**Joint Pathology Center**

**Veterinary Pathology Services**

## WEDNESDAY SLIDE CONFERENCE 2016-2017

# C o n f e r e n c e 6

**5 October 2016**

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**CASE I:** 2016A (JPC 4084736).

**Signalment:** 20-day-old domestic duck (*Anas platyrhynchos*).

**History:** The outbreak site was a duck farm with 4000 ducks in Japan. In January 2014, an increased mortality in the 14- to 21-day-old duck flock was reported to veterinary officials by the owner. The mortality rate was 16%, i.e. 316 out of 2020 birds. Clinical signs of some ducks included reduced movement, ataxia, and dorsal recumbency with leg paddling.

**Gross Pathology**: The surface of the heart and liver was covered by white fibrinous exudate.

**Laboratory results:** Bacteria were isolated from the brain, heart, liver, and spleen. Total DNA was extracted from the bacterial colonies, and PCR was performed for bacterial 16S ribosomal RNA. The genetic sequence of the PCR product revealed that isolates were 99.9% identical to *Riemerella anatipestifer* (RA) type strain.

**Histopathologic Description:** The epicardium is thickened due to the edema, hemorrhage, and abundant fibrinous exudate surrounded by heterophils, macrophages, and multinucleated giant cells. Heterophilic and fibrinous serositis are also observed in the liver, air sac, and intestine. Gram-negative stained bacterial colonies are rarely found in the fibrinous exudates in the body cavity.

**Contributor's Morphologic Diagnosis:** Heterophilic, granulomatous and fibrinous pericarditis with rare gram-negative bacterial rods**.**

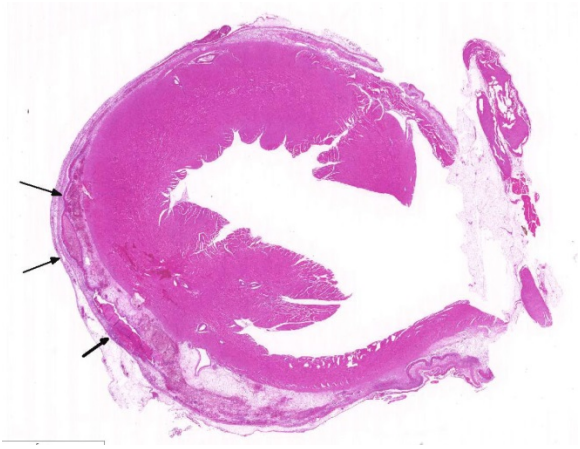
**Contributor's Comment**: *Riemerella anatipestifer* (RA) is a non-spore forming, non-motile, gram-negative rod.[14] *Riemerella anatipestifer* infection is a bacterial disease that primarily affects domestic ducks.[10] Young ducklings are more susceptible to the disease than adults.[10,14] *Riemerella anatipestifer* infection occurs as an acute or chronic form, characterized by fibrinous pericarditis, perihepatitis, air sacculitis, and meningitis.[10,14] *Riemerella anatipestifer* transmission can occur horizontally via the

***Pericardium, duck. The pericardium is diffusely covered with a whitish fibrinous exudate. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)***

respiratory tract and skin wounds,[10] whereas vertical transmission of RA via eggs is controversial. [7,8,11] Interestingly, some studies document that RA can be a part of the normal flora in the throat of some domestic and wild duck species.[3,15] At least 21 serotypes have been reported for RA, although there is some confusion regarding its classification.[12,13,14] Serotyping RA can be useful for epidemiological analyses and a vaccination strategy.[12,13]

Characteristic pathological findings, such as pericarditis and perihepatitis observed in the present case, are highly suggestive of RA infection. However, a definitive diagnosis of RA infection requires isolation and identification of RA from ducks suspected to be infected.[9,10,14] There are difficulties associated with the identification of RA.[9,10] *Riemerella anatipestifer* is characterized by the absence of species-specific biochemical properties.[5] Genetic sequencing of bacterial 16S ribosomal RNA and matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry are currently considered useful for the identification of RA.[4,8,13,16]

Other bacterial infections such as colibacillosis may cause gross lesions similar to those seen in RA-infected ducks.[6] The differential diagnosis also include salmonellosis, pasteurellosis, strept-ococcosis, and *Coenonia anatina* infection.[14]



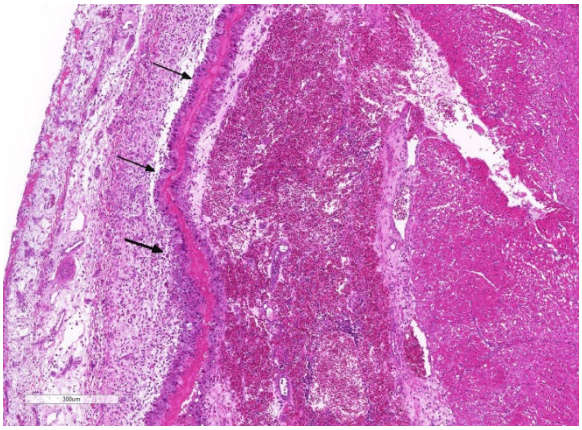
***Heart, duck. The epicardium is diffuse, expanded by linear granulomas (arrows), hemorrhage, fibrin and edema. (HE, 10X)***

**JPC Diagnosis:** Heart: Epicarditis, heterophilic and granulomatous, diffuse, severe with mild multifocal subepicardial necrotizing myocarditis, domestic duck, *Anas platyrhynchos*.

**Conference Comment:** *Riemerlla anatipestifer* (RA), also known as new duck disease or duck septicemia, is the causative agent of epizootic infectious polyserositis, a major problem of domestic ducks worldwide. It is also reported to be pathogenic for turkeys, chickens, pheasants, geese, and other waterfowl.[11] In geese, RA has been called goose influenza or septicemia anserum exsudativa.[5] This agent causes major economic loss in the duck industry due to high mortality rates of up to 75%, weight loss, and condemnations in ducklings under eight weeks old. Stress from adverse environmental conditions is the main predisposing factor for this disease.[4,10]

As mentioned by the contributor, fibrinous pericarditis and epicarditis are the most characteristic lesions of RA. This case represents the chronic form of the disease, with progression from fibrinous to granulomatous epicarditis with abundant granulation tissue on the epicardial surface. The pericardium was likely removed prior to tissue processing and is not present in the microscopic sections submitted for evaluation.

Many conference participants had little experience with this entity histologically, which led to a discussion of the differential diagnosis for epicarditis and polyserositis in avian species. While not a typical presentation for *Pasteurella multocida*, fowl cholera can cause similar lesions in poultry, chickens, and waterfowl.[4] *Salmonella pullorum* causes peritonitis and death in hatchling chicks; and peritonitis, arthritis,

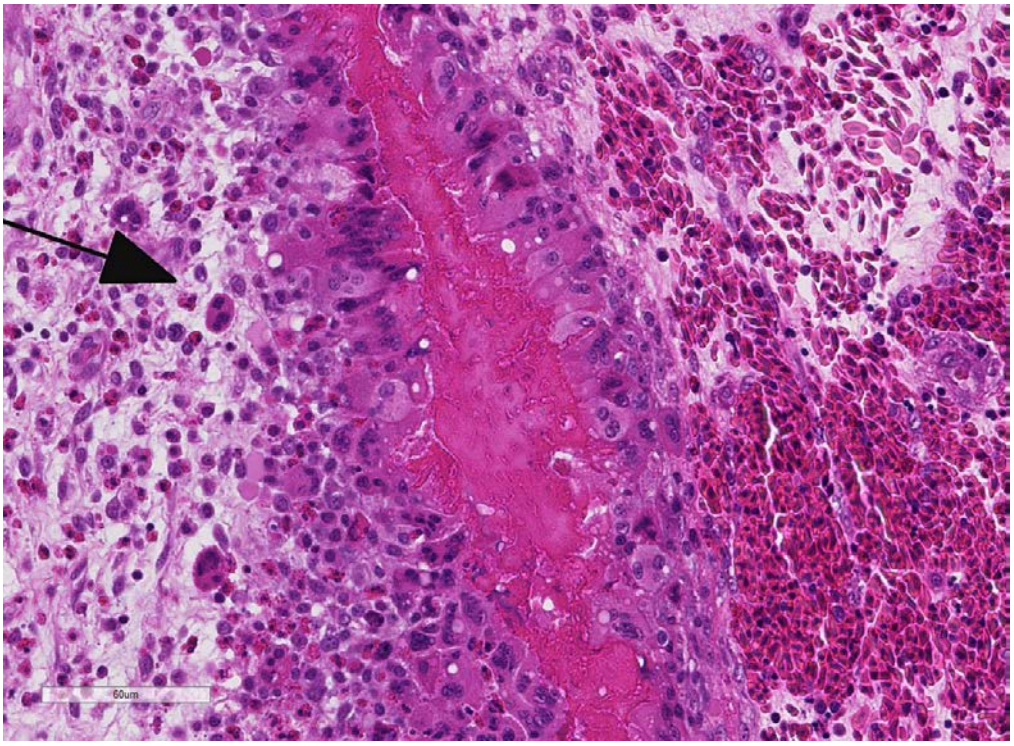


***Heart, duck. Higher magnification of the epicardium with a linear granuloma surrounding brightly eosinophilic material. There is extensive edema, heterophilic inflammation, and hemorrhage of the epicardium. (HE, 40X)***

and pericarditis in adults, and is often characterized by large hetrophilic granulomas in the myocardium. *Escherichia coli* and other coliform infections also can lead to fibrinous and heterophilic rmyocarditis, and should be considered as a differential in most avian species.[4] *Coenonia anatine*, described by the contributor and briefly discussed during conference, also causes exudative serositis in ducks and geese.[12] *Riemerella columbina* causes similar disease as RA in pigeons.[12] While not mentioned during conference, West Nile Virus, an arbovirus of the family *Flaviviridae*, should also be considered as a

differential for necrotizing myocarditis of many avian species, with young chickens and geese being most likely to develop clinical disease and mortality.[1]

Participants also discussed the three main rule-outs for polyserositis, pneumonia, and polyarthritis in pigs; among these include *Haemophilus parasuis*, *Mycoplasma hyorhinis*, and *Streptococcus suis*.[5] In horses, the most likely etiologies for polyserositis are *Streptococcus equi* and *Streptococcus zooepidemicus*.[2]




***Heart, duck. Higher magnification of the linear granuloma (arrow) with epithelioid and foreign body macrophages centered on a dense eosinophilic aggregate of fibrin and heterophilic debris. Numerous macrophages and heterophils are present in the surrounding tissue. (HE, 232X)***

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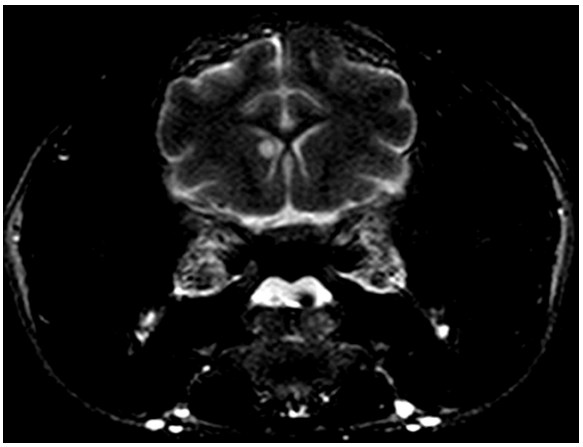
**CASE II:** 49587 (JPC 4084567).

**Signalment:** 8-year-old ovario-hysterectomized Siamese mix cat (*Felis catus*).

**History:** Two-week history of cluster seizures. No other previous medical history. Upon presentation, the patient was hypo-thermic (96 F), with a pulse of 120. She was exhibiting open-mouthed breathing with a respiratory rate of 45. Due to the anticipated duration of intensive care, euthanasia was elected. A postmortem MRI was performed, which revealed a focal lesion in the left caudate nucleus. Differential diagnoses included infectious causes, a vascular event, and neoplasia.

**Gross Pathology**: Central Nervous System: The distal aspect of the cerebellar vermis



***Cerebrum, cat. Results of a postmortem MRI. There is a focal lesion in the left caudate nucleus. (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org)***

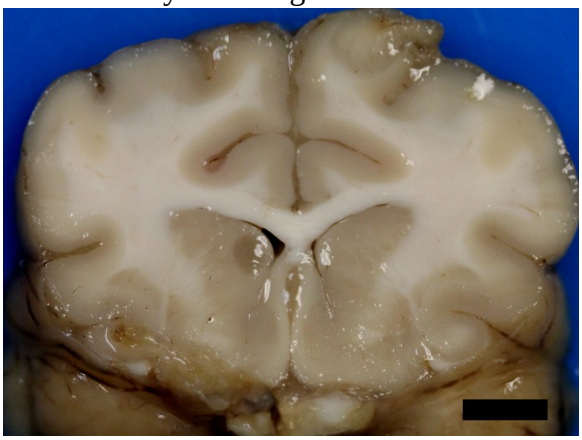
can be observed from the foramen magnum. Upon removal of the brain, the vermis is flattened over the brainstem (cerebellar coning). The brain is fixed whole and sectioned, which reveals diffuse, mild expansion of the leptomeninges and widening of sulci by yellow to brown, gelatinous material and a focal, 0.5 cm diameter, gelatinous mass at the left caudate nucleus.

Pulmonary: The lungs are mottled tan to medium red. Multiple nodules are present, and the tracheobronchial lymph nodes are markedly enlarged. The right cranial lung lobe has a large, firm to soft, smooth, tan to white mass that measures 2.5 x 2.5 x 1 cm. Within the left cranial lung lobe, at the junction between the cranial and caudal subsets, there is a focal, firm, 2 x 2 x 1 cm nodule. Smaller nodules are present at the distal aspect of the left cranial lung lobe that measure up to 1 cm in diameter. A large nodule is present with the accessory lobe that measures 2.5 x 1.3 by 1 cm.

Hemolymphatic: The mediastinal and tracheobronchial lymph nodes are markedly enlarged. All are soft to firm and pale tan to white. The largest of the mediastinal lymph nodes measures 1.7 x 2 x 1 cm and the tracheobronchial lymph nodes measure up to 1.5 x 1.0 x 1.0 cm.

Internal: In the omentum adjacent to the pancreas, there is a soft, smooth, tan to white mass that measures 1.6 x 1.6 x 1.3 cm. The regional omentum is hyperemic and thickened.

**Laboratory results:** Postmortem cytology of lung nodules, lymph nodes, omental nodule: Impression smears are similar and consist of mixed inflammatory cell populations including foamy macrophages, lymphocytes, and plasma cells. Inflammatory cells are intermixed with numerous yeast organisms which are

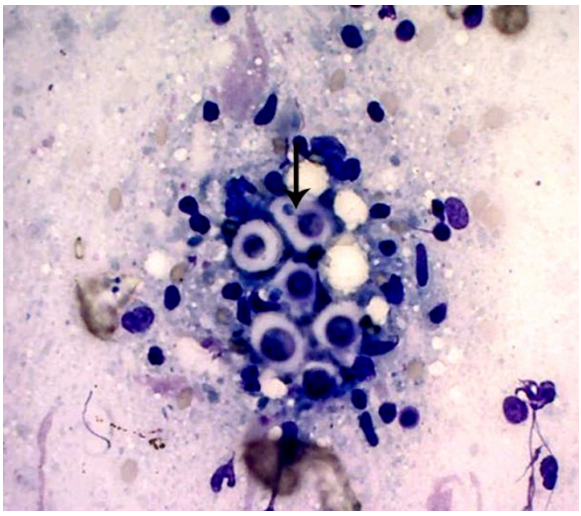


***Cerebrum, cat. A well-demarcated lesion is present in the caudate nucleus, and the leptomeninges are multifocally expanded by a gelatinous brown material. (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org)***

surrounded by a large, clear capsule, and occasionally exhibit narrow based budding (*Cryptococcus* spp).

**Histopathologic Description:** A section of the cerebral cortex at the level of the caudate nucleus or thalamus is examined. Diffusely, the leptomeninges are expanded by coalescing sheets of yeasts, interspersed by multifocal inflammatory populations. Sulcal widening is prominent. Yeasts are round to oval, approximately 5-10 um in diameter, with a thin, 1 um wall and are surrounded by

a large, 10-20 um diameter clear capsule. Occasional narrow-based budding is observed. Inflammatory populations interspersed by the yeasts are comprised predominantly of lymphocytes, plasma cells, and macrophages. The yeasts multifocally extend into the regional cerebral cortical parenchyma which exhibits white matter vacuolation and gliosis. In some of the sections, there is a focal nodule comprised of yeasts at the level of the caudate nucleus. Multifocally, scattered lymphocytes and plasma cells are present within and surrounding the nodule, both within Virchow-Robin spaces and less frequently, the regional parenchyma with mild accompanying gliosis. Occasional yeasts are observed within the lateral ventricles or Virchow Robin spaces in some slides. Throughout the cerebral cortex, there are increased numbers of branched, small caliber arterioles (edema) with increased numbers of glial cells, as well as mild vacuolation and gliosis of the centrum semiovale. Small numbers of vessels throughout the sections contain perivascular



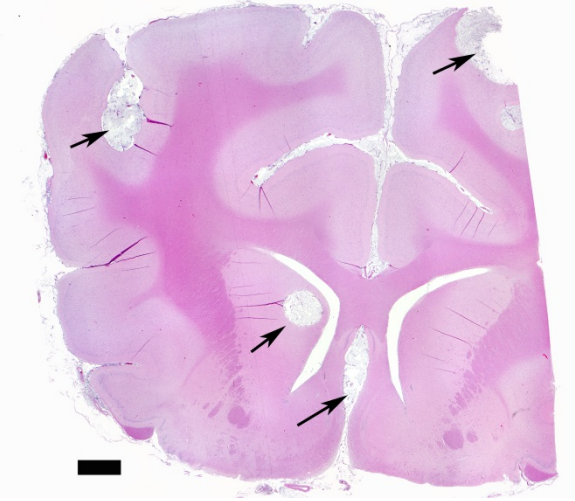
***Impression smear, cat. Impression smears of nodules from the lung, lymph nodes, and omentum revealed numerous yeasts with a clear capsule and narrow-based budding (arrow), admixed with macrophages, neutrophils, and cellular debris. (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org)***

aggregates of Gitter cells with intracytoplasmic brown pigment (lipofuscin). Neurons throughout the sample also contain intracytoplasmic brown pigment (lipofuscin).

Special stains: Gomori methenamine silver (GMS) and mucicarmine reveal positive staining of yeasts and highlights narrow based budding. The capsule is light pink with the mucicarmine stain.

**Contributor's Morphologic Diagnosis:** Brain: Meningoencephalitis, lymphoplasmacytic, histiocytic, multifocal to coalescing, severe with myriad encapsulated yeasts (etiology consistent with *Cryptococcus* spp), rare parenchymal in-filtration with regional spongiosis and gliosis; cerebral edema, diffuse, mild.

**Contributor's Comment**: The gross, cytologic and histologic findings are consistent with disseminated *Cryptococcus* spp. infection of the respiratory tract and central nervous system (CNS). Cryptococcosis is the most common systemic mycosis of cats,[3,5] infection of which is thought to be acquired from the environment.[5] Infection is not contagious or zoonotic, as is typical for the systemic mycoses.[3] Cryptococcosis in dogs and cats is primarily caused by two encapsulated, dimorphic, basidiomycetous fungi, *Cryptococcus neoformans* and *Cryptococcus gattii*.[5,7] The yeast phase is found under routine laboratory conditions and within mammalian host tissues.[5] Infection can occur from inhalation of the basidiospore stage or desiccated yeasts from the environment.[1] The infectious form can be found in soil, pigeon or other avian guano, and decaying organic matter.[3,5] The initial site of infection is hypothesized to be the nasal cavity, lungs, and/or gastrointestinal tract.[4,7] The skin, lymph nodes, CNS, and eyes are frequently affected.[7] Additional



***Cerebrum, cat. The meninges are multifocally expanded by aggregates of yeasts (arrows) and fewer inflammatory cells. There is also a focal circular lesion in the left caudate nucleus. (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org) (HE, 4X)***

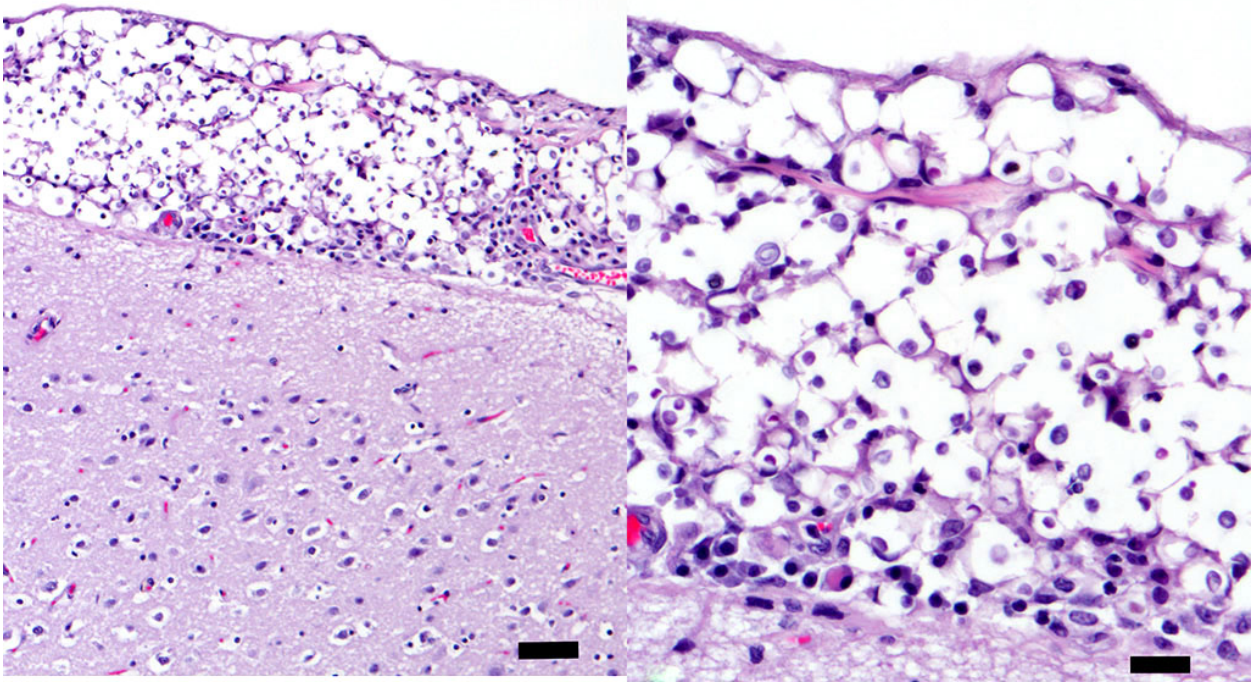
testing for species identification was not performed in this case.

Pathogenesis is dependent upon the amount of organism present upon exposure, virulence of the strain and immune status of the host. *Cryptococcus* has been called "a sugar-coated killer with designer genes",[5] due to its virulence factors.[5] The four main virulence factors include the ability to grow at 37 degrees C, its polysaccharide capsule, melanin production and secretion of degradative enzymes.[2,5] The polysaccharide capsule provides protection from the environment and host,[5] is comprised of negatively charged glucuronoxylomannan and enlarges after infection of the host.[5] The capsule is chemotactic for neutrophils, however, inhibits phagocytosis via its negative charge and blocks the antibody Fc receptor from communicating with host phagocytes. It interferes with leukocyte migration, can deplete complement and inhibits T cell responses. Resolution of infection in immunocompetent animals requires a T-helper cell 1 (Th1) pattern of cytokine and lymphocyte-mediated adaptive immune response.[2,4] Tumor necrosis factor, IL-12, IL-18, GM-CSF and IF-γ among others play a role in host defense.[2] Humoral factors are thought to aid in clearance via antibodies and complement.[2] Melanin or a melanin-like compounds are produced from diphenolic compounds via the enzyme laccase (phenoloxidase)[2,5] which help protect the organism from oxidative damage[7] and may modulate the host immunoinflammatory response.[3] Interestingly, rare *Cryptococcus* spp without a capsule can be easily phagocytized and incite a strong granulomatous response.[3]

Yeasts were more prominent than inflammation in the majority of the sample. *Cryptococcus* infection in cats has been shown to have relatively less inflammation than infections in dogs, which may reflect differences in infecting strains and/or underlying unrecognized defects in the immune or inflammatory response.[6-8] The cat, in this case, had no significant previous medical history, however, FIV/FeLv status was unknown. In one Australian study, Siamese, Birman and Ragdoll cats were found to be predisposed to *Cryptococcus* infection.[5] This cat was described as a Siamese mix.

After infection of the respiratory tract, fungal organisms are thought to spread hematogenously via macrophages, frequently to the CNS.[5] Extension of nasal cavity infection across the cribriform plate, frontal sinus or along cranial nerves into the brain may also occur.[5,7,8] Histologic evaluation of samples from the nasal cavity did not reveal any fungal organisms, and the frontal sinus was grossly normal. Ocular abnormalities can occur in up to one-third of affected cats,[5] however, no histologic evidence of infection of the eye or optic nerves was present in this case. Neurologic signs vary depending upon the location of

the lesions. Obtundation, behavioral changes, hyperesthesia, tremors, seizures, circling, head pressing, ataxia, paresis, head tilt, vestibular signs, and blindness are common clinical signs.[5] The cat, in this case, exhibited obtundation and cluster seizures. Meningeal involvement is common, as in this case, and is considered a predilection site.[6] Grossly, the leptomeninges may be unremarkable, or cloudy to thickened, with gelatinous mucoid material and sulcal widening.



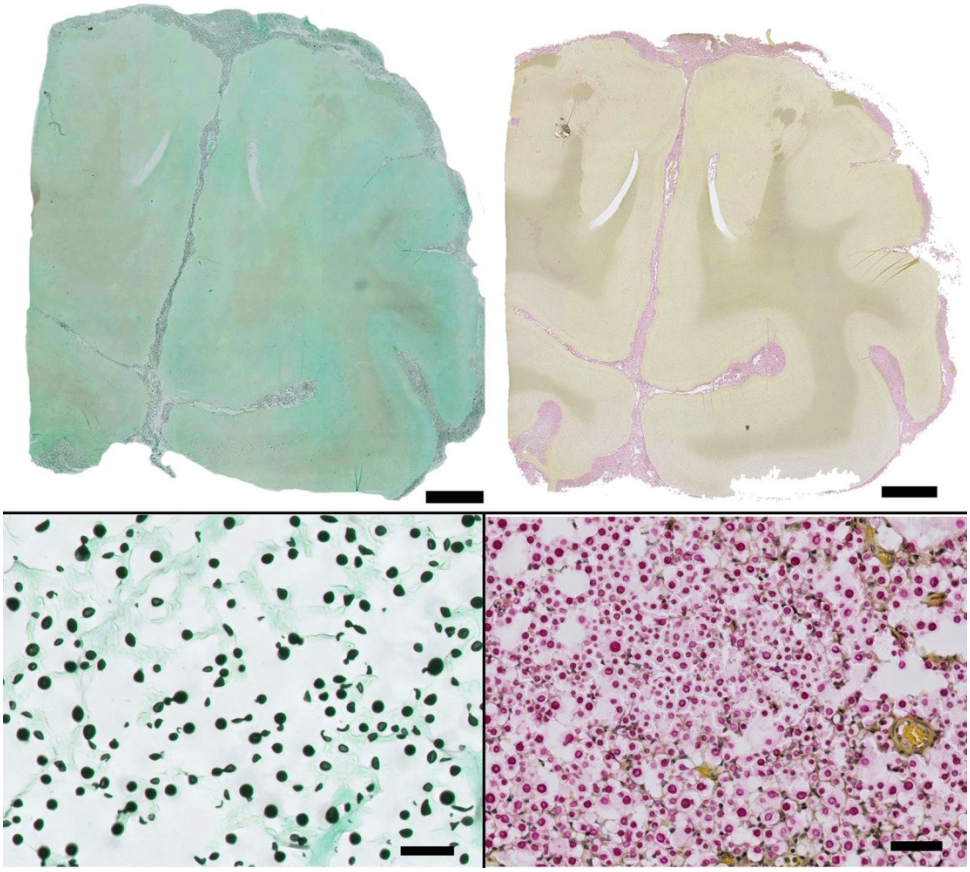
***Cerebrum, cat. There is marked expansion of the leptomeninges with numerous 3-6um budding yeasts with a prominent clear capsule. In the 400X image at left, there low numbers of plasma cells at the periphery, attesting to the chronicity of the lesion. (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org) (HE, 10X and 40X)***

Histologically, tightly packed yeasts give a "soap bubble" appearance.[3,6] The leukocytic response can consist of neutrophils, macrophages, multinucleate giant cells, lymphocytes, plasma cells and eosinophils, which is dependent upon host immune status.[8] In humans, CNS involvement manifests as meningitis, meningo-encephalitis, or Cryptococcomas, which are tumor-like intraparenchymal masses containing yeasts and inflammatory cells,[7] similar to the focal nodule observed with imaging and evaluation of the gross specimen in this case. Gelatinous pseudo-cysts are cystic extensions of Virchow-Robin spaces with collections of yeasts, and can also be detected with imaging modalities.[7] One study of cats and dogs with CNS *Cryptococcus* infection identified three histopathologic patterns: 1) Pseudocyst formation with expansion of cryptococcal organisms along Virchow-Robin spaces with multifocal intraparenchymal pseudocysts, 2) Diffuse meningitis only without pseudocysts or parenchymal involvement and 3) meningoencephalitis without pseudocyst formation.[7] These patterns were not found to correlate with the type of *Cryptococcus* sp. or treatment.[7]

**JPC Diagnosis:** Brain, cerebrum: Meningitis, lymphoplasmacytic and histiocytic, diffuse, moderate, with numerous narrow based budding and encapsulated yeasts, etiology consistent with

*Cryptococcus* sp., Siamese cat mix, *Felis catus*.

**Conference Comment:** The contributor provides an outstanding summary of the epidemiology, pathogenesis, clinical signs, gross and histologic patterns associated with disseminated cryptococcosis in dogs and cats. Cryptococcosis is a fungal disease with worldwide distribution and is the most common systemic mycotic disease in cats.



***Cerebrum, cat. Gomori methenamine silver (GMS) and Mucicarmine reveal positive staining of yeasts and highlights narrow based budding. The capsule is light pink with the Mucicarmine stain (Photo courtesy of the Animal Medical Center, 510 East 62nd St. New York, NY 10065 www.amcny.org) (HE, 4X)***

Dogs, horses, cattle, and humans are also affected by this dimorphic, basidiomycete, yeast-like fungi.[1,3,8,9] The diagnosis is typically based on identifying the organism and its characteristic thick capsule on histologic sections or cytologic preparations. *Cryptococcus* sp. is the only pathogenic mycotic organism with a thick capsule making the histopathologic diagnosis relatively straightforward; especially when there is a myriad of organisms, as in this case.[2] The thick polysaccharide capsule gives lesions a gelatinous appearance seen in the gross photograph and noted by the contributor. Distention of the leptomeninges by the organism is commonly observed in cats and occurs through a repetitive process of macrophage phagocytosis, cell lysis, and subsequent chemotaxis of additional macrophages allowing an expansive accumulation of the polysaccharide capsule.[3,8] Participants also identified multifocal narrow based budding in their sections further differentiating this fungus from *Blastomyces* sp, which have characteristic broad-based budding.[3]

The contributor noted that the distal aspect of the cerebellar vermis could be observed from the foramen magnum and upon removal of the brain, the vermis was flattened over the brainstem interpreted as cerebellar coning indicating cerebellar herniation. In humans, prognostic indicators for cryptococcosis include abnormal mental status, history of seizures, high antigen titers within serum and cerebral spinal fluid (CSF), poor host inflammatory response,

and high CSF pressure.[7] This animal likely had increased intracranial pressure secondary to infection leading to herniation of the cerebellum, altered mental state, and seizure activity. Increased intracranial pressure secondary to *Cryptococcus* sp. infection of the central nervous system is a negative prognostic indicator in humans and animals.[3,7]

Conference participants discussed different histochemical stains to highlight the thick capsule of this organism. The capsule can by highlighted by mucicarmine and in wet mounts, it stains with India ink. In addition to the periodic acid-Schiff and Grocott's methenamine silver stain run by the contributor, the yeasts also stain with Fontana-Masson stains due to their production of melanin via a virulence factor, laccase. A positive culture is required for definitive diagnosis.[3] Participants also noted the relative lack of inflammation within the neuropil of this animal. As mentioned by the contributor, in contrast to other mycotic infections, such as *Blastomyces* sp, *Coccidioides* sp, and *Histoplasma* sp, the host inflammatory reaction is often quite minimal, likely due the polysaccharide capsular component glucuronoxylomannan, preventing yeast recognition by phagocytes, induction of IL-10, and disruption of dendritic cell activation and maturation.[2,5]

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**CASE III:** 10471-11 (JPC 4003103).

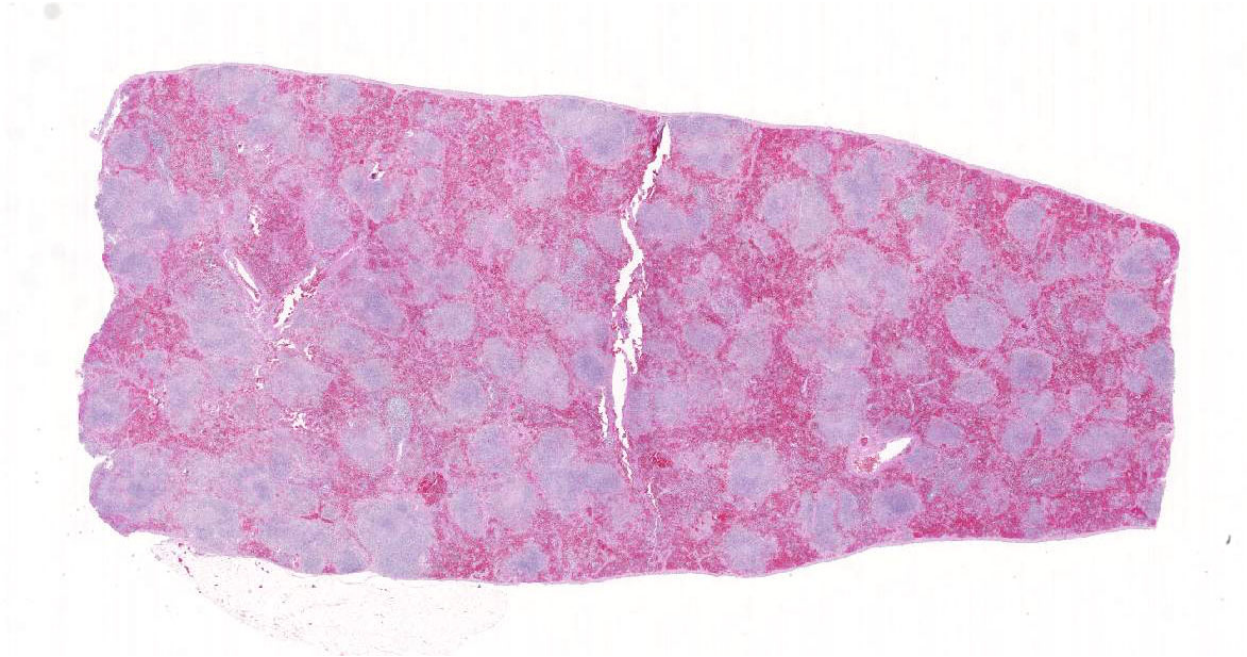
**Signalment:** 8-month-old female domestic shorthair cat (*Felis catus*).

**History:** Patient presented for lethargy, depression, and inappetence. Rectal temperature was 105.1F. Initial treatment consisted of antibiotics, anti-inflammatory drugs, and subcutaneous fluids. Five days later, icterus with hypoglycemia, hypo-albuminemia, and leucopenia were noted. Rectal temperature was 103F. The patient died the following day.

**Gross Pathology**: The spleen was enlarged with a roughened serosal surface. Multiple white pinpoint foci were observed on the serosal surface. The cut surface bulged and had a granular appearance. No other gross lesions were indicated by the submitting veterinarian.

**Laboratory results:** *Francisella tularensis* was isolated from the spleen and from a lymph node sample.

**Histopathologic Description:** In sections of spleen, there is disseminated and coalescing necrosis of germinal centers which extends into adjacent red pulp. Foci of necrosis are accompanied by an inflammatory response comprised of neutrophils and macrophages.



***Spleen, cat. At subgross magnification, white pulp is largely necrotic and areas of lytic necrosis coalesce throughout the slide. A few depleted lymphoid follicles retain their architecture. (HE, 5X)***

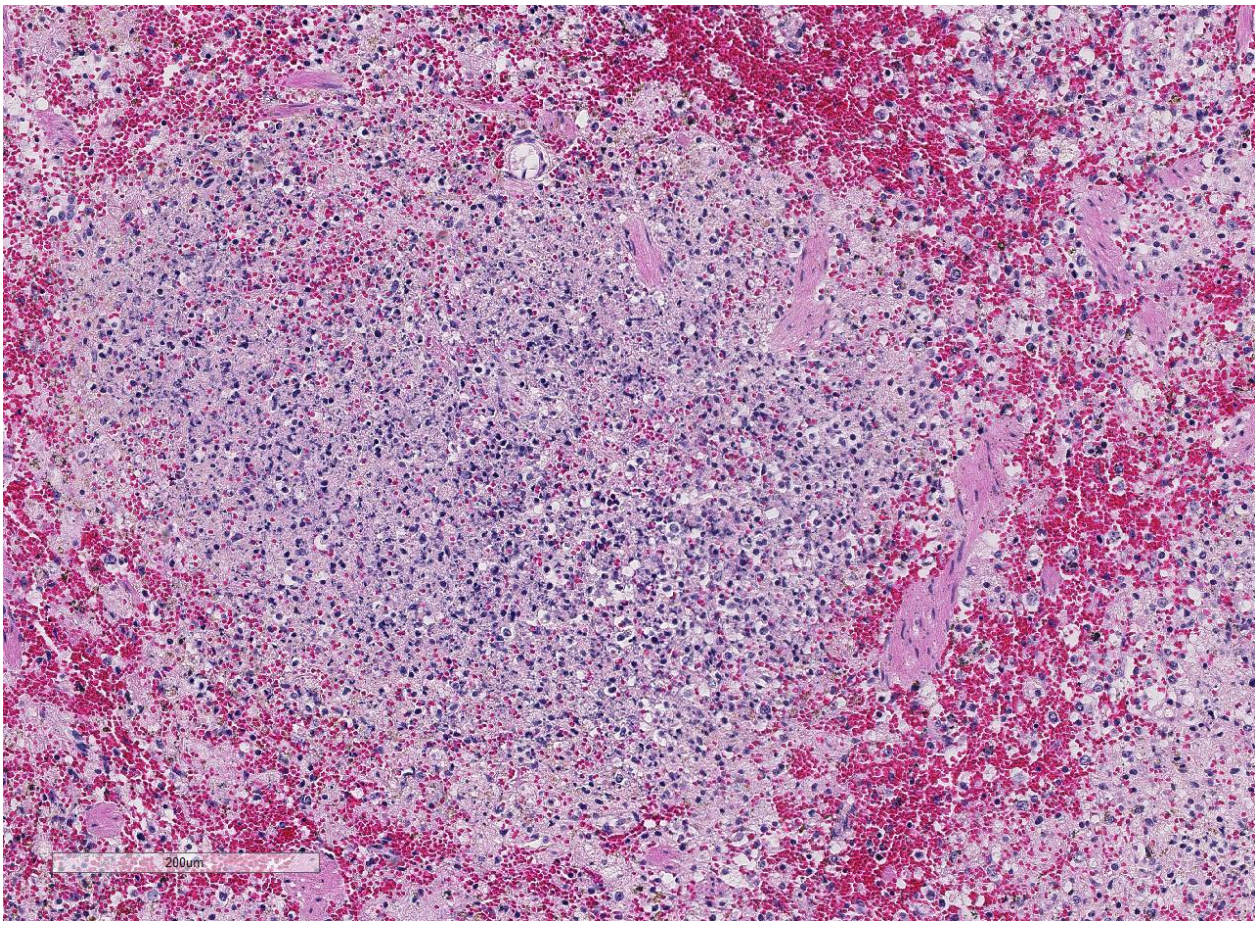
By use of special technique, numerous small gram-negative coccobacilli are seen within necrotic foci and the cytoplasm of individual inflammatory cells.

**Contributor's Morphologic Diagnosis:** Severe, subacute, multifocal, coalescing, necrotic, and pyogranulomatous splenitis

**Contributor's Comment**: *Francisella tularensis* is a gram-negative, facultative intracellular pathogen.[5] *F. tularensis* is subdivided into two subtypes. Type A is *F. tularensis subsp. tularensis* and has and infectious dose in humans of <10 CFU's, whereas type B is *F. tularensis subsp. holarctica* which as an infectious dose of <103 CFU and a milder form of tularemia in humans.[5] The organism is abundant in nature and infects many mammalian and arthropod species.[7] *F. tularensis* type A has been isolated from cats on numerous occasions and can be transmitted from cats and other animals (deer, personal experience) to humans.[1,3,7]

Diagnosis, in some cases, may be difficult, but culture appears to be more sensitive than immunohistochemistry.[7] Gross lesions consist of multiple pinpoint white foci on the spleen, liver, and lymph nodes. As a facultative intracellular parasite, it may persist for years as a latent infection.[7] The genes for several virulence factors have been identified and shown to share some features with the intracellular parasite, *Listeria monocytogenes*.[4] Tularemia in other mammalian species such as horses and sheep are often associated with heavy infestation by ticks such as *Dermacentor andersoni* and *Amblyomma americanum*.[7] One serologic survey indicated 12 – 24 percent of cats had antibodies to *F. tularensis* due to natural exposure.[6] Those serologically positive animals were negative for *F. tularensis* DNA, indicating infection may be may have been cleared naturally. Tularemia should be considered in a differential diagnosis of unexplained febrile illness in cats.

**JPC Diagnosis:** Spleen: Splenitis, necrotizing, multifocal to coalescing, severe, with mild lymphoid depletion and fibrin deposition, domestic shorthair cat, *Felis catus*.




***Spleen, cat. Higher magnification of a necrotic lymphoid follicle. The lytic necrosis extends into the surrounding tissue and there is hemorrhage within the marginal zone.***

**Conference Comment:** The contributor provides a great example of typical lesions of *Francisella tularensis*. In cats, there often is severe systemic disease and pathological manifestations are dependent on the dissemination of the pathogen.[1,8,9] As mentioned by the contributor, classic gross lesions for tularemia are miliary white foci 2mm or more in diameter in the liver, spleen, and lymph nodes. Histologically, the lesions are characterized by focal areas of severe necrosis, as seen in this case.[8] This gram-negative, intracellular bacillus can infect humans, wild rabbits, rodents, and over 100 species of wild and domestic mammals, birds, fish, and reptiles.[3,9] In the North America, the wild rabbit is the reservoir for the *biovar tularensis* (type A). *Biovar holarctica* (type B) is more common in aquatic species such as beavers and muskrats. *F. tularensis biovar mediasiatica* and *F. novicida* are restricted to central Asia.[7,8] Sporadic outbreaks of tularemia are known to occur in sheep and foals in association with heavy infestation with *Dermacentor andersoni* and *Amblyomma americanum* ticks. Typically, enlargement of the liver, spleen, and kidneys with miliary foci of necrosis are seen on post-mortem examination.[8] Dogs are generally highly resistant to natural infection, but there have been rare reports of mild disease in canines.[8]

The most common route of infection for humans originates from cleaning and skinning infected rabbits as well as arthropod bites. Humans can also be infected via contaminated water supplies and consumption of undercooked meat.[6] In addition to natural infection, *F. tularensis* is considered to be a serious potential bioterrorism agent, because it is one of the most infectious pathogenic bacteria known. As mentioned by the contributor, inhalation of as few as 10 organisms can cause severe pneumonic tularemia disease leading to serious illness and death.[1,6]

Experimentally-induced lesions from inhalation in African green monkeys included necrotizing pyogranulomatous lesions which targeted the lung and lymphoid tissue in addition to disseminated miliary necrotic foci on multiple organs and moderate to marked lymphoid depletion of the splenic white pulp and mediastinal lymph nodes.[6] Conference participants agreed that in this case, both red and white pulp of the spleen are affected by necrosis; however, lesions generally centered on the white pulp and extended into the red pulp in conjunction with severe lymphoid depletion and lymphocytolysis. There are currently no vaccines available to prevent disease.[6] As a result, conference participants discussed that extreme care needs to be taken when dealing with and shipping suspect tularemia cases.

These facultative intracellular organisms are most commonly located within macrophages, but may also be present extracellularly in exudates and necrotic debris. The organisms can also infect and survive in dendritic cells, neutrophils, hepatocytes, and lung epithelial cells. The ability of *F. tularensis* to infect macrophages, evade the immune system by preventing phagolysosome fusion, rapidly replicate within macrophages, and disseminate widely throughout the body is the key to its pathogenesis.[1,7,8]

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**CASE IV:** TAMU-02 2011 (JPC 4003102).

**Signalment:** Adult male coyote (*Canis latrans*).

**History:** This animal was found in a storeroom at a racetrack. Police were called because the animal was acting aggressively. The animal was shot twice with a shotgun, and presented for necropsy of suspected "chupacabra."

**Gross Pathology**: Over a dozen punctures in the right thoracic wall were associated with five fractured ribs and hemothorax with over seven pleural punctures (euthanasia procedure). An emaciated canid with severe alopecia and thickening of the skin sparing only sparse tan hairs on the dorsal midline and distal extremities was necropsied. Bilaterally, the pinnae had up to 3mm thick tan crusts (hyperkeratosis) with thick, white to tan exudate extending into the ear canals. Milder white to tan flaking and crusts extended along the dorsal midline.

**Laboratory results:** Rabies negative; Genetic testing: Coyote (*Canis latrans*)

**Histopathologic Description:** Ear pinna: Diffusely the epidermis has parakeratotic hyperkeratosis and thick serocellular crusting composed of keratin, eosinophilic karyorrhectic debris, erythrocytes, degenerate and nondegenerate neutrophils, large colonies of bacterial cocci and numerous embedded mites and their eggs. Mites have ~200 x 400um, characterized by jointed appendages, 3um chitinous exoskeleton, dorsal spines, striated muscle, intestinal and reproductive structures and a body cavity. Eggs are oval and thin-shelled, measuring ~30-60um. Diffusely, the epidermis is hyperplastic with prominent rete pegs, festooning, spongiosis, and multifocal areas of ulceration. Within the dermis is an inflammatory infiltrate composed of eosinophils, neutrophils, and plasma cells with fewer macrophages and

***Presentation, coyote. The presented carcass exhibited severe emaciation and diffuse alopecia, with evidence of a shotgun blast on the right thorax and shoulder. (Photo courtesy of: Dept of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University http://vetmed.tamu.edu/vtpb)***

lymphocytes. Multifocally, superficial and deep dermal vessels are congested and there is lymphangiectasia. Follicles are atrophic.

**Contributor's Morphologic Diagnosis:** Severe, seroexudative, hyperkeratotic, hyperplastic, and eosinophilic dermatitis with mites and bacteria.

**Contributor's Comment**: A story from the "south of the border down Mexico way." The media and locals really wanted this animal not to be a recognized species (see chupacabra website[1]). However, genetic testing identified it as a "plain, old-fashioned" coyote.

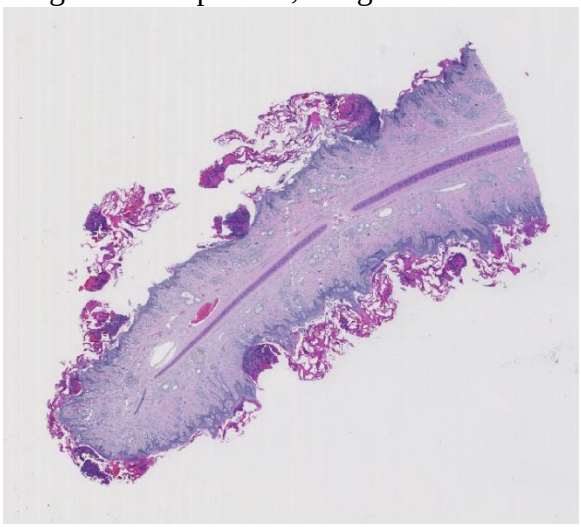
*Sarcoptes scabiei* has been reported to have occurred in 10 orders, 27 families, and 104 species.[2,8,9] Mites are highly contagious, but varieties may show high host specificity. Gross findings range from mild scaling and alopecia on the limbs and ventrum to near complete alopecia with hyperpigmentation, lichenification and severe crusting. Histologic changes include pronounced hyperkeratosis with extensive serocellular crusting, pronounced acanthosis, hyperplasia of the stratum spinosum, infiltration of neutrophils, lymphocytes, and plasma cells, and varying degrees of superficial dermatitis, vasodilation, and dermal edema.[9] It is surprising how thick macroscopic lesions may be characterized as epidermitis. The inflammatory reaction varies between species and individuals, and in the absence of mite detection, no one change is pathognomonic of sarcoptic mange.

Epizootics of sarcoptic mange caused by

***Ear pinna, coyote. The pinna were covered by dense plaque-like hyperkeratotic crusts with white exudate within the ear canal. (Photo courtesy of : Dept of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University http://vetmed.tamu.edu/vtpb)***

*Sarcoptes scabiei* in coyotes have been reported in Montana, Alberta, Wisconsin, Pennsylvania, New York, Kansas, Texas, Louisiana and South Dakota. Reports of mangy coyotes in south Texas go back to the 1920's. Adult males and particularly transient coyotes are significantly more likely to be infected. Severely affected animals have significantly less body fat. Infected individuals may be listless, show less fear of humans and are significantly more likely to seek shelter or food near human settlements. Mortality rates are higher in infected individuals; however, in southern climates, mange has not been shown to directly cause death.[2,8,9]

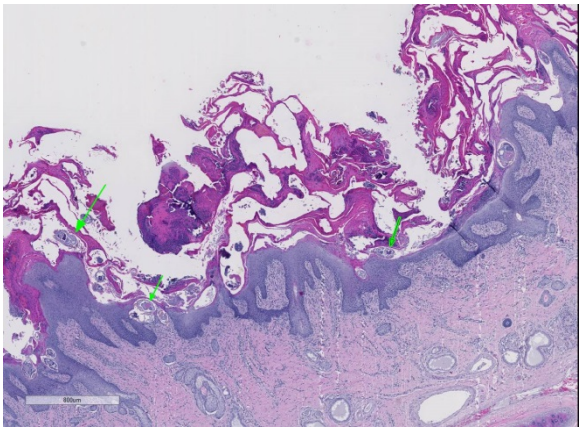
The myth of "El Chupacabra" appears to have arisen in Puerto Rico in the early 1990's and spread from there to South America, Mexico and the southern United States. Originally described as being ~3 feet tall with dorsal spines, leathery skin, a kangaroo-like posture, fangs and a forked



***Ear pinna, coyote. The submitted section of ear pinna is covered by a thick hyperkeratotic crust with scattered basophilic pustules (arrows.) (HE, 4X)***

tongue, the legend has morphed to include creatures resembling leathery dogs with pronounced spinal ridges and eye sockets. Be it the work of vampires, sadists, Santaría cultists or drug lords, chupacabra has provided a mythical explanation for unusual deaths of animals and humans.

The altered appearance and increased human contact of affected coyotes (the only animals identified as chupacabras and examined thus far in a scientific fashion) with sarcoptic mange provides a diagnosis, albeit banal, for the "chupacabra" phenomenon. Deflating the myth, it is hard to tell your kids that, "a mangy coyote will come after you if you don't do your homework or eat your supper."

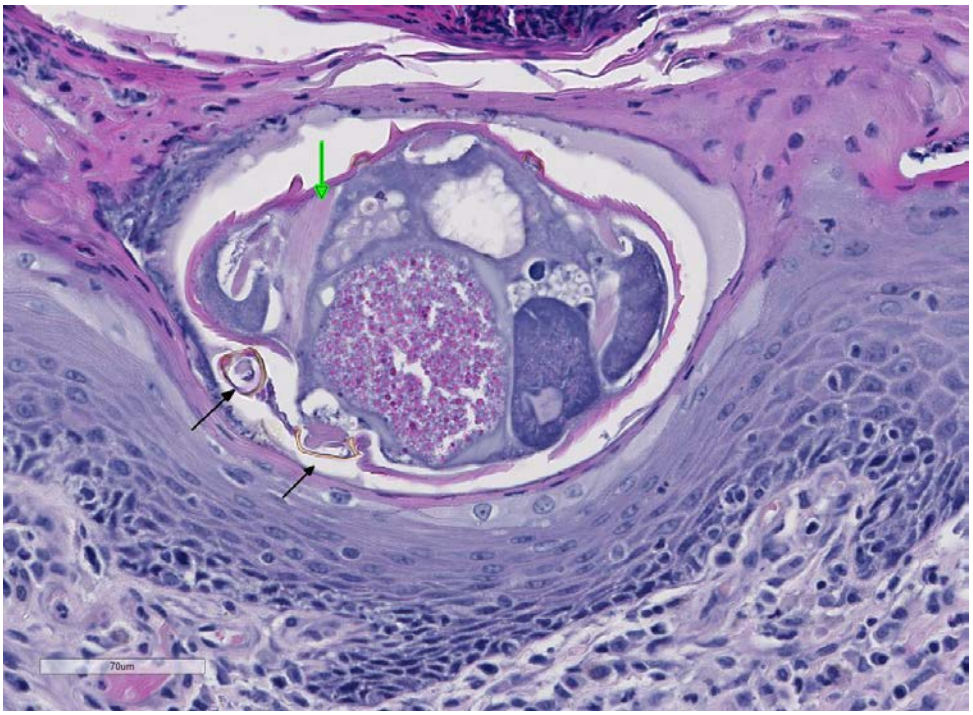


***Ear pinna, coyote. Higher magnification of the pinna demonstrating marked epidermal hyperplasia, superficial dermal inflammation, and numerous cross- and tangential sections of adult mites embedded within the hyperkeratotic crust. (HE, 10X)***

**JPC Diagnosis:** 1. Haired skin, pinna: Epidermal hyperplasia and hyperkeratosis, diffuse, severe, with mild eosinophilic dermatitis and numerous intracorneal mites, coyote, *Canis latrans*.
2. Lymph node, paracortex: Plasmacytosis, marked.

**Conference Comment:** The contributor provides a concise and insightful summary of the epidemiology, gross, and histologic lesions associated with *Sarcoptes scabiei* in a coyote. In this section of pinna, the mites are numerous and have a thin cuticle which is thickened by striated muscular attachments. The most striking and

diagnostic features are the numerous cuticular spines present on the chitinous exoskeleton of the adult female mites in this section. Male mites are approximately two-thirds the size of the females and do not have prominent cuticular spines.[2,4,6] *S. scabiei* mites are relatively host specific and different varieties are morphologically indistinguishable.[2,6] The mite is common in humans, pigs, dogs, and goats and uncommon to rare in cattle, sheep, horses, and cats. It is the most important ecto-parasite in swine causing maculopapular eruptions on the rump, flank, and abdomen of young growing pigs (hypersensitivity form) and thick crusts on the pinnae, head, neck, and legs of older multiparous sows (hyperkeratotic chronic form).[6]




***Ear pinna, coyote. Cross section of S. scabiei within the stratum corneum. Diagnostic features include a spiked chitinous exoskeleton, striated muscle (green arrow), and jointed appendages (black arrows). HE, 400X)***

Recently, there has been a suspected increase in host range of this ectoparasite with subsequent global diversification.[3,7] As a result, *S. scabiei* has been introduced to novel species, likely due to increased human interaction.[7] Most severely affected are the wild canids such as coyotes, red foxes, and grey wolves in North America; the southern hairy-nosed wombat in Australia; and the chamois, red deer, roe deer, and ibex in Europe. The introduction of this pathogen into new locations and hosts has been shown to produce high morbidity and mortality in these species.[3,6,7]

The pathogenesis involves direct damage due to the burrowing mite, irritation from mite excretions, and hypersensitivity to mite antigens in the cuticle, saliva, and feces. This leads to both an immediate (type I) and delayed (type IV) hypersensitivity reaction causing an intense pruritus and alopecia.[2,4,5,6] Pruritus causes decreased food intake (or ability to catch prey), dehydration, and severe weight loss along with secondary bacterial or fungal dermatitis. Eventually, animals succumb to the emaciation and dehydration if not properly treated. Poorly nourished or immunosuppressed animals develop massive mite burdens known as "Norwegian type scabies."[4,5,6]

Conference participants noted several examples of female mites burrowing into and under the stratum corneum forming "molting pockets" in the skin where the female will mate and lay eggs.[4]

Interestingly, many had difficulty finding eggs within their sections despite the presence of numerous molting pockets. Conference participants also discussed other types of burrowing mites that affect veterinary species such as *Notoedres* sp. and *Knemidocoptes* sp.[6] Additionally, participants noted mild reactive lymphoid hyperplasia with paracortical plasmacytosis in the adjacent lymph node. This reaction is relatively mild and likely related to chronic inflammation.

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