WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 8   26 October 2016


CASE I: 1525970 (JPC 4083864).

Signalment: 18-year-old, thoroughbred mare (*Equus caballus*).

History: The mare had a several year history of a polyp in the nasal cavity. The mass was surgically removed and submitted as an excisional biopsy.

Gross Pathology: The biopsy sample comprised a 2 x 1.5 x 1 cm smooth, pink-tan, spongy mass with multifocal ulcers and a narrow stalk at one edge.

Laboratory results: N/A

Histopathologic Description: Representative sections of the tissue comprise a polypoid proliferation of loose fibrovascular tissue covered by a hyperplastic, acanthotic stratified squamous epithelium with multifocal erosions and an eosinophilic exudate of mucin admixed with necrotic neutrophils, debris and erythrocytes. Numerous protistan organisms at varying stages of development are embedded within the surface exudate, epithelium, and submucosa, which is infiltrated by moderate numbers of lymphocytes, plasma cells, macrophages, fewer neutrophils, and
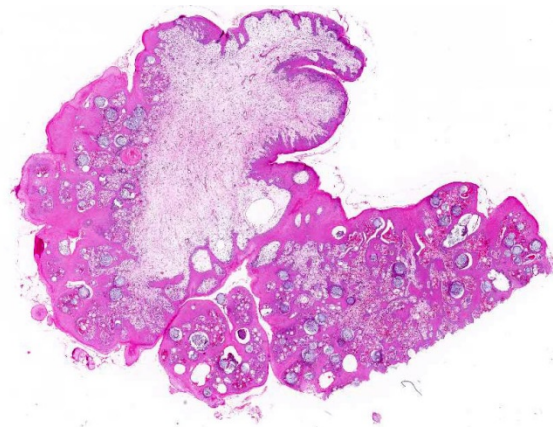
Nasal mucosa, horse. A polypoid proliferation of the nasal mucosa is submitted for examination. Large numbers of juvenile and mature sporangia are present within the submucosa, and few mature sporangia are present within the overlying epithelium. (HE, 4X)

variable amounts of hemorrhage and edema. Immature sporangia (trophocytes) are round and 10-100 µm diameter, with a 2 µm thick eosinophilic wall, lacy amphophilic cytoplasm, and a single, central, round karyosome (nucleus). Intermediate sporangia lack a nucleus and contain innumerable immature sporangiospores (endospores). Immature endospores are 1-4 µm diameter, with a thin wall, scant to moderate amounts of lightly basophilic cytoplasm, and irregularly round, para-central eosinophilic structures. Mature

sporangia located throughout the epithelium are spherical and 100-400 µm diameter, with a 2-5 µm thick, bilamellar, outer eosinophilic and inner amphophilic wall. The mature sporangia contain mature endospores or a mixture of mature and immature endospores. Mature endospores are 5-8 µm diameter spherical structures with a thin unilamellar wall, scant clear cytoplasm, and multiple, central, round eosinophilic bodies. Within mature sporangia, immature endospores are often located peripherally or emerge unilaterally from the wall of the sporangium. Mature sporangia are multifocally ruptured, releasing mature endospores through an apical pore in the epithelium or into the adjacent stroma, and surrounded by dense aggregates of neutrophils with fewer lymphocytes, plasma cells, and macrophages.

**Contributor's Morphologic Diagnosis:** Horse, nasal mass: Chronic polypoid and suppurative rhinosinusitis with protistan sporangia and endospores (consistent with *Rhinosporidium seeberi*)

Contributor's Comment: *Rhinsporiduim seeberi* is an uncommon cause of rhinitis and nasal polyps in humans, dogs, cats, horses, cattle and waterfowl.[3, 4, 7, 9, 10, 11, 12] The organism was originally identified in tissue extracted from nasal polyps of people in Argentina, and has since most commonly been diagnosed in tropical climates such as India and Sri Lanka.[3] It has been reported sporadically in horses from the Americas and Europe. [2, 9, 10, 11,]

Patients with nasal rhinosporidiosis may be asymptomatic, or present with clinical signs that include respiratory noise, sneezing, nasal discharge, and epistaxis.[2, 3, 4] If masses become large, obstruction of the nasal passage can occur.[1, 3] Examination of the nasal cavity reveals a unilateral polypoid mass protruding from the nasal mucosa,
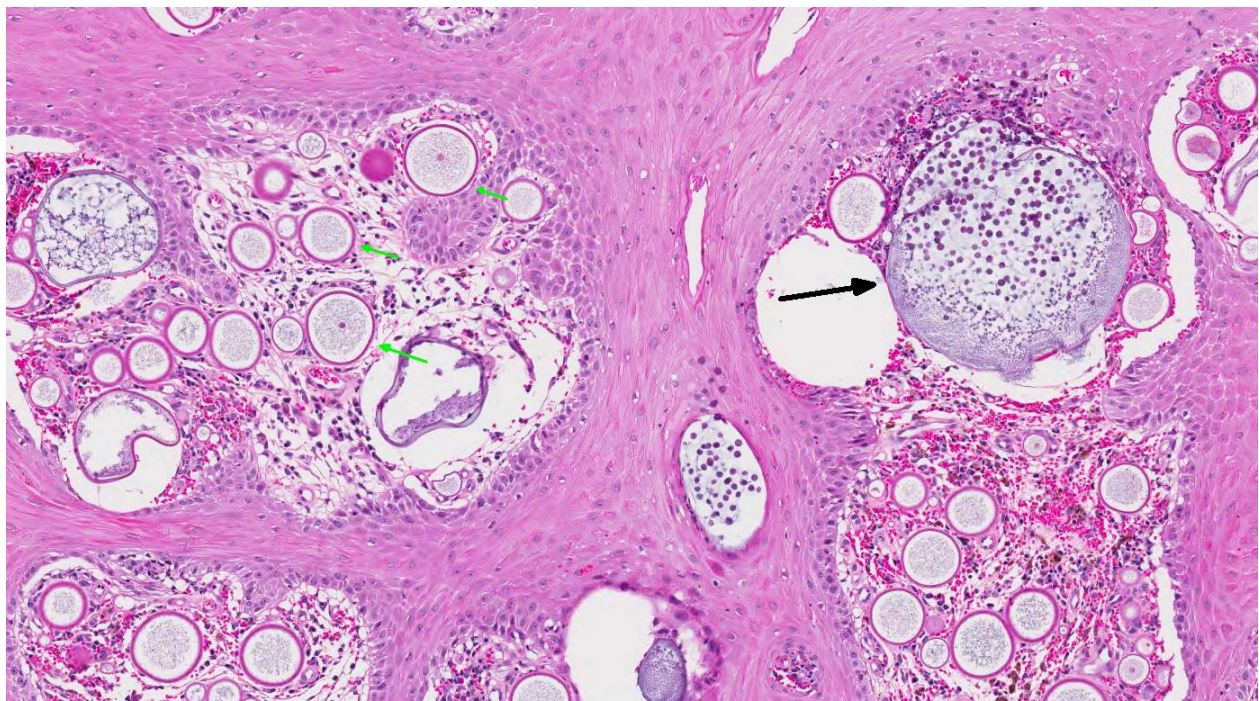
Nasal mucosa, horse. Numerous immature (green arrows) sporangia and few mature (black arrow) sporangia are scattered throughout the submucosa. (HE, 10X)
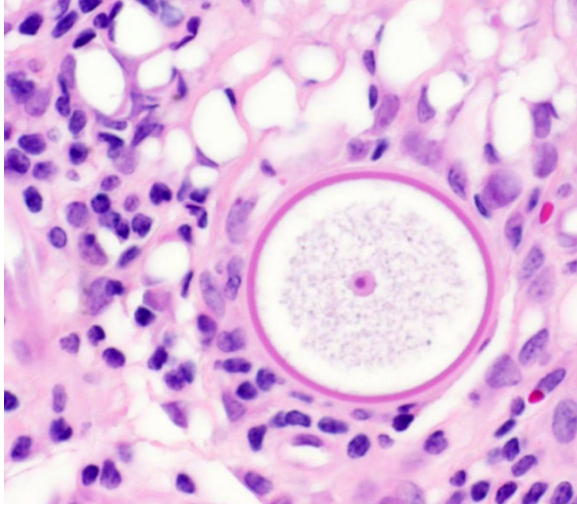
Nasal mucosa, horse. Immature sporangia have a 2 um thick eosinophilic wall, lacy amphophilic cytoplasm, and a single, central, round karyosome. (H&E, 40X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

though laryngeal masses in association with *R. seeberi* have been reported in horses.[2, 11] Close inspection of the surface of the mass may reveal small, white foci. In humans, there are reports of nasal, ocular, laryngeal and genital rhinosporidiosis.[3]

Histologic examination reveals a fibro-vascular polyp covered by stratified squamous to pseudostratified ciliated columnar epithelium. Sporangia in varying stages of development are observed throughout the fibrovascular and epithelial components of the polyp. Identification of sporangia with endospores in a polypoid nasal mass is diagnostic for *R. seeberi*.[7] Organisms are readily observed in H&E-stained sections; however, histochemical stains, such as GMS and PAS, can be used to highlight the walls of the sporangia.[5] Polymerase chain reaction (PCR) and DNA sequence analysis of tissue samples can be used to confirm the etiologic agent.[2]

The classification of *R. seeberi* has long been controversial. The organism was once considered to be protozoan, fungus, or a cyanobacterium. More recently, phylo-genetic analysis categorized *R. seeberi* as a eukaryote within the class *Mesomycetozoa*, a group of aquatic protistan parasites.[5, 6]

The pathogenesis of rhinosporidiosis is poorly understood, though stagnant water appears to be the reservoir for *R. seeberi*. The proposed mechanism of infection involves pre-existing defects in the epithelial barrier of the nasal passage, allowing mature endospores to enter the nasal mucosa from contaminated water.[3] Once within host tissues, endospores localize to the sub-epithelial connective tissues and mature into immature sporangia (trophocytes). Immature sporangia have thick, unilamellar eosinophilic walls, a central nucleus, and fine cytoplasm. As immature sporangia become intermediate sporangia, a bilamellar wall develops, the nucleus is lost, and the
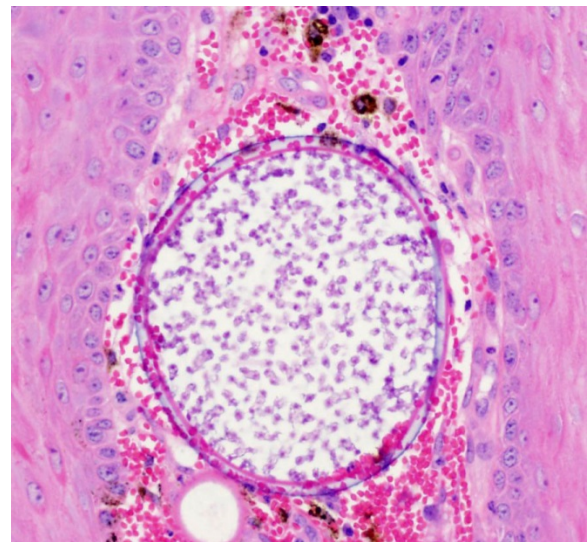
Nasal mucosa, horse. Intermediate sporangia lack a nucleus and contain innumerable immature sporangiospores (endospores). Immature endospores are 1-4 um diameter, with a thin wall, scant to moderate amounts of lightly basophilic cytoplasm, and irregularly round, paracentral eosinophilic structures. (H&E, 20X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

cytoplasm condenses to form immature sporangiospores (endospores). Intermediate sporangia eventually become larger, contain both immature and mature endospores, and are localized within the superficial epithelium. At this stage, the sporangia are considered mature and will continue to house developing endospores until release. Mature endospores contain multiple, central eosinophilic bodies, and are released from sporangia through apical pores in the epithelium into the nasal passage. Infective, mature endospores released into the nasal passage are then excreted into the environment along with nasal secretions.[7]
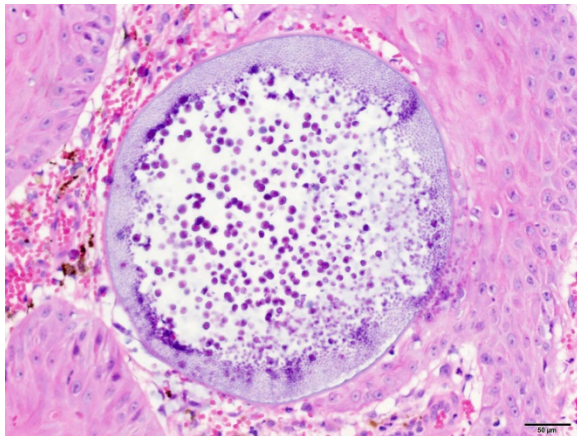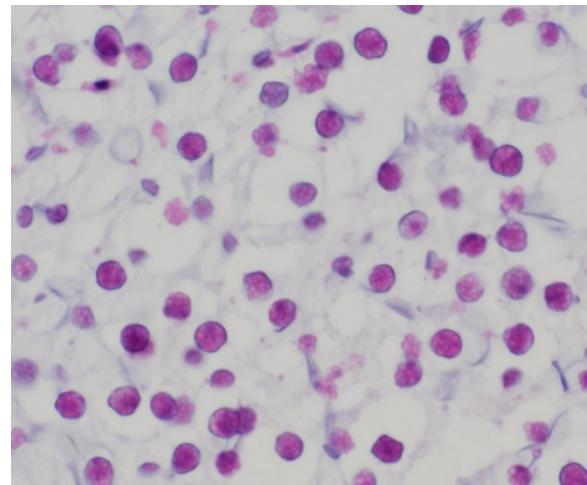

Nasal mucosa, horse. Mature sporangia are round, 100-400 um diameter, and have a 2-5 um thick, bilamellar wall (outer eosinophilic and inner amphophilic). The mature sporangia contain mature endospores, or a mixture of mature and immature endospores Within mature sporangia, immature endospores are often located peripherally, or emerge unilaterally from the wall of the sporangium. (H&E, 20X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

Surgical removal of any visible polyps is the treatment of choice for nasal rhino-sporidiosis. Excision is typically curative; however, recurrence is reported.[2, 3, 7] A diagnosis of concurrent nasal adeno-carcinoma and rhinosporidiosis was reported in a cat housed on a horse farm.[1]

In this horse, infectious organisms were embedded within a polypoid proliferation, grossly and histologically resembling a nasal polyp. Because rhinosporidiosis in humans is associated with polypoid proliferations, the authors speculate the proliferative (polypoid) response in this horse may be secondary to chronic infection and
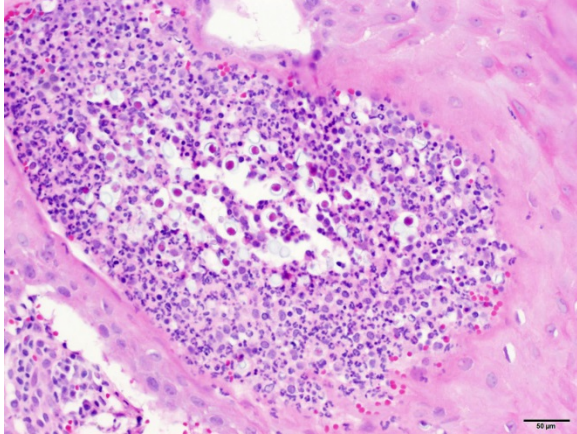

Nasal mucosa, horse. Mature endospores are spherical, 5-8 um diameter, with a thin unilamellar wall, scant clear cytoplasm and multiple, central, round eosinophilic bodies. (H&E, 40X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

inflammation, rather than represent a primary nasal polyp. Free endospores within the submucosa often elicit a pyo-granulomatous to granulomatous response; however, in this case, the predominant inflammatory infiltrates were neutrophils admixed with lymphocytes, plasma cells, and macrophages.

For this horse, travel history, clinical signs, laboratory findings and post-operative follow-up were not provided by the submitting veterinarians.

JPC Diagnosis: Nasal mass: Rhinitis, polypoid, chronic-active, diffuse, severe, with numerous protistan sprorangia and endospores, thoroughbred, *Equus caballus*

Conference Comment: The contributor provides an exceptional review of the epidemiology, clinical presentation, gross lesions, histopathologic findings, and differential diagnosis for *Rhinosporidium seeberi* in horses. Conference participants were impressed by the tremendous numbers of protistan endospores and striking variation in the size of the sporangia in the section. Despite slight slide variability,

Nasal mucosa, horse. Mature sporangia are often ruptured, releasing mature endospores through an apical pore in the epithelium, or into the adjacent stroma. Numerous neutrophils infiltrate the connective tissue surrounding ruptured sporangia, admixed with lesser lymphocytes, plasma cells, and hemosiderin-laden macrophages. (H&E, 20X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

conference participants noted chronic mixed inflammation with hemorrhage, edema, and granulation tissue in the subepithelial connective tissue and stalk of the nasal polyp.

Rhinosporidium seeberi is one of the few pathogens which reproduce by endo-sporulation.[3] The endospore is implanted in tissues and grows into much larger and variably sized sporangia. As sporangia grow and mature, endospores are produced and released into the surrounding tissue generating marked pyogranulomatous inflammation and effectively reinitiating the pathogen's lifecycle.[3] Conference participants discussed other endosporulators of veterinary significance including: *Prototheca wickerhamii*, *Chlorella* sp., *Coccidioides* sp., and *Batrachochytrium dendrobatidis*. Based on the size of the sporangia and observation of endo-sporulation, the differential diagnosis in this case includes *Coccidioides* sp. (see 2016-2017 WSC 4 Case 4). The endospores of *Rhinosporidum seeberi* contain inner granules, while *Coccidioides* sp. endospores do not. Also, *R. seeberi* endospores completely stain with Periodic acid-Schiff (PAS), while only the walls of the endospores stain in *Coccidioides* sp.[8] However, finding large sporangia in a polypoid nasal mass of a horse is typically considered diagnostic for rhinosporidiosis.[1,3]

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CASE II: 0398/15 (JPC 4086389).

Signalment: Young male Yorkshire/Hampshire cross pig (*Sus domestica*).

History: Two piglets (about three-weeks-old) had been growing slowly and showed respiratory symptoms with rapid breathing. They were both treated with antibiotics (penicillin) but died suddenly. The piglets were sent to necropsy. A few weeks later a small number of fattening pigs had reduced growth rate and were in poor body condition, the pigs were euthanized and sent to necropsy. All pigs were from the same specific pathogen-free farm (SPF). The submitted slide is from one of the fattening pigs.

Once a year tests are taken to show freedom from: swine influenza virus, *Actinobacillus pleuropneumoniae* (APP), *Mycoplasma hyopneumoniae* (SEP), *Sarcoptes scabiei*, Swine dysentery (*Brachyspira hyo-*

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dysenteriae), Intestinal spirochetosis (*Brachyspira pilosicoli)*, other Brachyspira-subspecies, toxin-producing *Pasteurella multocida*, *Salmonella* and *Lawsonia intracellularis*. The farm was not vaccinated against PCV-2.

Gross Pathology: At necropsy, the fattening pig was in poor body condition and presented with a mottled myocardium,
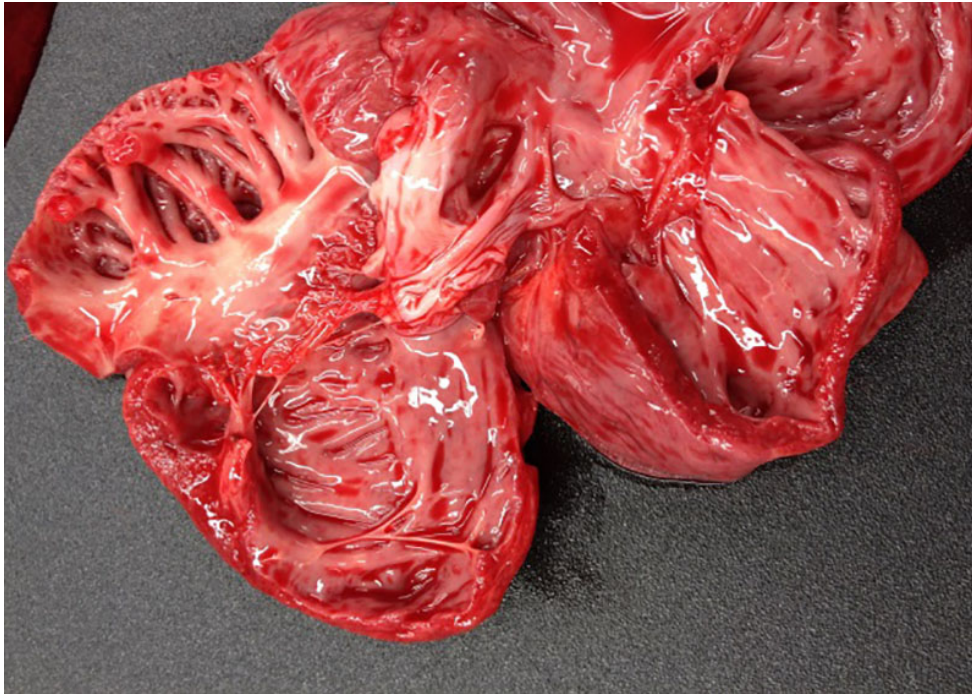
Heart, piglet. The heart of the submitted piglet had marked dilation of the right and left atria and ventricles.

enlarged lymph nodes, multiple white foci scattered throughout the kidneys, firm cranioventral lung, and mild ascites. The two piglets were in poor body condition, the hearts had marked dilatation of left and right ventricle and atrium (dilated cardio-myopathy) and the piglets presented signs of cardiac failure with acute stasis in liver, ascites, and pulmonary edema.

Laboratory results: Immunohistochemistry heart: positive for PCV-2 (piglet)

PCR lymph node: Positive for PCV 2 (fattening pig).

Histopathologic Description: Heart: Multifocal to coalescing chronic severe granulomatous interstitial inflammation with loss of myocytes is present. The inflammatory cells are dominated by lymphocytes, plasma cells, and macrophages with fewer neutrophils and eosinophils. Few multinucleated giant cells are also seen. Multifocally, the myocytes are degenerated or necrotic with loss of striations and eosinophilic swollen cytoplasm, loss of nuclei, and occasionally pyknotic nuclei, scattered degenerated Purkinje fibers are also seen. Multifocally, there are small foci of basophilic homogenous material (mineralization). The outer walls of medium and large sized vessels are infiltrated by varies amount of mononucleated inflammatory cells and the vessels have marked thickened tunica adventitia due to inflammation and edema, multifocal small sized vessels have degenerated walls with infiltration of mixed inflammatory cells (vasculitis). The endocardium is moderately thickened with abundant mixed inflammatory cells, reactive fibroblasts and angiogenesis (granulation tissue).
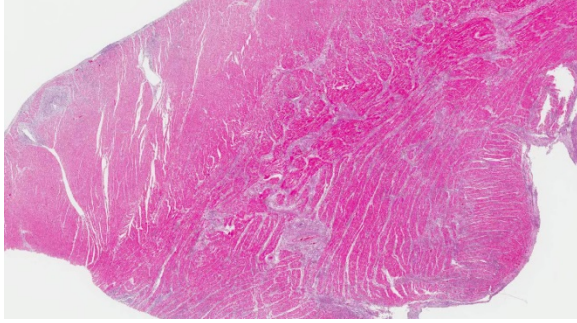
Heart, piglet. Subgross magnification of the submitted section of left ventricle reveals a significant inflammatory infiltrate throughout the myocardium, epicardium, and endocardium, which replaces myofibers. (HE, 4X)

On the Masson trichrome stain, there is a moderate amount of extracellular collagen present in areas with myocyte loss and with reactive fibroblasts.

Contributor's Morphologic Diagnosis: Heart: Myocarditis and endocarditis, multifocal to coalescing, interstitial, severe, chronic, lymphoplasmacytic and histiocytic with multinucleated giant cells (granulomatous) and vasculitis.

Contributor's Comment: Porcine circovirus (genus *Circovirus*, family *Circoviridae*) is a small (17nm diameter), non-enveloped virus that contains circular single-stranded DNA.[3,8] The virus was initially discovered in 1998 and was first isolated from pigs with *post weaning multisystemic wasting syndrome* (PMWS). The virus is highly prevalent in the domestic pig population all over the world and PCV-2 infection is common in herds as a subclinical disease. PCV-2 infection has been associated with several disease complexes including PMWS, enteric disease, respiratory disease, porcine dermatitis and nephropathy syndrome and reproductive disorders.[3,8,10] Vaccine against PCV-2 has been used since 2006, which have been effective controlling and preventing PCV-2 associated diseases. Although the majority of the conventional pigs entering the market are now vaccinated, PCV-2 still remains an important differential diagnosis for various disease manifestations in pigs.[8] In PMWS, pigs exhibit a systemic
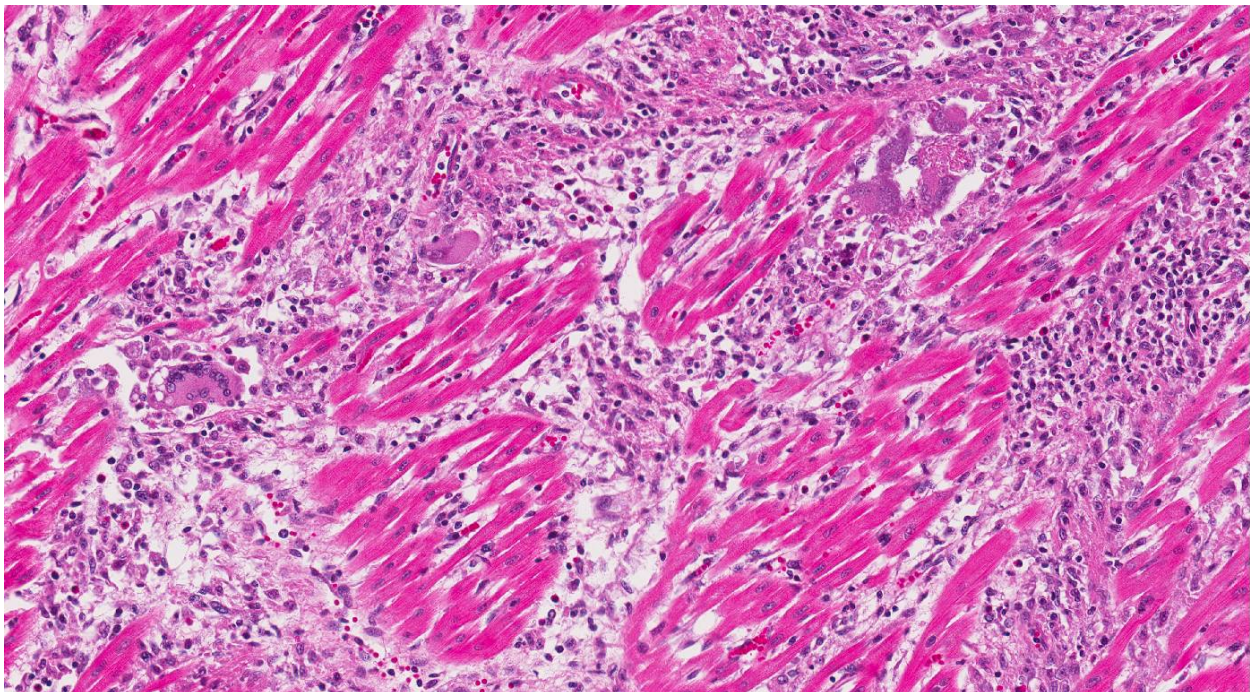
Heart, piglet. A granulomatous infiltrate separates, infiltrates, and replaces myofibers, which contains small amount of wispy collagen. Multinucleated giat cells are scattered throughout the infiltrate. (HE, 200X)
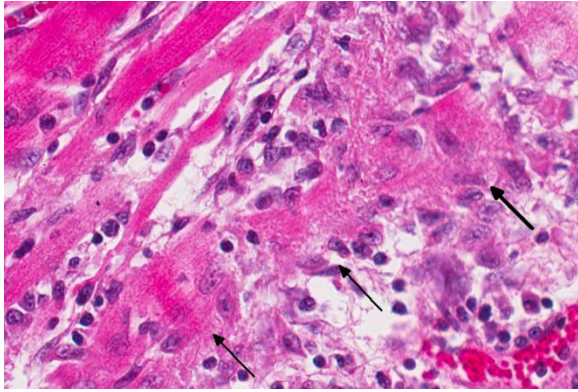
Heart, piglet. In areas of inflammation, degenerate myofibers (black arrows) exhibit loss of cross striations and fragmentation. (HE, 400X)

infection involving several organ systems and the disease is characterized by loss of weight or wasting in combination with various other clinical signs such as dyspnea, diarrhea, pallor, and jaundice. Other clinical signs include coughing, fever, central nervous signs, or sudden death.[3,8] On gross necropsy, lymphadenopathy is the most consistent feature of PMWS, but the gross lesions and severity are highly variable.[8] Diagnosis is mainly based on clinical findings and typical gross and histological lesions, and is supported by demonstration of active PCV2 infection by immuno-histochemistry or PCR.[3]

Porcine circoviral antigen and nucleic acid are most consistently present in the cytoplasm of monocytes, macrophages and dendritic cells throughout the body. Vascular smooth muscle and endothelial cells occasionally express circoviral antigen.[3] PCV2 induces characteristic lesions in the lymphoid system which includes lymphoid depletion and granulo-matous inflammation with multi-nucleated giant cells, lesions are commonly observed in the tonsils, spleen, Peyer´s patches, and lymph nodes. In the respiratory system, bronchinterstitial pneumonia may be a feature and the virus can cause

meningoencephalitis and vasculitis in CNS. In the digestive system, the most frequent lesion is granulomatous enteritis characterized by increased number of macrophages and scattered multinucleated giant cells in the mucosa and submucosa of the ileum and occasionally the colon and cecum. In the kidney, lesions are characterized by lymphoplasmacytic or granulomatous interstitial nephritis. In the vascular system, PCV2 antigen has been demonstrated in endothelial cells and in inflammatory cells in the arterial walls and chronic lymphoplasmacytic vascular lesions has been described among pigs infected with PCV2.[8,10] In foetuses, lesions are most prominent in the cardiovascular system, especially in the heart where cardio-myocytes are degenerated, or lost and replaced by fibrous connective tissue. In addition, there is non-suppurative myo-carditis and occasionally multinucleated giant cells.[2,6, 8,11] Heart failure and dilated cardiomyopathy is described as a feature among aborted foetuses and very young animals secondary to myocarditis.[2,6,8,11]

The presented case from the pig-farm showed typical gross and histological lesions of PCV2 infection that are compatible with the described cases in the literature. The fattening pig had lymphoid depletion and granulomatous inflammation in lymph node and granulomatous inflammation with vasculitis in kidney and heart. A purulent bronchopneumonia was seen in the lungs; however, the material was not cultured but secondary bacterial infection is most likely. The two young piglets with dilated cardiomyopathy had chronic granulomatous myocarditis at on histopathology. The diagnosis was supported by demonstrating active PCV2 infection with PCR and immuno-histochemistry.

JPC Diagnosis: Heart: Pancarditis, transmural, lymphohistiocytic, multifocal to coalescing, marked, with vasculitis, myocardial loss, and fibrosis, York-shire/Hampshire cross pig, *Sus domestica*.

Conference Comment: The contributor provides an excellent summary of porcine circovirus-2 (PCV-2) infection in swine. There are two separate genotypes of PCV have been implicated in this species. PCV type 1 (PCV-1) is generally thought to be nonpathogenic and does not cause disease in pigs, although it has been implicated as a potential cause of congenital tremors in newborn piglets.[3] PCV-2 is pathogenic in pigs and causes post weaning multisystemic wasting syndrome (PMWS) discussed by the contributor above. PMWS is a multifactorial systemic disease and clinically manifests at 25-150 days of age with most cases occurring between 7 and 15 weeks.[3-6,9] The six fundamental clinical signs include wasting, dyspnea, lymphadenopathy, diarrhea, pallor, and jaundice. Coughing, fever, gastric ulceration, and meningitis have also been reported. Serum antibodies to PCV-2 are very common in swine herds around the world, and positive antibody titer does not necessarily equate to clinical PMWS.[3] However, co-infection of PCV-2 with porcine reproductive and respiratory syndrome virus (PRRSV), and/or porcine parvovirus (PPV), produces more severe clinical disease; although, PCV-2 has been shown to be sufficient to produce disease in susceptible animals without co-infection.[3,4,6,9,10]

Conference participants discussed that lymphoplasmacytic and histiocytic myo-carditis with varied numbers of multi-nucleated giant cells and myocardial degeneration, loss, and replacement by fibrosis are typical histologic changes associated with infection by PCV-2.[3,4] In addition, necrotizing vasculitis, a key histologic feature in this case, has been implicated as a hallmark lesion in PCV-2 infection, as well as the severe form of
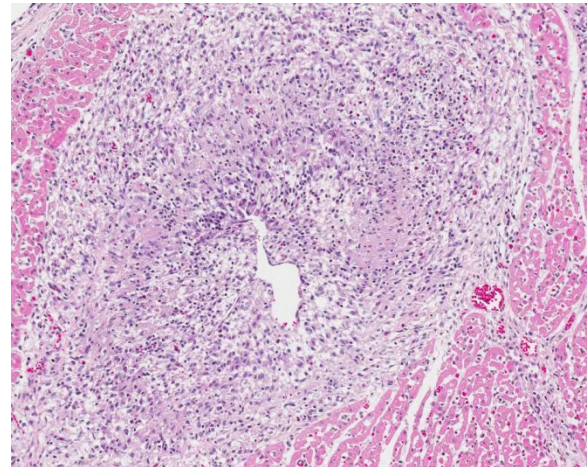
Heart, piglet. The walls of coronary vessels are effaced by granulomatous inflammation which extends into and replaces surrounding myofibers. (HE, 200X)

systemic porcine circovirus-associated disease (PCVAD), discussed below.[1-3,9] Participants also noted the lack of unique and characteristic intracytoplasmic baso-philic botryoid viral inclusion bodies, typically associated with PCV-2 infection. Inclusion bodies may be present within macrophages; however, they are not always identified. Definitive diagnosis of PMWS is based on a combination of typical clinical signs of disease, typical histologic lesions, and detection of PCV-2 in affected tissues via immunohistochemistry, as demonstrated by the contributor in this case.[3]
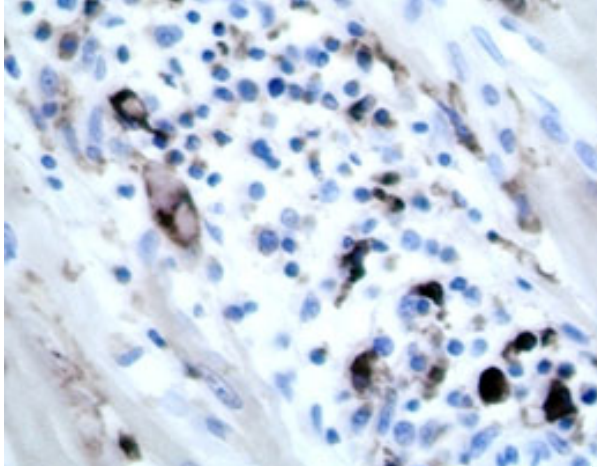
Heart piglet. Macrophages within the interstitial infiltrate exhibit strong cytoplasmic immunopositivity for porcine circovirus-2 viral antigen. (anti-PCV-2, 400X)

As mentioned above, infection with PCV-2 alone can cause disease in pigs; however, it is more commonly identified in a complex of multiple pathogen infection, known as PCVAD. This syndrome results from co-infection of PCV-2 with PPV, PRRSV, encephalomyocarditis virus (EMCV), swine influenza virus, or *Mycoplasma hyo-pneumonia*, or a combination of these agents.[1,3,4,10] It can also occur from PCV-2 infection in association with recent vaccination.[3,4] Despite the economic losses caused by PCV-2, PMWS, and PCVAD, the pathogeneses underlying the clinical findings remain largely unclear; however, they are likely related to macrophage activation prior to infection and subsequent endothelial cell modulation.[7] Conference participants discussed that antigens for PCV-2 can be found in macrophages, monocytes, and dendritic cells, as well as endothelial cells and vascular smooth muscle.

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CASE III: S16-0248 (JPC 4085382).

**Signalment:** Adult male and female Guinea pigs (*Cavia porcellus*).

History: In a collection of guinea pigs, three adult animals died within two weeks. All of them showed signs of dyspnea and two of them had been treated with antibiotics. Two animals were dissected.

Gross Pathology: The thoracic cavity of both animals contained approximately 2 ml of a red and turbid fluid. The cranial lung lobes and the accessory lobe of both animals were diffusely red and heavy. In parts of the caudal lobes, there are multifocal firm areas.

Laboratory results: Bacteriological examination of lung tissue (cultivation): No bacteriological or mycological agent was detectable.

Histopathologic Description: Depending on the cut-section, up to 100% of the lung tissue is affected. Diffusely within the bronchi and bronchioles are abundant degenerate and non-degenerate neutrophils, sloughed hypereosinophilic necrotic epithelial cells, admixed with fewer macro-phages and lymphocytes. Epithelial cells of

the bronchi and bronchioles are usually detached and necrotic, exhibiting a hypereosinophilic cytoplasm and pyknotic nucleus. Epithelial cells often contain a 7-10 µm, amphophilic, round to oval, intranuclear inclusion body which peripheralizes the chromatin. Depending on the cut-section, around the bronchi and bronchioles, as well
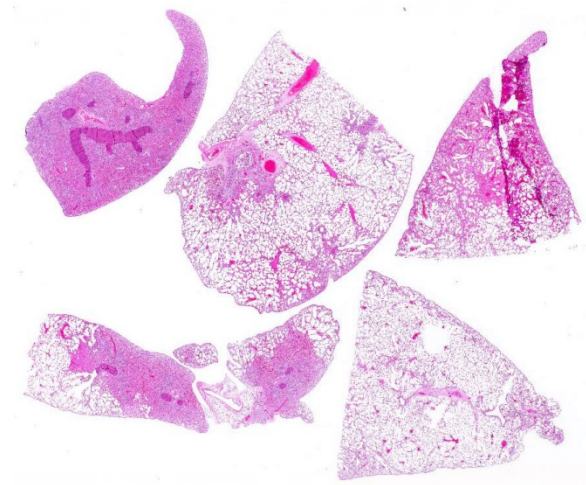
Lung, guinea pig. Multiple sections of lung exhibit variable degrees of consolidation. Airways are multifocally filled with a dense brightly eosinophilic exudate. (HE, 4x)

as perivascular, there is a cuff of a variable number of inflammatory infiltrates mainly composed of lymphocytes and fewer plasma cells. In some alveolar lumina, a small number of macrophages and lesser neutrophils are recognized. The interstitium is multifocal markedly thickened by inflammatory cells, mainly consisting of neutrophils and fewer lymphocytes. In addition, there is multifocal activation and hyperplasia of pneumocytes type 2. In some areas, the alveolar lumina are filled with eosinophilic, beaded fibrillar material (fibrin) admixed with a moderate amount of neutrophils. Adjacent to these areas, multiple vessels are filled with eosinophilic, amorphous material (thrombi). Multifocal the alveolar lumina are filled with homogeneous, eosinophilic, proteinaceous material (alveolar edema) and the lumina of
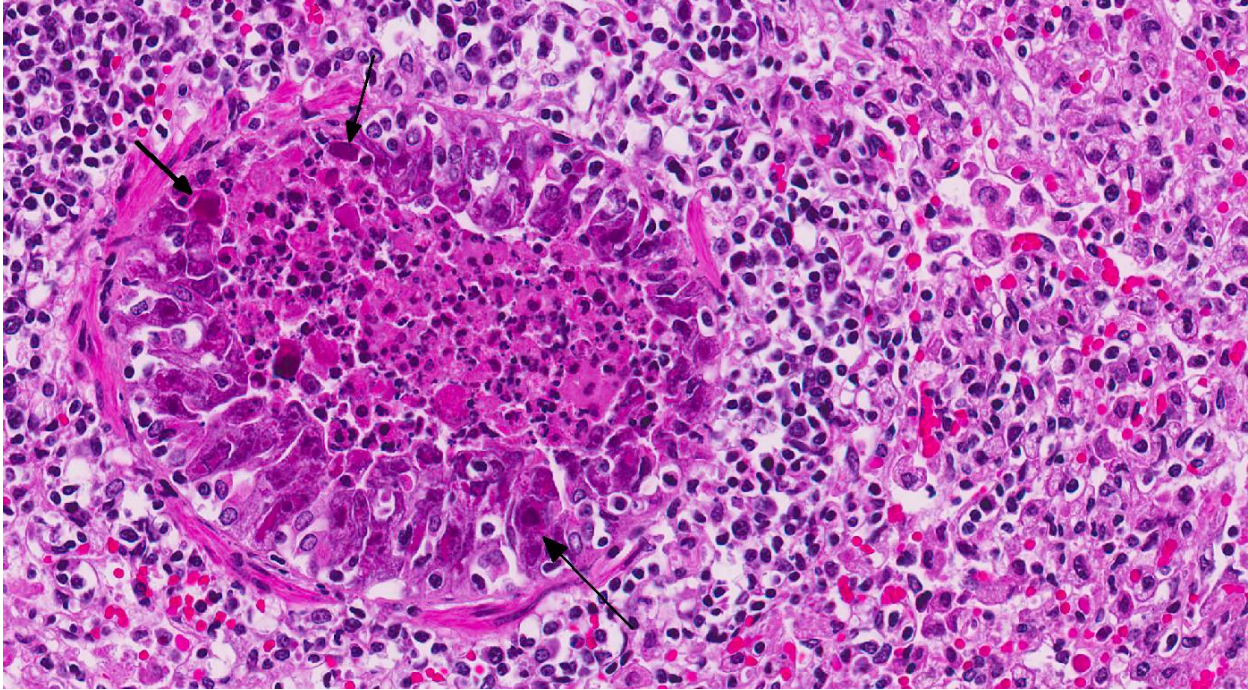
Lung, guinea pig. There is diffuse necrosis of airway epithelium. Necrotic epithelial cells are hypertrophic and nuclei are expanded by a deeply basophilic viral inclusion. (HE, 400X)

the vessels are distended and filled with numerous free erythrocytes (hyperemia). Multiple alveoli are collapsed (atelectasis).

The inclusions are typical for adenoviruses and the diagnosis was confirmed by subsequent transmission electron microscopy.

Contributor's Morphologic Diagnosis: Bronchitis and Bronchiolitis, diffuse, subacute, necro-suppurative, severe with epithelial amphophilic intranuclear inclusion bodies and mild acute bronchointerstitial pneumonia

Contributor's Comment: More than 100 antigenic types of adenoviruses have been identified that infect mammals and birds. In addition, there are 47 human adenovirus types are classified and five more candidate types are presently being studied.[2] The family of *Adenoviridae* is divided into four serological genera: *Mastadenovirus,* viruses

that infect only mammalian species (e.g. human adenovirus 2); *Aviadenovirus*, viruses that infect only birds (e.g. fowl adenovirus 1); *Atadenovirus* that includes viruses with a wider host range, including snakes, lizards, ducks, goose, chickens, possums, and ruminants; *Siadenovirus*, which includes frog adenovirus 1 and turkey adenovirus 3, as well as several others of raptors, budgerigars, and tortoises.[3]

Adenovirus virions are non-enveloped, hexagonal, and have an icosahedral symmetry, 70-90 nm in diameter, with one (genus *Mastadenovirus*) or two (genus *Aviadenovirus*) fiber projections from each vertex of the capsid.[2,3]

The genome consists of a single linear molecule of double-stranded DNA, 26 to 45 kbp in size with inverted terminal repeats. Infection may be productive, abortive, or latent. In productive infections, the viral genome is transcribed in the nucleus, mRNA

is translated in the cytoplasm, and virions self-assemble in the nucleus. In latent infections and in transformed and tumor cells, viral DNA is integrated into the host genome.[2,3] Intranuclear inclusion bodies are formed, which contain numerous virions in paracrystalline arrays.[2,4]

Adenoviruses have a narrow host range and many adenoviruses cause persistent infections. Reactivation of a latent infection can take place by immunosuppression, e.g. Equine adenovirus causes severe disease in immunocompromised hosts.[3]

In dogs two types of canine adenoviruses are well known, type 1 and 2. Type 1 (CAdV-1) causes infectious canine hepatitis, a potentially fatal disease involving vasculitis and hepatitis. Type 1 infection can also cause respiratory and eye infections. Canine adenovirus 2 (CAdV-2) is one of the potential causes of kennel cough. Core vaccines for dogs include attenuated live CAdV-2, which produces immunity to CAdV-1 and CAdV-2. CAdV-1 was initially used in a vaccine for dogs, but corneal edema was a common complication.[3]

In horses, adenoviral infections are usually asymptomatic or cause a mild upper respiratory disease. In Arabian foals with a primary severe combined immunodeficiency disease, equine adenovirus 1 can cause bronchopneumonia and generalized disease, involving pneumonia and destruction of pancreatic and salivary gland tissue.[3]

In ruminants, bovine adenovirus-3 causes mild respiratory disease. Occasionally calves suffer from a severe respiratory or enteric disease.[3]

In sheep, adenoviral infections occasionally cause cytomegalic bronchiolar and alveolar epithelial cells similar to cytomegalovirus.

Lambs can suffer from a severe respiratory or enteric disease.[3]

In non-human primates (NHPs) it can cause mild to moderately severe respiratory and enteric disease and keratitis/conjunctivitis.[3]

The avian adenoviral infections are divided into groups. Group I causes inclusion body hepatitis, respiratory and enteric infections in chickens, turkeys, and geese. Group II causes turkey hemorrhagic enteritis, marble spleen disease. And group III causes egg drop syndrome in chickens and ducks.[3]

In humans, an adenoviral infection causes a respiratory disease, pharyngitis, and follicular conjunctivitis.[3]

Some adenoviruses have shown to be oncogenic in laboratory animals. Cells from a number of rodent species and humans can be transformed in culture by adenoviruses. The frequency of malignant transformation is extremely low. Some adenoviruses, such as Ad2 and Ad5, have not been shown to be oncogenic in animals. Tumorigenic potential has been attributed to the capacity of some adenoviruses to turn off the expression of
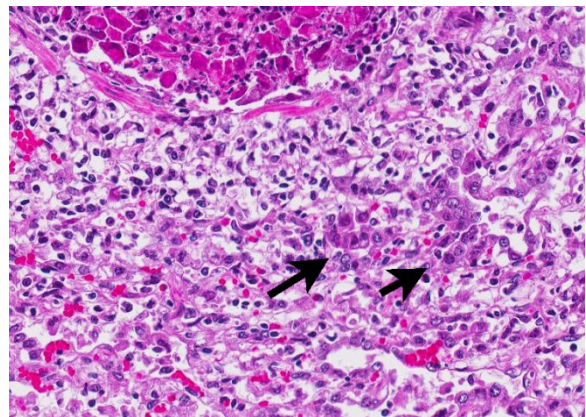
Lung, guinea pig. Alveolar septa in the adjacent parenchyma are expanded by fibrin and edema and often lined by type II epithelium. Alveolar spaces contain increased numbers of macrophages, as well as fibrin and edema.

genes of the major histocompatibility complex and thus to allow the transformed cells to overcome host defenses and grow into solid tumors. [2,5]

Viral pneumonia caused by adenovirus is one of the most common infections in breeding colonies and collections of guinea pigs. Guinea pigs are susceptible to a respiratory adenovirus that causes pulmonary disease and inclusions in respiratory epithelium. Guinea pig adenovirus (GPAdV-1) has been reported in Europe, North America, and Australia. The infection is characterized by a low morbidity, but drastically high (up to 100%) mortality rate.[1] Incubation time varies between 5 to 10 days. The animals die within 24 to 48 hours after showing the first clinical signs.[3] Clinical disease most often appears in young animals. Affected animals may show no clinical signs or can be severely dyspneic. The characteristic macroscopic lesions involve a consolidation of the cranial lobes of the lungs and hilus.[1,4]

A diagnosis may be confirmed by immunocytochemistry, serology, PCR, or the presence of adenoviral particles in affected cells by electron microscopy. Recently, a homotypic antigen has been developed using the GPAdV hexon gene incorporated into replication-defective adenovirus vector. This assay has proven to be more sensitive than the mouse adenovirus (MAV), which was used in the past for serological testing of GPAdV.[1]

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, necrosuppurative, multifocal, acute, severe with necrotizing bronchitis, type II pneumocyte hyperplasia and epithelial intranuclear inclusion bodies, Guinea pig, *Cavia porcellus*.
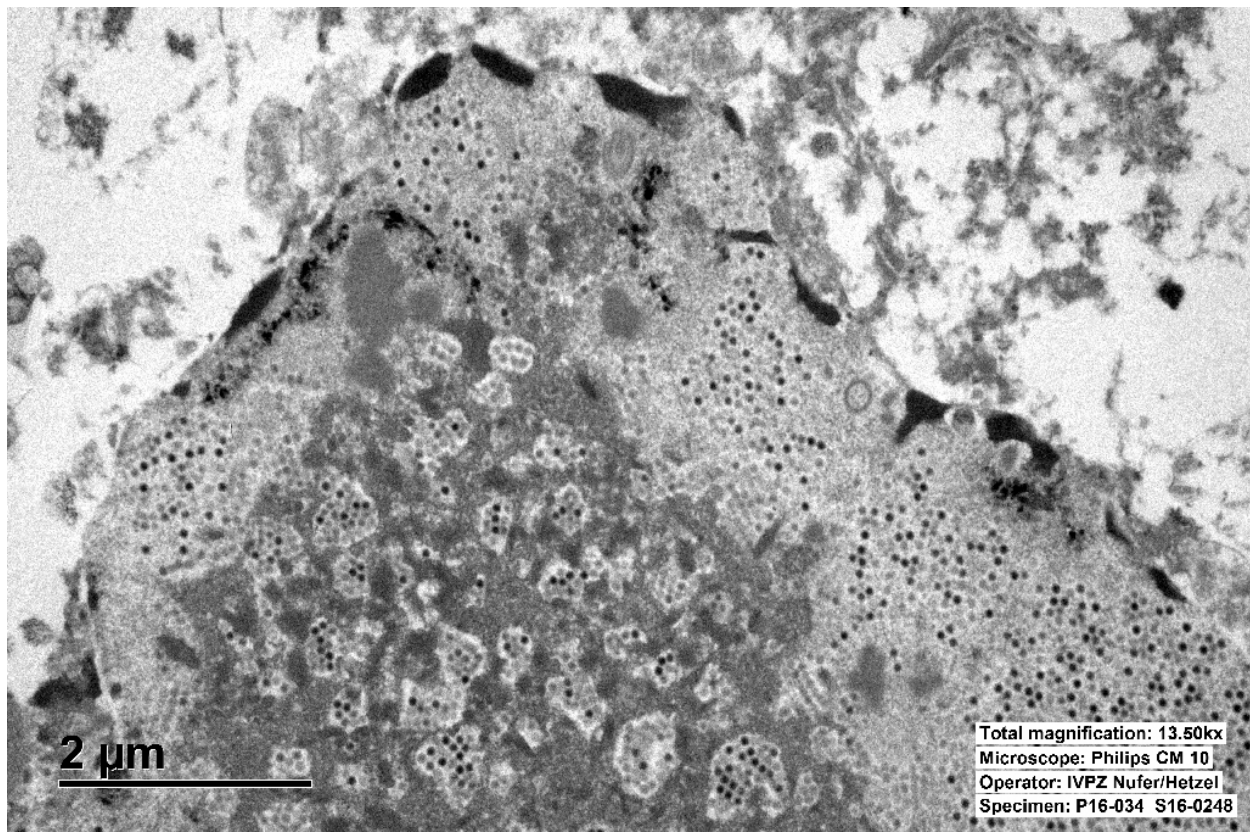
Lung, guinea pig. Electron micrograph of a bronchiolar epithelial cell nucleus showing crystalline arrays of hexagonal adenovirus virions. (Mag=13,500) (Photo courtesy of: Institute of Veterinary Pathology, Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich)

Conference Comment: The contributor provides a good summary of different adenoviruses in various affected species. As mentioned above, the most common adenoviral diseases of veterinary importance are the equine adenovirus-1 (inclusions within intestinal epithelial cells of SCID foals), canine adenovirus-1 (inclusions in hepatocytes and endothelial cells of puppies), canine adenovirus-2 (inclusions in alveolar macrophages of dogs with kennel cough), and adenoviral infection of rhesus macaques (inclusions in pancreatic acinar cells of non-human primates).[3] A novel adenovirus in domestic pigeons has recently been identified (pigeon adenovirus 2), however, it has not yet been implicated in causing clinical disease in this species.[6]

In this case, conference participants readily identified the prominent and highly characteristic intranuclear amphophilic inclusions within the exfoliating bronchial and bronchiolar epithelial cells, along with moderate to severe peribronchial leukocyte infiltration. Typically, adenoviruses are associated with prior immune suppression.[2,3] In Guinea pig adenoviral infection (GPAdV-1), the mortality rate in clinically affected animals is nearly 100%, with young and immunosuppressed animals being the most affected. Typical lesions have been observed in the airways of clinically normal animals, emphasizing the importance of immune suppression in this disease entity. Some participants posited that there may be a secondary bacterial infection that contributed to the pulmonary changes in this Guinea pig.[5] Viruses are known to damage the respiratory epithelium, which plays a role in the pathogenesis of bacterial pneumonia by inhibiting bacterial clearance and facilitating adhesion.[2,3,5] In this case, there is clear epithelial targeting of the bronchial and bronchiolar epithelium with
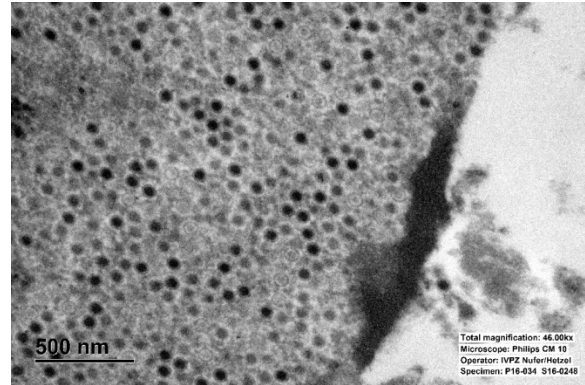
Lung, guinea pig. Hexagonal viral particles in lattice arrays are found in the nucleus and cytoplasm of bronchiolar epithelial cells. (Mag=46,000) (Photo courtesy of: Institute of Veterinary Pathology, Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich)

suppurative exudate filling airways and extending into the surrounding interstitium. However, no additional agents were detected by the contributor. Conference participants discussed the differentials for severe respiratory disease in Guinea pigs, including: parainfluenza virus, Guinea pig cytomegalovirus, and bacterial pneumonia of the lower respiratory tract, such as *Bordetella bronchiseptica*.[5] Definitive diagnosis is made by immunohisto-chemistry, PCR, or the demonstration of the adenovirus particles in affected cells by transmission electron microscopy (TEM).[5]

Ultrastructurally, adenoviral inclusions are composed of prominent intranuclear paracrystalline arrays of virions and unassembled viral capsid proteins, demonstrated nicely by the TEM image provided by the contributor. Adenovirus is not the only virus that will form hexagonal paracrystalline arrays in affected tissue. Conference participants discussed other viruses that form ultrastructural para-crystalline arrays. These include poly-omavirus, papillomavirus, picornavirus, iridovirus, and circovirus.[3]

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<u>**CASE IV:**</u> 15144 (JPC 4084218).

Signalment: Two-month-old male African green monkey (*Chlorocebus aethiops sabaeus*).

History: This animal was found dead in the enclosure on the morning of the day of necropsy.

Gross Pathology: Approximately 75% of the left and 50% of the right lung lobes were replaced by randomly scattered, multifocal to coalescing, umbilicated, 3x3x3 mm to 1.5x3x2cm, firm, off-white to tan nodules, surrounded by 1-5 mm wide dark red zones. The remainder of the left lung was dark red, heavy and wet, and the lateral aspect was adhered to the adjacent costal pleura. Neither side collapsed upon opening the thoracic cavity, and the left lung sank in formalin.

Laboratory results: Aerobic bacterial culture positive for *Yersinia enterocolitica.*

Histopathologic Description: Most of the architecture is effaced and replaced by fragmented eosinophilic cellular and karyorrhectic to streaming nuclear debris (necrosis), innumerable neutrophils, many degenerate, and large foamy macrophages. Colonies of small bacterial rods are prominent in areas of necrosis. The wall of the left bronchus is transmurally effaced by similar necrotic inflammatory exudate which extends into the lumen and also lies within
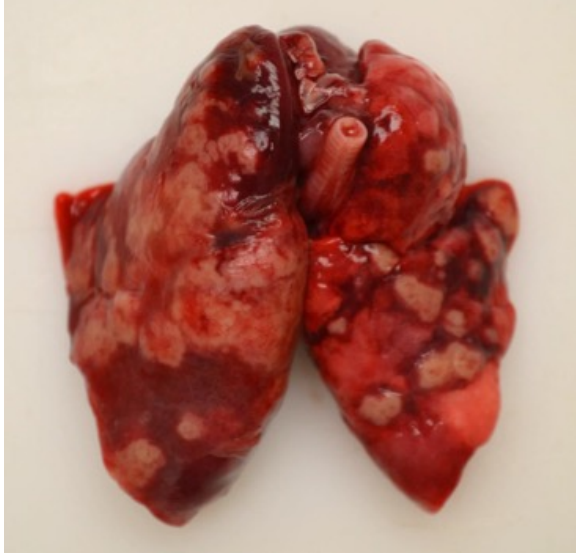
Lung, African green monkey. The pulmonary parenchyma is replaced by multifocal to coalescing, umbilicated, tan nodules. (Photo courtesy of: Wake Forest School of Medicine, Section on Comparative Medicine, Medical Center Boulevard \ Winston-Salem, NC 27157 www.wakehealth.edu)

adjacent bronchioles. The remaining alveolar spaces are filled with homogeneous proteinaceous material (edema fluid), frequent neutrophils and foamy alveolar macrophages. Connective tissue fibers surrounding the pulmonary vasculature are separated by clear space (edema).

Contributor's Morphologic Diagnosis: Pneumonia, multifocal, chronic, severe, necrotizing with bacilli (consistent with *Yersinia enterocolitica*).

Contributor's Comment: *Yersinia enterocolitica* is a short gram-negative bacillus within the family *Entero-bacteriaceae*. It is an important food-borne pathogen that is commonly found in the gastrointestinal tract of humans and other species. Pigs and rodents are sources of human infection, and transmission occurs

through contaminated food and water. This pathogen most often produces necrotizing gastrointestinal lesions with large colonies of bacteria in humans and animals, but can also affect the urinary, respiratory, musculo-skeletal, integumentary and cardiovascular systems in humans.[1,7]

Yersinia enterocolitica is classified into 6 biotypes and 57O group strains. Among the 6 biotypes, all except the 1A biotype (1B and 2-5) are pathogenic. 1B/O:8, 2/O:5, 2/O:9, 3/O:3 and 4/O:3 are most commonly isolated from humans. 1B/O:8 is considered the "new world" strain and is highly pathogenic to humans and predominantly present in the United States, while 4/O:3 and 2/O:9 are found in the Europe and Asia. Strain 4/O:3 has frequently been isolated from pigs and 1B/O:8 has been isolated from rodents. [1,3,7]

Pathogenic strains of *Y. enterocolitica*
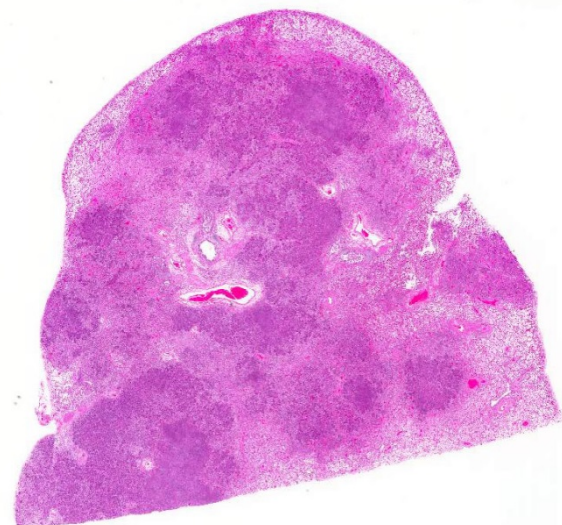
Lung, African green monkey. Approximately 80% of the pulmonary parenchyma is effaced by coalescing areas of lytic necrosis. (HE, 4X)

contain the virulence plasmid pYV/pCD which encodes *Yersinia* adhesin A (yadA), Ysc-Yop type III secretion system (TTSS), chromosomally encoded virulence genes ail, myfA, ystA, ysa, and the high pathogenicity island (HPI-) that aids in iron acquisition. The expression of virulence genes depends on temperature and calcium availability *in-vivo*. Virulence proteins produced by PYV plasmid resist phagocytic killing and complement mediated lysis of the bacteria by the host.

Successful infection begins with colonization of the distal small intestinal mucosa. Attachment and entry into the intestinal epithelial cells are facilitated by yadA and *INV,* respectively. Upon entering the epithelial cells the bacteria preferentially enter M cells in the Peyer's patches where macrophages internalize them and transport them to the mesenteric lymph nodes (MLN).
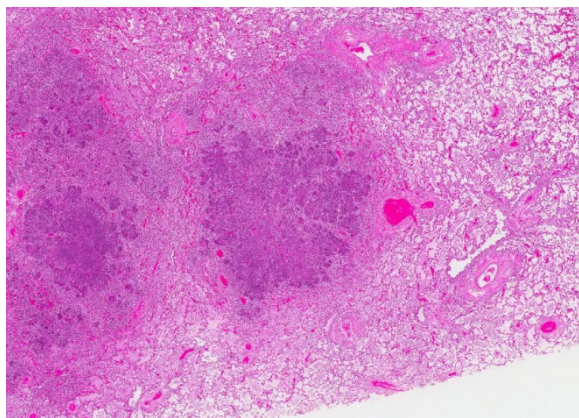
Lung. African green monkey. Higher magnification of areas of lytic necrosis and surrounding parenchyma.

Yersinia bacteria multiply within the macrophages and also extracellularly in the lymphoid organs leading to abscess

formation, often bacteremia and frequently, hepatitis. [1,3,7]

This case was the first in an outbreak involving more than 20 *Y. enterocolitica* fatalities in 2015 – 2016, and was unique in that the pneumonia occurred in the absence of intestinal and MLN lesions. The finding of necrotizing tracheitis and large airway orientation in the lung suggests a respiratory route of infection. Some reports in humans have also suggested primary infection by inhalation, without gastrointestinal involvement. [2,9]

JPC Diagnosis: Lung: Pneumonia, necrosuppurative, multifocal to coalescing, subacute, severe, with fibrin thrombi, hemorrhage, edema, and numerous colonies of coccobacilli, African green monkey, *Chlorocebus aethiops sabaeus*.

Conference Comment: The contributor provides a rare case of pneumonia caused by *Yersinia enterocolitica* in an African green monkey, as well as an excellent discussion of the virulence factors and pathogenesis of the bacterium. Despite the highly usual presentation, this case illustrates the characteristic appearance of the gram-negative coccobacilli forming multifocal to coalescing bacterial microcolonies surrounded by intense infiltrates of viable and degenerate neutrophils and necrotic cellular debris filling and replacing pulmonary architecture. *Yersinia* sp. Micro-colonies are readily distinguishable from other large colony-forming bacteria such as *Actinomyces, Actinobacillus, Coryne-*

bacterium, Staphylococcus and *Streptococcus* spp.[9] Other species of *Yersinia (pseudotuberculosis* and *pestis)* have the same histologic appearance in tissue section as is present in this case.
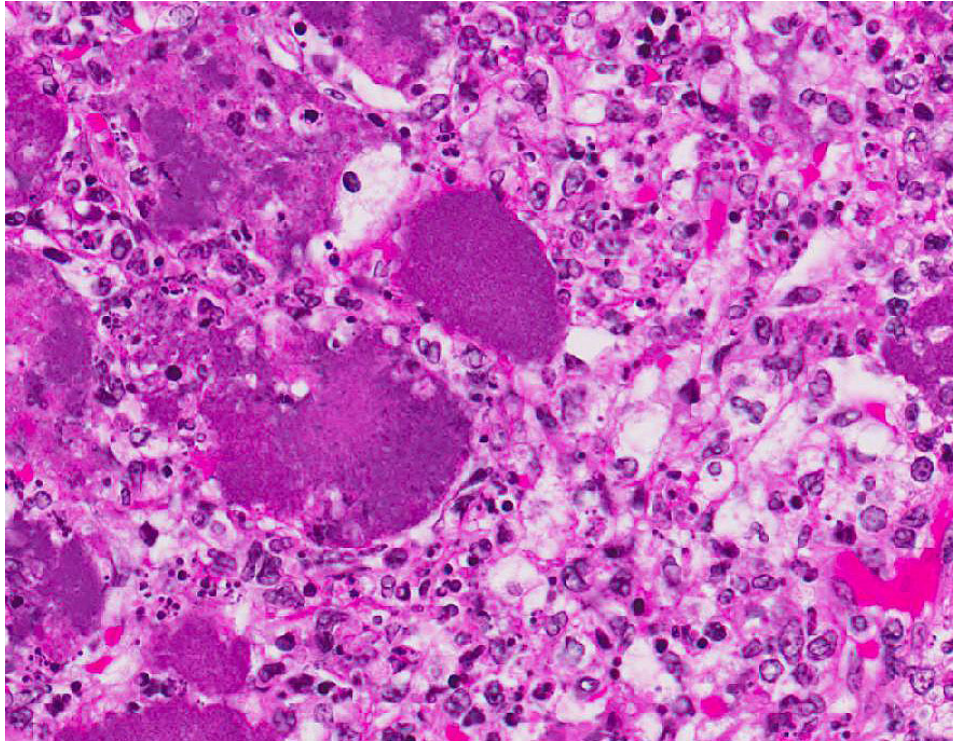
Lung, African green monkey. Areas of lytic necrosis contain large colonies of coccobacilli admixed with numerous degenerate neutrophils admixed with abundant cellular debris. (HE, 400X)

Speciation of the bacteria requires culture or bacterial isolation.[1,9]

Yersinia enterocolitica has a worldwide distribution and affects a many different animal species and humans.[1,2,7,9] As mentioned by the contributor, the most common mode of transmission is caused by foodborne outbreaks in humans. In nonhuman primates, infections are typically acquired by the fecal-oral route.[8,9] The organism can be shed in the feces by asymptomatic animals in the collection, as well as by rodents and birds in the environment where it can survive and grow at low temperatures. Disease is often secondary to stress, poor nutrition, environmental flooding, and cold weather.[9] The contributor suggests that the route of infection for this animal is via inhalation of the organism, perhaps via aerosolized feces, causing the development of necrotizing tracheitis and pneumonia without gastrointestinal lesions.

Many conference participants noted the presence of numerous large fibrin thrombi within peribronchial veins and lymphatic vessels. Gram-negative bacteria, such as *Yersinia* spp, commonly cause sepsis and its associated coagulopathy. During gram-negative septic shock, lipopolysaccharide (LPS, endotoxin) induces increased tissue factor (TF) and factor XII expression.[4,5,6] TF (factor III) then forms a complex with plasma factor VII as part of the extrinsic coagulation pathway. This complex activates factor X of the common pathway and factor IX of the intrinsic pathway, leading to the formation of thrombin (factor II) which generates fibrin from fibrinogen (factor I). Fibrin is cross-linked and polymerized via factor XIII
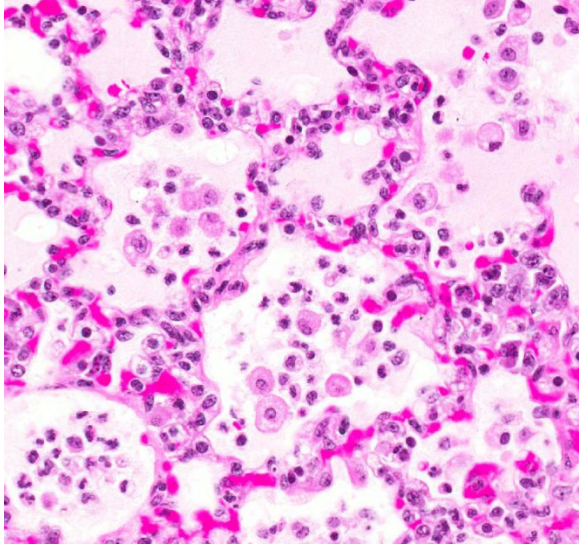
Lung, African green monkey. Alveoli within the remaining lung contains abundant edema and moderate numbers of macrophages and neutrophils. Alveolar septa are expanded by edema, and circulating neutrophils and macrophages. (HE, 400X)

and deposited as a fibrin clot. During sepsis, procoagulant activity predominates over anticoagulation and fibrinolysis due to inhibition of tissue factor pathway inhibitor (TFPI), thrombomodulin, and protein C as well as activation of plasminogen activator inhibitor (PAI-1), and previously mentioned TF.[5,6] This leads to disseminated intravascular coagulation (DIC) and exuberant fibrin deposition. DIC eventually consumes critical blood-clotting factors leading to hemorrhage and shock.[5,6] However, a recent study in mice has shown that fibrin can also perform critical protective functions during infection with *Yersinia enterocolitica*. These studies suggest that fibrin may be an attempt by the host to physically trap bacteria limiting dissemination, in addition to facilitating the recruitment and activation of neutrophils and macrophages within infected tissues via interleukin 6 (IL-6) and monocyte chemotactic protein-1 (MCP-1).[5] In fact,

mice treated with the anticoagulant warfarin prior to inoculation with *Yersinia enterocolitica* had a markedly higher systemic bacterial burden and shortened survival time compared to untreated mice.[5]

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