC 4035592).

Signalment: 10-12-week-old Lohmann Brown laying hens from a breeder in Nigeria, (*Gallus gallus domesticus*).

History: The chickens were vaccinated for pox, with what was discovered later on as the wrong dosage. They started to show crusts in the eyelids, which slowly involved mucocutaneous junctions. The animals became anorectic. There was gradual spread into a large portion of the flock until revaccination was recommended.

Gross Pathology: Not available. Only eyes, including eyelids were submitted in formalin.

Histopathologic Description: Part of a sagittal section from an eye including feathered skin from eyelids.

There is locally extensive, marked epithelial and epidermal hyperplasia of the conjunctiva and feathered skin. The epithelium is up to 8-10 times its normal thickness and has extensive serocellular crusting. There are foci of heterophilic infiltration and hemorrhage. Many keratinocytes have marked cytoplasmic vacuolation (ballooning degeneration) and many are expanded by 10-30 µm eosinophilic/red intracytoplasmic inclusions (Bollinger bodies). The dermis is moderately infiltrated by lymphocytes, macrophages and heterophils.

Contributor's Morphologic Diagnosis: Feathered skin: Lymphohistiocytic and heterophilic dermatitis with epidermitis, epidermal hyperplasia and intracytoplasmic inclusions, findings

typical of fowl pox.

Contributor's Comment: The submitted samples reflect a classic histological picture of fowl pox.

Avian or fowl pox are diseases caused by a variety or viruses of the genus *Avipoxvirus*, a member of the poxviridae. This DNA virus group has very large viral particles (250 to 300 nm). *Avipoxvirus* induces intracytoplasmic, lipophilic inclusion bodies, Bollinger bodies, in the epithelium of the integument. Their appearance is pathognomonic.[3,9,10] The virus is recognized in more than 232 species of wild birds representing 23 orders, however, it is likely that many more bird species are susceptible to *Avipoxvirus*.[1]

Fowl pox is a slow spreading disease which may present in two forms: 1) the cutaneous form is characterized by the development of discrete nodular proliferative skin lesions on the non-feathered parts of the body, and 2) the diphtheritic form with fibrinonecrotic and proliferative lesions in the mucous membranes of the upper respiratory tract, mouth and esophagus. Flock mortality is usually low if the cutaneous form prevails, but it may be high with generalized infection, with the diphtheritic form, or when the disease is complicated by other infectious or poor environmental conditions.[1,3,9] In canaries and other finches, mortality can reach 80 to 100%.[1,3,9]

Avian pox is not of public health significance and it doesn't generally affect mammals.[9]

The mode of transmission is through virus carriers and contaminated environment. Mosquitoes and mites are the main vectors. Aerosols generated from infected birds, or the ingestion of contaminated food or water have also been implicated as a source of transmission.[10] The disease may have a seasonal incidence, occurring during late summer and autumn, which in some countries coincides with the peak of the mosquito season. The virus can survive in the vector's salivary glands for 2 to 8 weeks. Spread of the virus within a given bird population is triggered by close contact between birds and the formation of small traumatic lesions due to pecking on one another. During latent infection or after recovery from clinical disease, the virus is intermittently shed via the feces or the skin and feather quills.[3,9]

The clinical signs seen in the cutaneous form of pox are very characteristic, with formation of nodules that first appear as small white foci and then rapidly increase in size and become yellow. Initially there is formation of papules followed by vesicles and thickened areas. Adjacent lesions may coalesce and crust, acquiring a gray, or dark brown color. Later on, there is development of inflammation with hemorrhage and formation of a scab that sloughs off and leaves a pink scar.[3]

In the diphtheritic form, slightly elevated, white, opaque nodules develop in the mucous membranes. Nodules rapidly increase in size and often coalesce, becoming a yellow, cheesy, necrotic, pseudo-diphtheritic or diphtheritic membrane. The inflammation process may extend into the sinuses and also in the esophagus, pharynx and larynx resulting in respiratory disturbances.[9]

Histopathology usually reveals epithelial hyperplasia (in both diphtheritic and cutaneous forms), with cell swelling, associated inflammatory changes, and typical large, solid or ring-like, eosinophilic intracytoplasmic inclusions known as Bollinger bodies.[3,9,10] Transmission electron microscopy (TEM) may also reveal definite proof of *Avipoxvirus* infection, demonstrating the typical particles within inclusion bodies. Avipoxvirus identification may also be carried out by negative staining electron microscopy with 2% phosphotungstic acid (PTA) on infected cells.[10]

JPC Diagnosis: Feathered skin and mucocutaneous junction: Conjunctivitis and dermatitis, proliferative and necrotizing, multifocal, marked, with epithelial intracytoplasmic viral inclusion bodies (Bollinger bodies).

Conference Comment: The family *Poxviridae* is composed of epitheliotropic DNA viruses that cause cutaneous or systemic disease in many species of animals, including wild and domestic mammals, birds, and humans (see table 1).[4,6] This case provides an excellent example of the cutaneous ("dry") form of avian pox, which is summarized comprehensively by the contributor. Readers are also encouraged to review WSC 2012-2013, conference 3, case 4 for an example its diphtheritic ("wet") manifestation in a wild turkey.

Conference participants briefly discussed the differential diagnosis for gross findings associated with the diptheritic form of avian pox, including Gallid herpesvirus-1 (infectious laryngotracheitis), *Capillaria annulata* or *C. contorta*, *Trichomonas gallinae*, *Candida albicans*, *Aspergillus* spp., and vitamin A deficiency. Rule outs for the cutaneous form include dermatophytosis (*Trichophyton megninii* or *T. simii*), papillomavirus and the mite *Knemidokoptes gallinae*.[3,4] These conditions can generally be differentiated microscopically; histochemical staining with giemsa highlights the prominent intracytoplasmic Bollinger bodies associated with avipoxvirus, while herpesviral infection results in intranuclear viral inclusions. Identification of yeast, hyphae or pseudohyphae (in cases of candidiasis or aspergillosis), arthrospores (dermatophytosis), trichomonads, nematode adults, larvae or eggs (capillariasis), arthropod segments (knemidokoptosis), or squamous metaplasia of glandular epithelia (vitamin A deficiency) is suggestive of one of the alternative etiologies enumerated above.[2,5,7-9,11]

Table 1: Select genera of the family *Poxviridae*.[4,6]

Genus	**Virus/Disease**	**Major Hosts**
Orthopoxvirus	Vaccinia virus Buffalopox/Rabbitpox virus*	Numerous: cattle, buffalo, swine, rabbits
	Cowpox*	Rodents (reservoir), cattle, cats, elephants, rhinos,
	Camelpox	Camels
	Ectromelia (Mousepox)	Mice, voles
	Monkeypox*	NHPs, squirrels, anteaters
Capripoxvirus	Goatpox	Goats, sheep
	Sheeppox	Sheep, goats
	Lumpy skin disease virus	Cattle, cape buffalo
Suispoxvirus	Swinepox virus	Swine (vector= *Hematopinus*

		suis)
Leporipoxvirus	Myxoma virus	Rabbits (*Oryctolagus* & *Sylvilagus* spp.)
	Rabbit fibroma virus, Hare fibroma virus	Rabbits
	Squirrel fibroma virus	Grey and red squirrels
Avipoxvirus	Fowlpox, canarypox, quailpox, etc	Chickens, turkeys, peacocks, etc.
Parapoxvirus	Caprine parapoxvirus (Orf; contagious ecthyma)*	Sheep, goats
	Bovine parapox (bovine papular stomatitis virus)*	Cattle
	Pseudocowpox*	Cattle
	Sealpox*	Seals
	Parapoxvirus of red deer	Red deer
Molluscipoxvirus	Molluscum contagiosum virus*	NHPs, birds, dogs, kangaroos, equids
Yatapoxvirus	Yabapox virus & tanapoxvirus*	NHPs
Unclassified	Squirrel poxvirus, fish (carp edema), horsepox	

* zoonotic

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CASE IV: 11-1195 (JPC 4033515).

Signalment: Age-unspecified female Yorkshire pig, (*Sus scrofa*).

History: This Yorkshire pig was one of several animals belonging to a hemorrhagic shock study. This pig (animal number 30962), age unknown, was hemorrhaged over a 15-minute period until 55% of its estimated blood volume (EBV) was lost via the femoral artery catheter. At time 15 minutes, the pig received initial resuscitation therapy. Oxycyte (5 mL/kg) was given as a continuous infusion over 10 minutes via the external jugular vein. There was no reported history of clinical illness.

Gross Pathology: There were no reported gross findings at necropsy.

Laboratory Results:
- *Mycoplasma hyopneumoniae PCR: NEGATIVE*
- *Mycoplasma hyopneumoniae* IHC: NEGATIVE

Histopathologic Description: Lung: Diffusely, filling bronchi and bronchioles is an exudate composed of moderate numbers of viable and non-viable neutrophils, macrophages, fewer lymphocytes and plasma cells, admixed with edema, and rare fibrin. Multifocally, both bronchial and bronchiolar epithelium are mildly hyperplastic. A similar infiltrate extends into interlobular septa, expands and obscures alveolar septa, and fills alveolar spaces. Rarely, there is mild type II pneumocyte hyperplasia. Multifocally surrounding bronchi, bronchioles, and blood vessels (often lined by hyperplastic endothelium) are variably sized lymphoid follicles with vague germinal centers (BALT hyperplasia), with mild lymphocytolysis. Multifocally, the pleura is separated from the subjacent lung parenchyma by clear space (suspected artifact).

Contributor's Morphologic Diagnosis: Lung: Pneumonia, bronchointerstitial, suppurative and histiocytic, chronic, diffuse, severe, with lymphoid (BALT) hyperplasia, mild lymphocytolysis, type II pneumocyte hyperplasia, bronchiolar and bronchial epithelial hyperplasia, and edema, Yorkshire pig, porcine.

Contributor's Comment: This is a case of suspected *Mycoplasma hyopneumoniae*, which is a common cause of non-fatal pneumonia in young pigs. The disease is prevalent in grower-finisher pigs; however, animals as young as 5-weeks may be affected.[1] Also known as porcine enzootic pneumonia, disease progression is often insidious in endemic areas where subclinical carriers serve as key sources of infection for naïve herds.[1]

Gross lesions associated with mycoplasmal pneumonia are discolored, collapsed, firm lungs affecting the cranioventral lung lobes.[1] Acute histologic lesions are characterized by alveoli

containing macrophages and neutrophils along with edema.[1] Chronic infections in swine are similar to other species (e.g., rats, mice) where peribronchial/peribronchiolar (BALT), and perivascular lymphoid hyperplasia is a dominant histologic feature.

Although the exact pathogenesis is not completely understood, *Mycoplasma hyopneumoniae* firmly adheres to the cilia of the respiratory tree resulting in ciliostasis. Attachment to the respiratory epithelium invokes the following:

(1) Influx of neutrophils into the tracheobronchial mucosa
(2) Extensive loss of cilia (deciliation)
(3) Broncho-alveolar lymphoid tissue (BALT) hyperplasia
(4) Influx of mononuclear cells into the peribronchiolar, bronchiolar, and alveolar interstitium[3]

Diagnosis of *Mycoplasma hyopneumoniae* infection in the porcine lung is based on results of three methods: isolation of the organism by culture, immunofluorescence (IF) testing, or immunohistochemistry using polyclonal antibodies.[3]

Despite our inability to definitively diagnose *Mycoplasma hyopneumoniae* as a causative agent for the lung lesions in this case, the histologic lesions are highly suggestive of this entity.

JPC Diagnosis: Lung: Bronchopneumonia, pyogranulomatous, diffuse, chronic, severe, with BALT hyperplasia.

Conference Comment: In conference, the moderator led a brief review of the morphologic patterns of pneumonia. Bronchopneumonia is the most common type of pnuemonia in domestic animals. It is characterized by inflammatory lesions arising primarily within the airways (with occasional spread into the surrounding interstitium), and there is usually cranioventral consolidation of the lungs. Bronchopneumonias are typically caused by many types of inhaled bacteria, including *Mycoplasma* spp. Fibrinous bronchopneumonia in particular has a propensity for depositing on pleural surfaces, thus some pathologists refer to it as pleuropneumonia. In contrast, inflammatory lesions develop within alveolar walls and the bronchiolar interstitium in cases of interstitial pneumonia. The portal of entry can by hematogenous or aerogenous. Interstitial pneumonia tends to be diffuse and is generally associated with viruses, toxins, sepsis, or protozoa such as *Toxoplasma* spp. The term bronchointerstitial pneumonia describes pulmonary lesions with features of both interstitial and bronchopneumonia and is specifically associated with viruses that cause necrosis in both bronchiolar and alveolar epithelial cells (e.g. small ruminant respiratory syncytial virus, canine distemper and porcine/equine influenza). With embolic pneumonia, sterile or septic (e.g. from vegetative valvular endocarditis), thromboemboli are delivered hematogenously to the lung; inflammation is random and multifocal and it centers upon pulmonary arterioles and alveolar capillaries. Verminous pneumonia typically exhibits a caudodorsal distribution.[3] Based on this broad classification scheme, the microscopic lesions in this case are most consistent with bronchopneumonia secondary to *Mycoplasma hyopneumoniae*.

The contributor provides an excellent summary of *M. hyopneumoniae*. See WSC 2013-2014, conference 7, case 1 for further discussion of *Mycoplasma* spp., and table 1 for an abbreviated list of *Mycoplasma* species important in veterinary medicine.

Table 1. Select *Mycoplasma* species of veterinary importance.[4]

***Mycoplasma* species**	**Hosts**	**Disease**
M. mycoides subsp. *mycoides* (small colony type)	Bovine	Contagious bovine pleuropneumonia
M. bovis	Bovine	Mastitis, pneumonia, arthritis, otitis
M. agalactiae	Ovine, Caprine	Contagious agalactia (mastitis)
M. capricolum subsp. *capripneumoniae*	Caprine	Contagious caprine pleuropneumonia
M. capricolum subsp. *capricolum*	Ovine, Caprine	Septicemia, mastitis, polyarthritis, pneumonia
M. mycoides subsp. *capri* (includes strains previously classified as *M. mycoides mycoides* large colony type)	Ovine, Caprine	Septicemia, pleuropneumonia, mastitis, arthritis
M. ovipneumoniae	Ovine, Caprine	Pneumonia
M. pulmonis	Rodents--rat and mouse	Colonize nasopharynx and middle ear; affect respiratory and reproductive tracts and joints
M. hyopneumoniae	Swine	Enzootic pneumonia
M. hyosynoviae	Swine (10-30 weeks of age)	Polyarthritis
M. hyorhinis	Swine (3-10 weeks of age)	Polyserositis
M. suis	Swine	Mild anemia, poor growth rates
M. ovipneumoniae		mild pneumonia
M. haemofelis	Feline	Feline infectious anemia
M. cynos	Canine	Implicated in kennel cough complex
M. haemocanis	Canine	Mild or subclinical anemia; more severe signs in splenectomized animals
M. gallisepticum	Turkeys and Chickens	Chronic respiratory disease; infectious sinusitis
M. synoviae	Turkeys and Chickens	Infectious synovitis

M. meleagridis	Feline, Equine	Conjunctivitis in cats, pleuritis in horses
M. equigenitalium	Equine	Abortion

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http://wrair-www.army.mil

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