Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 25

1 May 2019

CASE I: N261/13 (JPC 4037902).

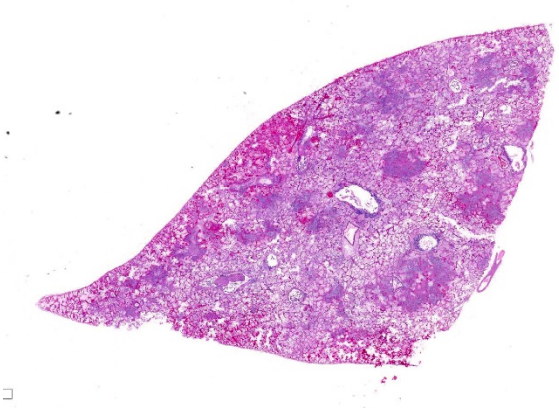
Signalment: 5 month old, female, Devon rex, *Felis domesticus*, feline.

History: This young, fully vaccinated, pedigree Devon rex cat with no previous history of illness became acutely ill with pyrexia and severe dyspnea, the morning following its attendance at a cat show where it had won both classes of competition for which it had been entered. Despite immediate veterinary attention and antibiotic treatment, the animal died within 36 hrs and was submitted for necropsy.

Gross Pathology: The carcass is well preserved with adequate body fat reserves. Periocular and perinasal regions are encrusted with light brown discharge. Tracheal and bronchial lumens filled with frothy mucopurulent exudate. There is multifocal consolidation in all lung lobes: on sectioning coalescing pale firm foci are present. The kidneys are bilaterally congested at the cortico-medullary junction. The stomach contains mucus only. Feces of normal color and consistency in rectum. The urinary bladder is empty.

Laboratory results: Both *Bordetella bronchiseptica* and *Mycoplasma felis* were cultured from samples of lung.

Microscopic Description: Multifocally, there is extensive necrosis of contiguous alveolar walls with filling of associated airspace with cell debris, protein-rich fluid/fibrin, and admixed intact and degenerate inflammatory cells (predominantly neutrophils with fewer macrophages and lymphocytes). Myriad loosely scattered to clumped basophilic coccobacilli (bacteria), and small numbers of



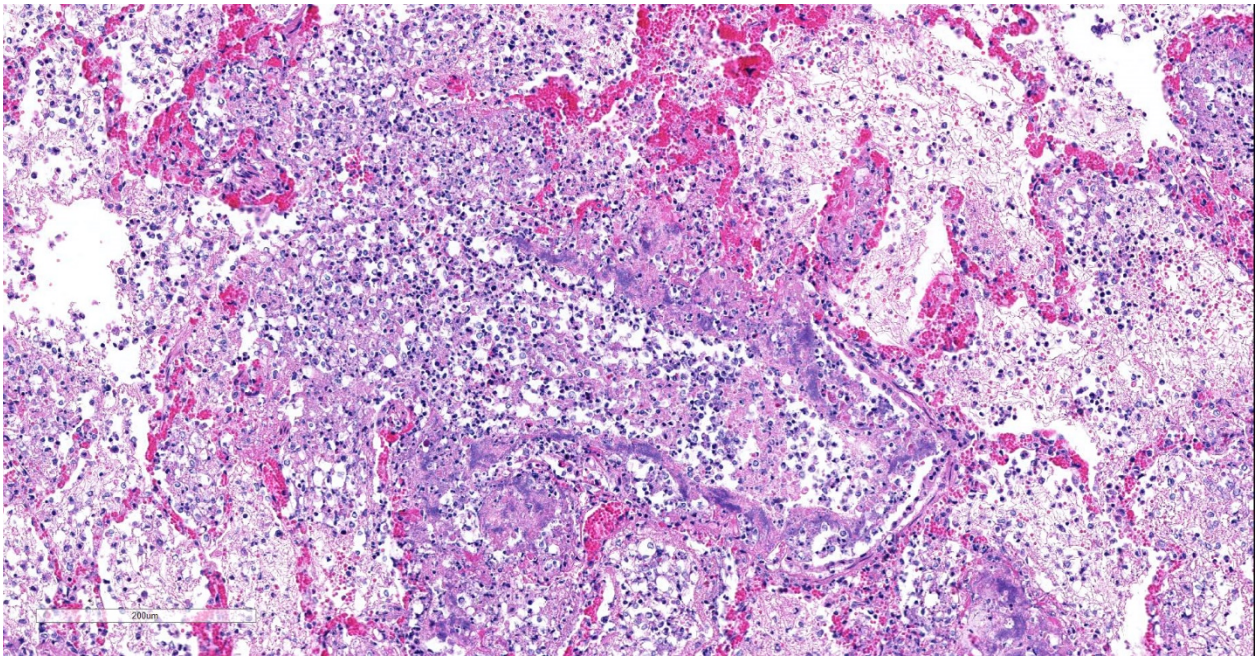
Lung, cat. Approximately 33% of the section is effaced by areas of hyper\cellularity centered on small airways, and there are multifocal, largely subpleural areas of hemorrhage and edema. (HE, 4X)

erythrocytes also noted in these areas. Bronchioles frequently contain similar inflammatory debris and bacteria, and focally, necrosis of bronchiolar epithelia is detected. Widespread hyperemia with small number of medium-caliber blood vessels variously exhibiting fibrinoid necrosis and leucocytoclastic vasculitis. Occasional thrombi noted (not visible in all sections).

Contributor's Morphologic Diagnoses: Lung: necrotizing bronchopneumonia, acute, severe with myriad bacteria (identified as *Bordetella bronchiseptica* on culture).

Contributor's Comment: *Bordetella bronchiseptica* is a gram-negative coccobacillus commonly carried in the nasopharynx of healthy cats. In a survey of respiratory pathogens in multi-cat (≥5 cats) households, *B. bronchiseptica* was detected by PCR in 5% of cats from households with disease, and in 1.3% of cats without.[6] Considered a somewhat uncommon pathogen in this species, infection typically manifests when pulmonary defenses are impaired by feline calicivirus or herpes virus infections, or by stressful environmental conditions (while occasional structures possibly suspicious of basophilic intra-nuclear inclusions were observed in bronchiolar gland epithelium in this case, these were considered equivocal: immunohistochemistry was not performed).[2,4,10] The clinical consequences on infection can vary dramatically: from mild pyrexia, coughing and sneezing to severe pneumonia and death as seen in this case.

Details of the pathogenesis of infection in the cat are inferred from studies in other species. Bacterial virulence is regulated by the *Bordetella virulence gene (bvg) operon*, which orchestrates the expression of the adhesin filamentous haemagglutinin (FHA), pertactin, and the fimbriae that facilitate adherence to the ciliated epithelium, as well as to macrophages and neutrophils within the



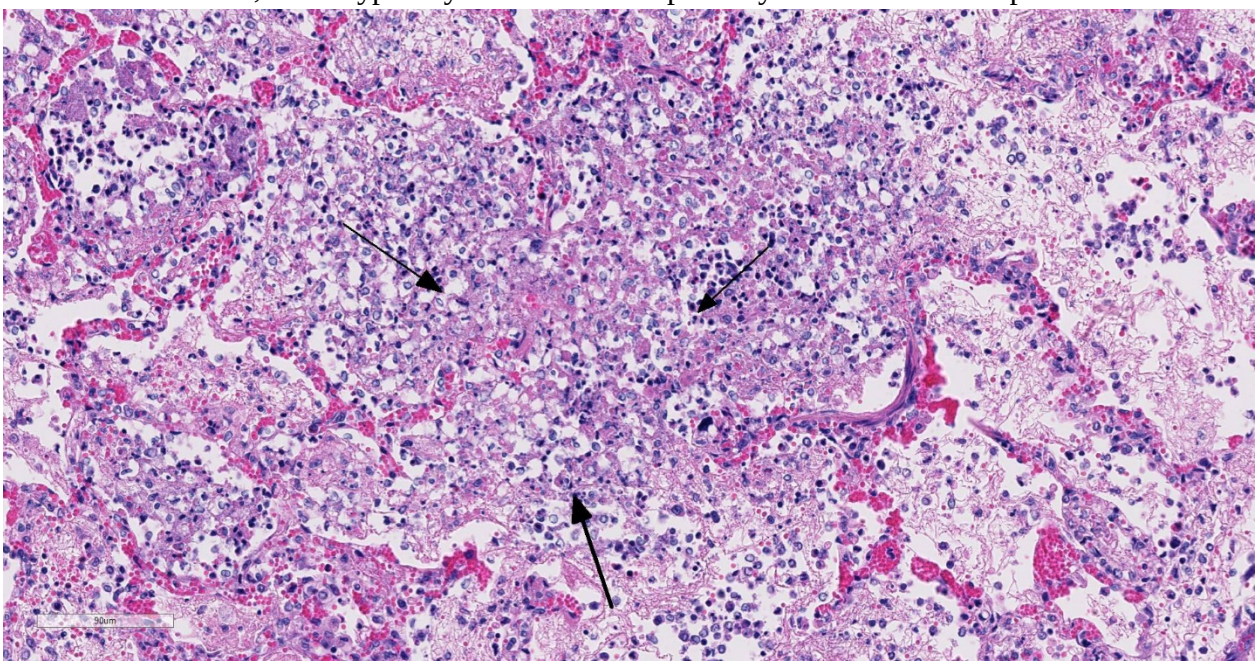
Lung, cat. Areas of inflammation are centered on necrotic airways, which are filled with numerous viable and degenerate neutrophils, cell debris, fibrin, and bacterial colonies. The exudate has ruptured through the wall and spilled into the surrounding alveoli. (HE, 163X)

lungs.[2,6] Following attachment the pathogen s*ecretes the RTX* adenylate cyclase toxin (hemolysin) that results in impaired leucocyte phagocytosis and oxidative burst, and may induce their apoptosis. A lipooligosaccharide with endotoxin activity and a soluble peptidoglycan-derived tracheal cytotoxin are also produced that induce ciliostasis and the apoptosis of ciliated epithelial cells.[2,6]

Bacterial culture (as in this case) and PCR are used to conform the diagnosis, although both lack sensitivity.[6,9] In regions where vaccines against *B. bronchiseptica* are available, it is recommended that cats should not be routinely vaccinated, given the typically mild disease that occurs. However, vaccination is encouraged in units such a shelters where populations are in continuous turnover, and thus at increased risk of infectious disease.[6] Organisms are shed in oral and nasal secretions of infected cats and infected dogs are considered an infection risk for cats.[5,6] Rare zoonosis, typically in immunocompromised individuals, have been reported.[8,11]

Mycoplasma felis can colonize the upper respiratory tract of cats, but evidence suggests it is not a significant primary pathogen. Molecular detection techniques have found *Mycoplasma* spp in the lower respiratory tract of 15.4% of cats with respiratory disease, with *M. felis, M. gateae* and *M. feliminutum* the species identified. However, the pathogenic significance of their presence remains unclear.[7] It is likely that *M. felis* contributed to the severity of the pneumonia in this case through opportunist secondary involvement.

This case represented a sudden and highly traumatic loss for the owner concerned as they witnessed the rapid clinical deterioration and death of their prize-winning animal over a period of 36 hrs. We may speculate that contact with other cats at the cat show provided a source of the infection, and how possibly the stress of transport or attendance



Lung, cat. Within affected areas there are discontinuous alveolar septa, characteristic of septal necrosis (black arrows.) (HE, 313X)

at this event could have precipitated disease. In any event, the rapidity of disease progression suggests the strain of *B. bronchiseptica* involved was particularly virulent.

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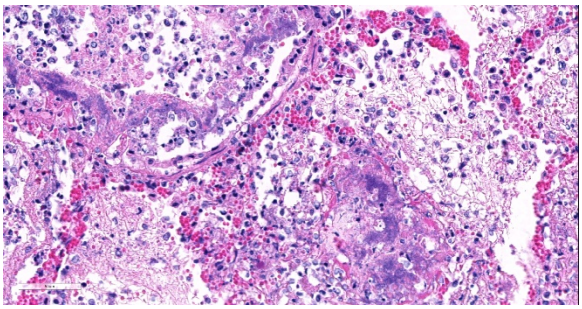
http://www.ucd.ie/vetmed/

JPC Diagnosis: Lung: Bronchopneumonia, fibrinosuppurative and necrotizing, multifocal to coalescing, severe, with numerous bacterial colonies.

JPC Comment: The contributor has provided an excellent review of *Bordetella bronchiseptica* in this case, as well as the pathogenesis of the disease across species lines. *Bordetella bronchiseptica* is an important, if sporadic, pathogen of the respiratory system in a wide range of mammalian species. Previous WSC cases include *Bordetella bronchopneumonia* infection in a chinchilla (WSC 2014-2015, Conf 18, Case 4), an African green monkey (WSC 2011-2012, Conf 22, Case 3), squirrel monkeys (WSC 2007-2008, Conf 9, Case 3, pigs (WSC 1996-1997, Conf 20, Case 2), a rabbit (WSC 1995-1996), among others.

There are three species of *Bordetella* of veterinary importance, including *B. bronchiseptica* (whose dermonecrotic toxin also causes atrophic rhinitis in pigs and rabbits), *B. hinzii* which causes limited respiratory disease in turkey poults and rare septicemia in humans, and *B. pertussus* a potent pathogen in humans which may cause lower respiratory disease in lambs.

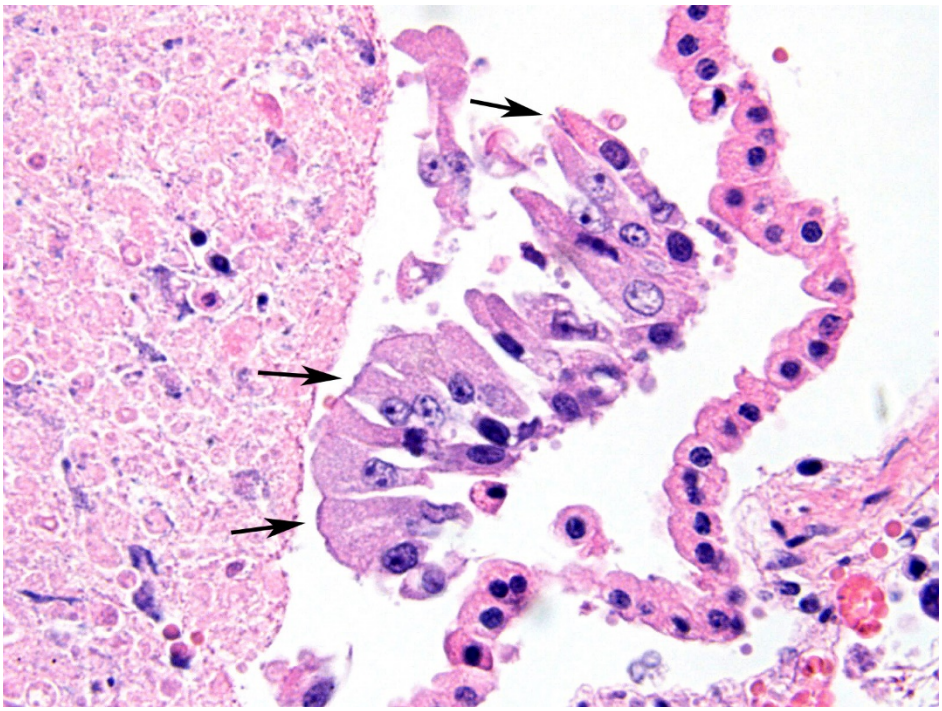
Well known for its involvement in non-fatal tracheobronchitis (aka "kennel cough"), a recent publication[9] has detailed *Bordetella*'s



Lung, cat. Large colonies of bacillii are present within airways and alveoli in close proximity. (HE, 400X)

role in fatal pneumonia as exemplified in this case. *B. bronchiseptica* has been identified in between 12 and 78% of dogs with lower respiratory tract infections, and 5-13% of cats.[9] At a major university veterinary school, a retrospective study identified *B. bronchiseptica* via immunohistochemistry and/or bacterial culture in 8 of 36 of canine and 14 of 31 feline cases of fatal bronchopneumonia. Upon close review, bacteria could be visualized among the cilia in 4 of 36 canine cases and 5 of 31 feline cases.[9] Close review of columnar epithelium in this case also disclosed the presence of cilia-associated bacteria; it was seen only on intact cells, as necrotic or attenuated epithelium has probably already lost its cilia. This may be an important finding in such cases, as the lesions associated with the bronchopneumonia in these cases, in the authors' opinion, were not specific for any particular pathogen.

Bordetella bronchiseptica can be an opportunistic pathogen in humans as well, particularly immunosuppressed individuals and cystic fibrosis patients. *B. bronchiseptica* and *B. pertussis*, (the causative agent of whooping cough) exhibit little genetic variation,[1] with *B. pertussis* being a more recent derived, human adapted bacterium derived as a consequence of gene deletions and loss of genetic regulatory functions. Both organisms possess the *Bordetella* virulence gene as discussed



Lung, cat: Careful evaluation of the HE slide may disclose the presence of bacilli lining cilia of columnar airway epithelium (arrows). Necrotic or attenuated epithelium likely lack cilia, so are not worthy of inspection. (HE, 600X)

above. In a large children's CF center, 7 patients had multiple repeated isolates of *B. bronchiseptica* from their airways. All patients had documented exposure to pets or lived on a farm or an operating kennel, resulting in an overall infection of as many as 12% of children in the center at any time.[1]

One of the issues associated with persistence of *Bordetella* infections in human patients leading to vaccine failures and re-emergence of disease has been recently elucidated – the ability for this bacterium to produce biofilms.[3] This is yet another feature of the BvgAS gene, which regulates the expression of genes encoding surface membrane, secreted, and regulatory proteins from host cells, as well as filamentous hemagglutinin, pertactin, fimbriae and various toxins which contribute to the extracellular matrix comprising the *Bordetella* biofilm.[3]

The moderator reviewed the general patterns of pneumonia in the lung across animal species. In this particular case, attendees were split on whether the appropriate morphologic diagnosis would be bronchopneumonia (based on the traditional pathogenesis of this aerogenous bacteria and the obvious necrosis within airways) or bronchointerstitial, based on the widespread necrosis of alveolar septa, pulmonary vessels, and exudative alveolitis in much of the section

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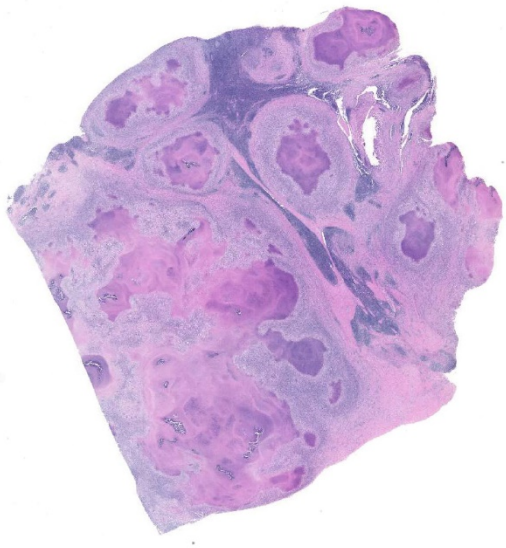
CASE II: 2014 Case 2 (JPC 4050143).

Signalment: Juvenile (<30 months), unreported gender, unreported breed, *Bos taurus*, bovine.

History: The bovine was presented for slaughter at a United States Department of Agriculture (USDA) federally inspected slaughter facility and passed antemortem inspection. The carcass was retained for further diagnostic testing after lesions suspicious for bovine tuberculosis were observed in lymph nodes of the head, thoracic, and abdominal cavities and lungs during postmortem inspection.



Lymph node, ox: The node is effaced by multiple, often coalescing granulomas. (HE, 5X)

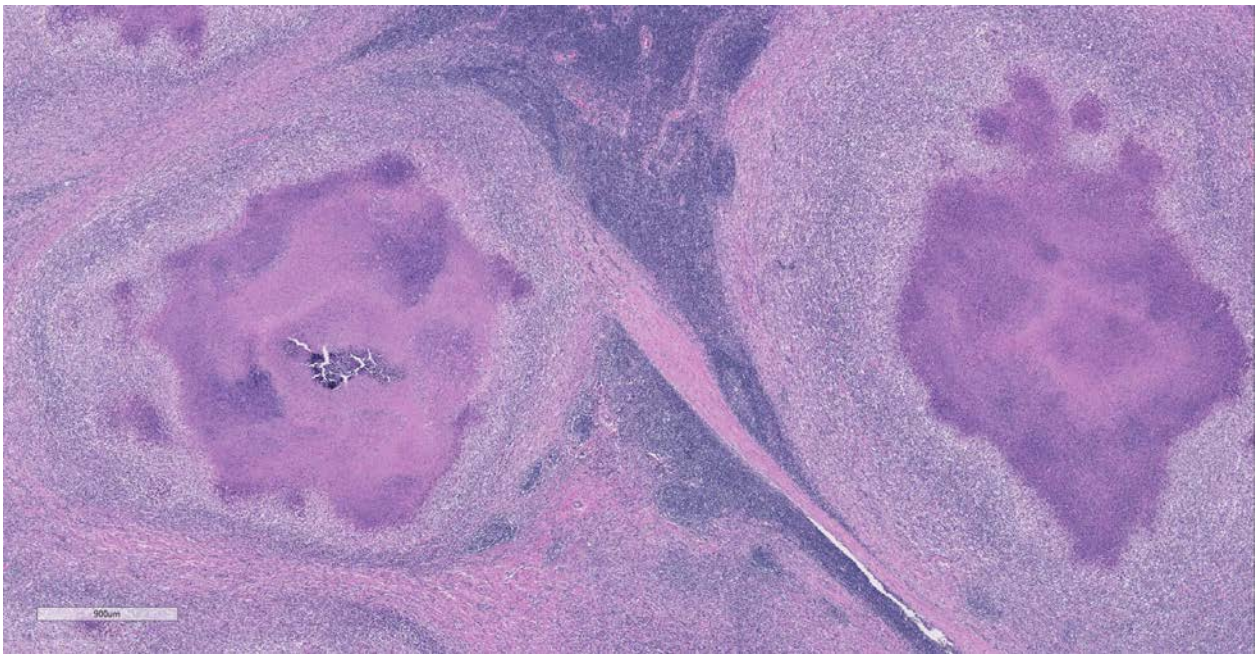
Gross Pathology: None

Laboratory results: PCR from formalin-fixed, paraffin-embedded tissues were positive for IS*6110* (*Mycobacterium tuberculosis* complex) and negative for IS*900* (*M. avium paratuberculosis*) and 16S rDNA (*M. avium* complex). Culture recovered *M. bovis*.

Microscopic Description:
Lymph node. Up to 80% of the parenchyma (there is some slide variability) is effaced by coalescing to locally extensive granulomas. Granulomas are composed of central areas of caseous necrosis and variable amounts of basophilic granular material (mineral) rimmed by large numbers of epithelioid macrophages and multinucleated giant cells surrounded peripherally by lower numbers of lymphocytes, plasma cells, fibroblasts, and fibrous connective tissue. Giant cells have up to 10 peripheral nuclei (Langhans type). There are rare, ~5µm long, acid-fast bacilli within areas of necrosis and the cytoplasm of macrophages and giant cells.

Contributor's Morphologic Diagnoses: Lymph node: Lymphadenitis, necrogranulomatous, locally extensive, chronic, severe, *Bos taurus*.

Contributor's Comment: *Mycobacterium bovis* causes tuberculosis in many mammals, including cattle and is a zoonotic disease. Cattle slaughtered for consumption in the United States in federally inspected abattoirs undergo inspection by personnel from the Food Safety Inspection Service (FSIS) of the USDA to insure they are safe and wholesome for entry into the market. In accordance with the USDA's Bovine Tuberculosis Eradication Program, FSIS personnel retain carcasses when lesions resembling tuberculosis are identified. Suspect granulomas from these cattle are collected and submitted to the National Veterinary



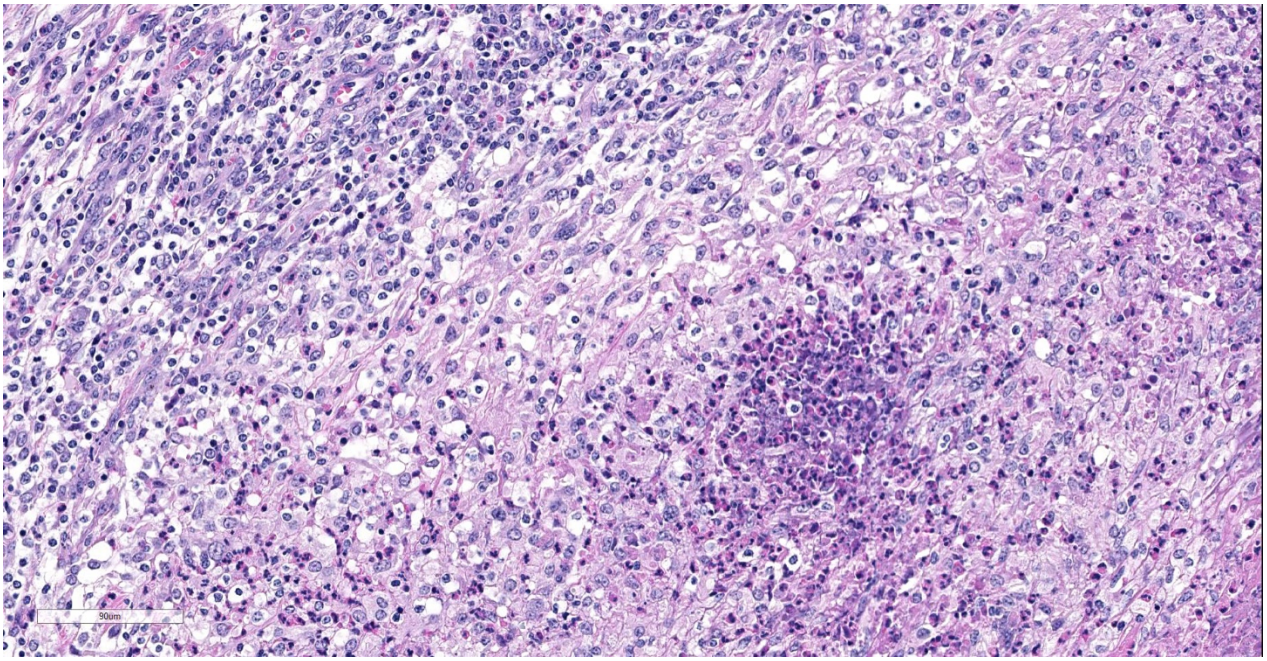
Lymph node, ox: Granulomas have a thick, often central core of cellular debris, which is occasionally mineralized. The intervening remnant node is hyperplastic. (HE, 23X)

Services Laboratories (NVSL) for histopathology and culture.8

Microscopic lesions consistent with tuberculosis in cattle are often multicentric and coalescing with central areas of caseous necrosis and mineral. Epithelioid macrophages with small to moderate numbers of Langhans-type multinucleated giant cells surround the necrosis with smaller numbers of peripheral lymphocytes, plasma cells, and occasional neutrophils. [2] The typical tuberculous lesion caused by *Mycobacterium bovis* has rare to occasionally moderate numbers of acid-fast bacteria present within the cytoplasm of macrophages and giant cells as well as in areas of necrosis [2]

Bovine cases that are histologically compatible with tuberculosis undergo additional testing at NVSL by means of PCR on formalin-fixed, paraffin-embedded (FFPE) tissue to test for mycobacterial DNA. Because the scope of the TB eradication program is focused on identifying *M. bovis*, primers used in the PCR are limited to those for *M. tuberculosis* complex (MTBC, of which *M. bovis* is a member), *M. avium* complex (MAC, common environmental mycobacteria) and *M. avium paratuberculosis* (MAP, the bacterium that causes Johne's disease in cattle). A recent report[7] of mycobacteria cultured from clinical samples submitted to the NVSL stated that the majority of mycobacteria cultured from cattle were *M. bovis* (32%) followed by *M. avium* complex (25.5%). The next most common species, *M. fortuitum*/*M. fortuitum* complex, comprised 10.1%.[7]

The microscopic features of the current case were consistent with bovine tuberculosis and FFPE tissue was tested by PCR for mycobacterial DNA using our primers for MTBC, MAC, and MAP. PCR was positive for the MTBC primer sets and negative for MAC and MAP. False negative results for mycobacterial DNA can occur in some cases. The more common reasons for false negative



Lymph node, ox: The periphery of the node contains numerous epithelioid macrophages and aggregates of neutrophils, and more peripherally (HE, 238X)

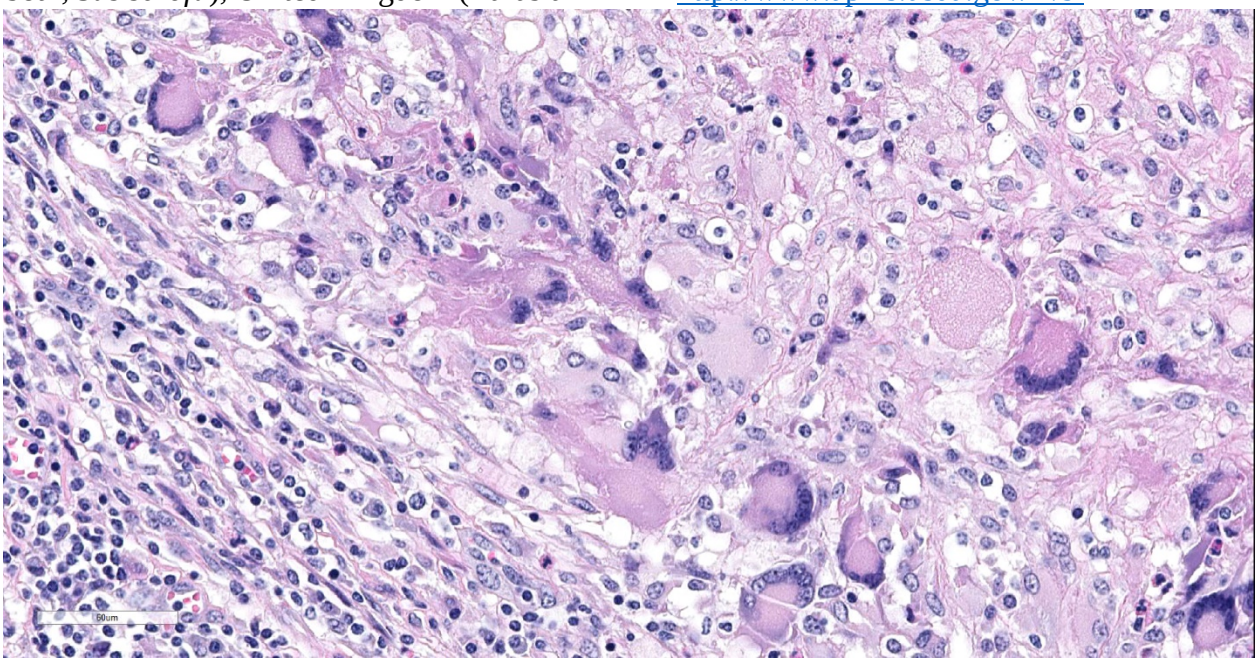
PCR results include tissue being fixed in formalin for an extended period of time (>7 days) and and/or extremely low numbers of AFB present in the lesion or tissue section. Formalin fixation causes irreversible cross-linking between DNA and protein, which becomes worse as the tissue fixes over time.[3]

Culture, which is considered the gold standard for definitive diagnosis of bovine tuberculosis[2] and can take up to 10 weeks to complete with slow-growing mycobacteria, recovered *M. bovis*.

Mycobacterium bovis is a member of the *M. tuberculosis* complex, which includes *M. tuberculosis*, *M. africanum*, *M. canettii*, *M. pinnipedii*, *M. caprae*, *M. microti*,[5] and the newly described *M. mungi*.[1] *M. bovis* can cause disease in cattle as well as humans and other domesticated and wild mammals. Currently, *M. bovis* is endemic in various populations of wildlife and are a source for re-infection of domesticated animals in regions of the United States (white-tailed deer, *Odocoileus virginianus*), Spain (wild boar, *Sus scrofa*), United Kingdom (Eurasian badgers, *Meles meles*), and New Zealand (brushtail possums, *Trichosurus vulpecula*).[6]

Disease manifestation can vary, but *M. bovis* typically causes granulomatous inflammation in the lungs and lymph nodes of the head (retropharyngeal), chest (tracheobronchial and mediastinal) and/or abdomen (mesenteric), often reflecting the route of transmission (inhalation or ingestion).[2,7] Grossly, the classic tubercle is encapsulated, pale yellow, and often has a caseous core that may be variably mineralized.[2] Clinically, the only sign of infection may be chronic weight loss (wasting), weakness, loss of appetite, fluctuating fever, cough, exercise intolerance, and lymphadenomegaly.[9] Most animals that are infected with *M. bovis* do not develop clinical disease.[2]

Contributing Institution:
National Centers for Animal Health, Ames, IA
http://www.ars.usda.gov/main/site_main.htm?modecode=36-25-30-00
http://www.aphis.usda.gov/nvsl



Lymph node, ox: Numerous Langhans giant cells are present at the periphery of the granulomas. (HE, 238X)

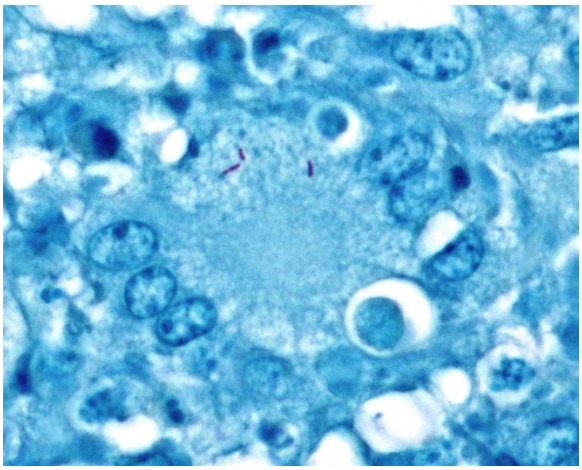
JPC Diagnosis: Lymph node: Lymphadenitis, granulomatous, multifocal to coalescing, severe, with diffuse moderate follicular and paracortical hyperplasia.

JPC Comment: The contributor does an excellent job reviewing the thorough protocol used to investigate tissue suspected of - *Mycobacterium bovis* infection at the National Veterinary Services Laboratory, an important link in food safety in the United States.

M. bovis is a member of the growing *M. tuberculosis* complex as described above. A recent publication[4] noted the divergent pathogeneses in non-bovine hosts in several countries of the world which have been incriminated in maintaining infection in local livestock.

In the North America, the white-tailed deer, and to a lesser extent, elk harbor the infection and potentiate the infection though contact with cattle in their pasturing area. Infected lymph nodes in deer often have soft centers, resembling abscesses; however neutrophils, as seen in cattle, are not a feature of the lesion. [4] Approximately 30-35% of infections exhibit spread to the thoracic viscera, and often the pleural surfaces, where they form pearlescent nodules reminiscent of mesothelioma.

In England and Ireland, the Eurasian badger (*Meles meles*) has been indicted as a major factor in outbreaks of cattle with bovine tuberculosis. In the badger, respiratory infection is most common with 50% cases showing pulmonary infection, and 35% of cases involving lymph nodse. Elongate radial lesions may be seen in the kidneys, and miliary lesions may be seen in the liver and spleen. Many infected badgers show no visible gross lesions.[4]



Lymph node, ox. Three acid-fast bacilli, approximately 5-7µm, within the cytoplasm of a multinucleated giant cell. Modified Ziehl-Neelsen., 1000X)

In western Europe, wild boars serve as wildlife reservoirs of *Mycobacterium bovis*. This species, well known as highly susceptible to *M. bovis*, has been used as a sentinel species to screen for *M. bovis* in Hawaii and New Zealand. Disseminated infection is the rule in this species, with lesions in multiple anatomic regions, including cranial lymph nodes and mandibular lymph nodes, lungs, liver, and spleen.[4]

In New Zealand, the brushtail possum (*Trichosurus vulpecula)* is considered a source of infection for cattle. Highly susceptible, they manifest the disease by infection of subcutaneous lymph nodes, forming draining fistulous tracts. Terminally ill possums attract cattle by wandering erratically across pastures, and curious cattle may sniff and lick them, thereby receiving exposure to the bacteria. In these regions, infected wild ferrets may also be infected, but their role in spreading the infection to cattle is controversial – a reduction in possum population in these areas results in a proportional decrease in infection of this carnivore, suggesting that the ferret is a spillover host at best.[4]

The participants agreed that an alternate JPC diagnosis of multiple lymph node granulomas would be acceptable as well. As this was the last conference of the 2018-2019 year, the box containing the age-old discussion of "granulomatous versus granuloma" was quickly slammed shut.

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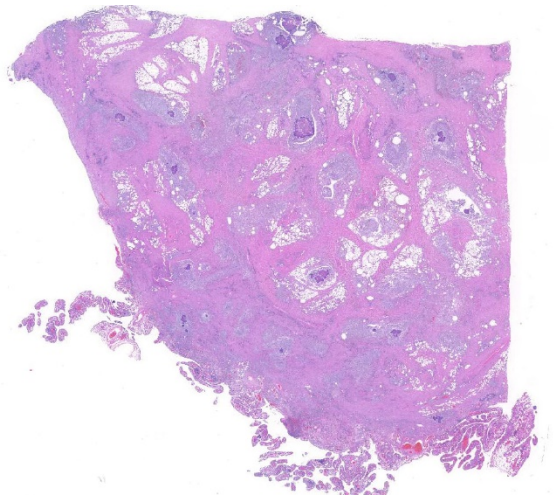
CASE III: S-2017-20 (JPC 4100993).

Signalment: 6-year and 5-month old female neutered crossbreed dog (*Canis lupus familiaris*).

History: A 6-year 5 months-old female neutered Springer Spaniel Cross presented for investigation of pyrexia of unknown origin of 5 months duration. The owner also noted lethargy and mild gastrointestinal signs during this time (inappetence and diarrhoea). Previously the patient had responded well to oral prednisolone and potentiated amoxicillin, however symptoms recurred



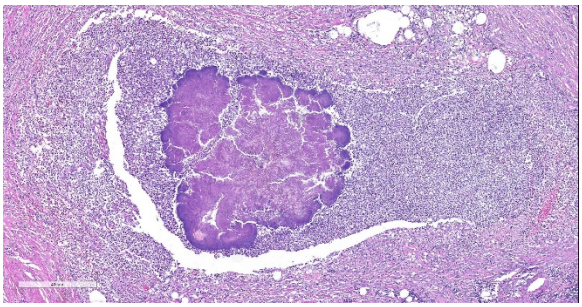
Omentum, dog. The omentum is markedly expanded, and largely effaced by multifocal to coalescing pyogranulomas. (HE, 4X)

after the treatment was stopped. On physical examination, marked pyrexia of 40.3C was noted, with tachycardia of 160 beats per minute and normal synchronous pulses. A normal respiratory rate of 32 breaths per minute with normal respiratory effort was noted. Auscultation of the thorax was unremarkable. Mucous membranes were pink and moist, with CRT <2s. Mild discomfort was noted on deep palpation of the cranial abdomen, which was subjectively mildly bloated. Rectal examination was unremarkable and peripheral lymph nodes were within normal limits. Abdominal ultrasound showed free fluid within the abdomen. At exploratory laparotomy the abdomen was diffusely filled with creamy pink fluid and there were multiple masses in the omentum and mesentery of the jejunum, which were sampled for histopathology.

Gross Pathology: In a multifocal to coalescing pattern, the greater omentum was expanded by irregular, tan to brown to dark red and moderately firm mass-like lesions with cream to tan, multifocally red cut surfaces. The adjacent omentum was tan to brown, and soft.

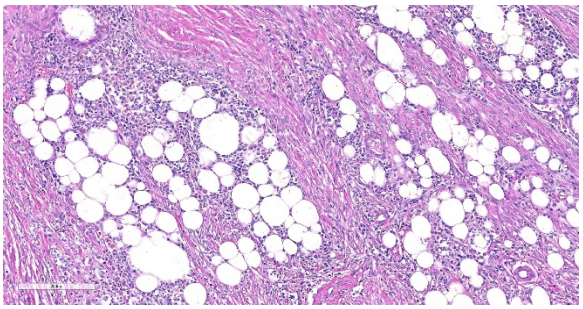
Laboratory results: Haematology: WBC count 18.2x109/L (ref 6-17), otherwise unremarkable. Biochemistry: C-reactive protein 26.3mg/L (ref 0-8.2) and hypoalbuminaemia 17g/L (ref 25-41). ALT was mildly decreased and AST was mildly increased. Abdominal fluid sample: Cytology of aspirated abdominal fluid showed neutrophilic macrophagic inflammation with phagocytosed bacteria and was consistent with a septic exudate. Culture of omental masses: *Actinomyces* spp. was cultured.

Microscopic Description:
Expanding and effacing the collagenous connective tissue and adipose tissue of the omentum, in a multifocal to coalescing



Omentum, dog. Pyogranulomas are centered on large colonies of filamentous bacilli which range up to a mm in diameter. (HE, 63X)

distribution, are large numbers of macrophages, neutrophils and lesser numbers of lymphocytes, which are often forming moderately sized to large groups. Small to moderate numbers of plasma cells are present multifocally, often at the periphery of these groups and multifocally within the connective tissue and adipose. Large numbers of macrophages and neutrophils often surround large colonies of filamentous Gram-positive bacteria, measuring approximately 1x3-7µm. Bacteria are admixed with large (approximately 500µm diameter) accumulations of finely granular basophilic to eosinophilic material which radiates outwards from a central focus (Splendore-Hoeppli material; "sulphur granules"). These colonies often show a rosette-like arrangement and are visible subgrossly. Small amounts of eosinophilic material, cellular debris, haemorrhage and small numbers of haemosiderin-laden macrophages (haemosiderophages) multifocally surround the colonies. Further to the periphery of these foci are haphazardly arranged, hypertrophied fibroblasts embedded in fibrous connective tissue which often progresses to thick bands of mature fibrous connective tissue, containing scattered to moderate numbers of neutrophils, lymphocytes, plasma cells, and fewer macrophages.



Omentum, dog. There is mild granulomatous inflammation focused on areas of remnant adipose tissue. (HE, 150X)

The omentum is lined by flattened to multifocally plump mesothelial cells and exhibits multifocal ulceration. Underlying ulcerated areas there are frequent plump reactive fibroblasts, aligned in parallel to each other and perpendicular to adjacent, newly formed blood vessels (angiogenesis) and to the ulcerated surface (granulation tissue formation). At the edges of the section the omentum exhibits a multifocal folded appearance and contains numerous, frequently congested, blood vessels and is expanded by variable numbers of macrophages, lymphocytes, neutrophils, plasma cells, and fibroblasts.

Contributor's Morphologic Diagnoses: Greater omentum: Peritonitis, pyogranulomatous, lymphocytic, and plasmacytic, chronic, locally extensive, severe; with:

a. Numerous accumulations of Splendore-Hoeppli material with intralesional microcolonies of Gram-positive filamentous rod bacteria, morphology consistent with *Actinomyces* spp.;
b. Granulation tissue formation, chronic, multifocal, marked; and
c. Fibrosis, chronic, multifocal, marked.

Contributor's Comment: The histopathological findings in this case were consistent with abdominal actinomycosis, a diagnosis that was supported by the microbiology results. The patient had an abdominal drain placed at the time of exploratory laparotomy and was subsequently hospitalized for 6 days. Antibiotic treatment with amoxicillin-clavulanic acid commenced following receipt of the histopathology and culture results, and treatment resulted in good clinical improvement and a positive outcome.

Actinomycosis is caused by filamentous, gram-positive bacteria from the family Actinomycetaceae, genus *Actinomyces*, and to a lesser extent to the related genus *Arcanobacterium*. These bacteria are normally present in the mucous membranes, especially in the oropharynx, and in the genital and gastrointestinal tracts, having however the potential to cause opportunistic infection when inoculated into tissues in association with other bacteria when there is mechanical disruption of normal mucosal barriers (such as deeply penetrating wounds or migration of foreign material).[7,14]

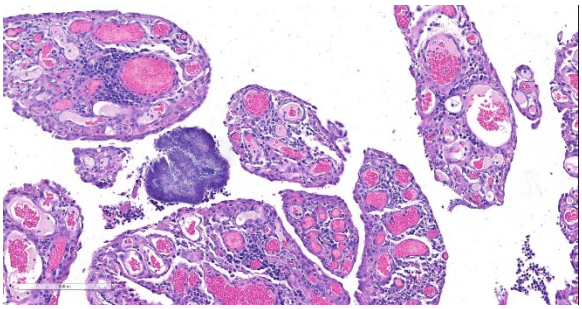
Canine actinomycosis is most common in young adult to middle-aged large breed dogs (median age of 5 years old) that have outdoor access, especially retriever and hunting breeds. The development of actinomycosis in outdoor dogs is frequently related to exposure to plant penetrating material, such as grass awns.[7,14] After ingestion or inhalation, plant awns become contaminated with *Actinomyces* spp. and other bacteria from the oropharynx, and then migrate to various sites.[6,7,14]

Actinomyces spp. can also be inoculated into tissues by bite injury. Other co-infecting aerobic and anaerobic bacteria from the oral cavity or intestinal tract impair normal host

defenses and reduce oxygen tension, which allows *Actinomyces* spp. to endure.[7,14] Abdominal actinomycosis, as seen in this case, may develop when ingested foreign bodies penetrate the gastrointestinal tract, which leads to the formation of intra-abdominal mass lesions and ascites or it can also occur due to direct extension from subcutaneous tissues or hematogenous spread of the organism to abdominal organs such as the liver.[9,14]

Actinomyces spp. that have fimbriae can bind to specific cell surface receptors on other bacteria, especially streptococci, and this bacterial co-aggregation prevents the capacity of neutrophils to phagocytize the organisms.[13,14] Dense colonies of *Actinomyces* spp. form, and these ´Sulfur' granules can be macroscopically visible in exudates in actinomycosis as small yellow free granules.[15] The colonies are progressively encircled by concentric accumulations of neutrophils, macrophages, and plasma cells, with the ensuing development of pyogranulomatous inflammation[14] and are often bordered by star-like or club-shaped-like amorphous eosinophilic material (Splendore-Hoeppli material).

The connective tissue is destroyed by proteolytic enzymes from the associated bacteria, macrophages and degranulated neutrophils, which allows the inflammatory process to extend through normal tissue planes. Less frequently *Actinomyces* spp. spreads hematogenously to distant sites. In some cases, the inflammatory reaction is accompanied by mass formation and extensive fibrosis, as in this case. The most common clinical forms of actinomycosis in dogs and cats involve the cervicofacial region, thorax, abdomen, and subcutaneous tissue, but central nervous system infections including meningitis and meningoencephalitis,[5,14] and ocular infections such as keratitis and endophthalmitis,[3] may also occur.



Omentum, dog. At the edge of the section, there are mesothelial lined papillary projections of fibrous connective tissue, and bacterial colonies ("granules") within the extracellular space. (HE, 150X)

In cattle, actinomycosis, caused by *Actinomyces bovis*, often affects the bones of the jaw, with development of granulomatous and fibrosing osteomyelitis ('lumpy jaw'). Less commonly it can also affect the musculature of the tongue, leading to the development of chronic fibrosing nodular myositis.[16] *Actinomyces* spp. may also cause hepatic abscesses in different species.[4] In swine, *Actinomyces suismastidis* has been associated with the development of pyogranulomatous mastitis[10] and *Actinomyces hyovaginalis* has been associated with necrotic pulmonary lesions in the same species.[2] In horses, *Actinomyces* spp. can cause abscesses, which are most commonly located in the submandibular and retropharyngeal regions[8] and *Actinomyces denticolens* has been associated with the development of submandibular lymphadenitis.[1]

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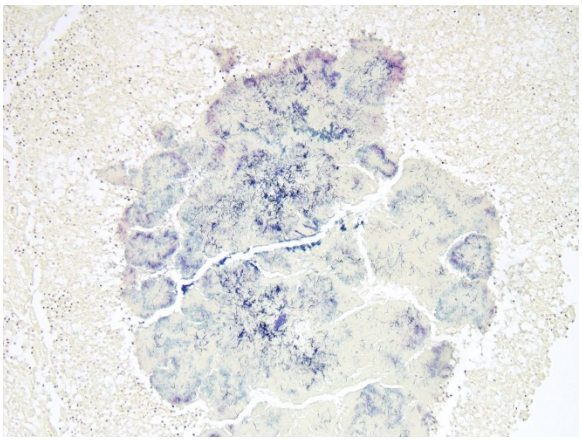
JPC Diagnosis: Omentum: Peritonitis and

steatitis, chronic-active and pyogranulomatous, multifocal to coalescing, severe, with large colonies of filamentous bacilli and marked mesothelial hyperplasia

JPC Comment: The contributor has provided an excellent review of actinomycotic infection in the dog and a wider range of species. *Actinomyces* is a genus of gram-positive bacteria, with new species being identified on a regular basis in species as diverse as mammals and mollusks.

Members of the genus *Actinomyces* are well documented in health and disease in humans as well. A number of species of *Actinomyces*, including *A. odontolyticus, A. oris, and A. naeslundii*, are common commensals in the oral cavity, and are components of the biofilm on teeth at all ages. A number of commensal actinomycetes colonize the gastrointestinal tract, where they are proposed to help in the breakdown of complex sugars as well as producing antibiotics to maintain balance of the bacterial flora.[12]

The pathology of actinomycosis in humans was first identified in 1892 by Kruse, when the bacterium was known as *Streptomyces israelii* (now *A. israelii*).[11] Twenty-five species of pathogenic actinomycetes have now been identified in humans. These species of *Actinomyces* are endogenous inhabitants of mucosal membranes which are introduced into deeper tissues by trauma, surgery, or the introduction of foreign bodies. In humans, actinomycosis is classified as orocervicofacial, thoracic, and abdominal.



Omentum, dog: Filamentous bacilli stain positively with a tissue gram stain. (Brown and Brenn, 400X)

More than half of all cases of human actinomycosis are orocervicofacial, an unsurprising fact considering the ubiquity of multiple species in the oral cavity, and many infections are polymicrobial in nature. Unlike dogs, the basis for thoracic infection is usually aspiration of oropharyngeal secretions, and results in pulmonary abscessation. Extrapulmonary extension into the thorax (common in small animals) is relatively uncommon in humans, with sepsis being a more common result. Abdominal infections are often the result of abdominal surgery or invasive infection such as appendicitis; pelvic infections have been associated with prolonged use of intrauterine devices for contraception. Less common manifestations are cutaneous and musculoskeletal infections (generally caused by traumatic implantation, and cerebral and disseminated actinomycosis (usually resulting from sepsis). An excellent review of human infection, to include specific forms of infection and associated actinomycotic species was published by Kononen et al. in 2015.[11]

The moderator discussed the nature of Splendore-Hoeppli material and various theories concerning its makeup, and possible mechanisms of infection the dog and cat, as well as actinomycotic infections in other species.

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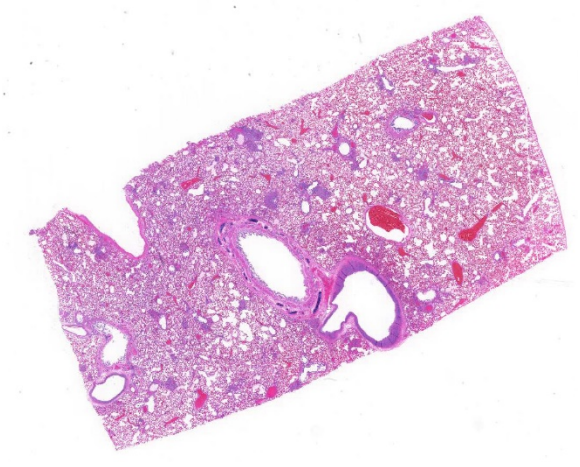
CASE IV: 12H5674 (JPC 4032439).

Signalment: A six year old, spayed female Pembroke Welsh Corgi dog (*Canis lupus familiaris*).

History: The dog presented with tachypnea, increased respiratory effort, cardiomegaly, panhypoproteinemia, mild anemia.

Gross Pathology: The dog had areas of ecchymoses around the peripheral veins and clotted blood in the right nasal cavity and ethmoid conchae. Feces were black and tarry. Lungs were diffusely firm and dark red. There were pinpoint multifocal white circular structures diffuse across the lung surface. Other findings included: subendocarial



Lung, dog. At low magnification, there are areas of hypercellularity around small arterioles. (HE, 7X)

petechiae and petechiae cranial to the trigone of the bladder.

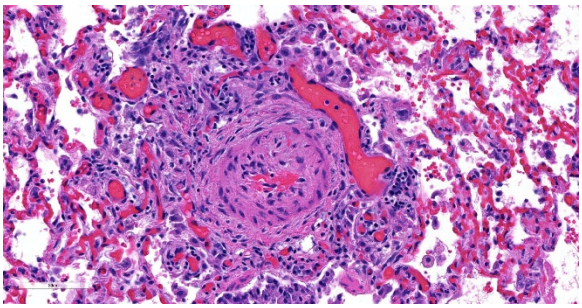
Laboratory results: The dog had a slight increase in neutrophils (11.81; range 3.0-11.4 x 10^{-3}/ul), monocytes (2.35; range 0.15-1.35 x 10^{-3}/ul), reduced hematocrit (36.9; range 37.0-55%), reduced platelets (43; range 200-500 x 10^{-3}/ul), increased ALT (223; range 24-90 IU/L), BUN (>180; range 10-30 mg/dl), decreased calcium (8.6; range 9.7-11.3 mg/dl), increased phosphorus (10.7; range 3.2-6.0 mg/dl), increased glucose (202; range 68-115 mg/dl), decreased sodium (122; range 141-151 mEq/L), decreased total protein (4.6; range 5.2-7.1 gm/dl).
v variable numbers of lymphocytes (plexiform pulmonary arteriopathy). Multifocal alveoli are variably-filled with seroproteinaceous fluid and/or hemorrhage along with increased numbers of alveolar macrophages. There is moderate multifocal to diffuse vascular congestion.

Contributor's Morphologic Diagnoses: Lung, arteriolitis and arteritis, proliferative, lymphocytic and fibrosing with plexiform change, chronic moderate to severe

Lung, hemorrhage, congestion, and edema, multifocal, moderate

Contributor's Comment: The arterial lesions coupled with the clinical and laboratory results are suggestive of plexiform pulmonary arteriopathy.[2,6] The dog lacked heartworms and lacked evidence of a left to right cardiac shunt and thus the changes are consistent with idiopathic pulmonary arterial hypertension (IPAH).[6] In a limited number of dogs (six) with pulmonary arteriopathy and hypertension 67% were female, as in this case. The pulmonary vascular changes likely led to or were caused by pulmonary hypertension resulting in right heart failure suggested by radiographic and ultrasound findings that included right ventricular dilation and hepatic centrilobular congestion. Hemorrhage and congestion are present in the lung and are likely due to pulmonary hypertension possibly exacerbated by the thrombocytopenia. The hypoproteinemia and azotemia could not be fully explained. Right heart failure can lead to renal dysfunction but typically not glomerular leakage.

Some cases of IPAH have a mild inflammatory component in the arterioles which was evident in this case as well.[6] It could not be determined if the inflammation was secondary or incidental to the



Lung, dog. There is intimal hyperplasia and expansion of the tunica medial by smooth muscle hyperplasia and increased amounts of collagen and extracellular matrix. Surrounding affected areioles, there is a proliferation of thin-walled arteriolar branches. (HE, 282X)

proliferative change or if there is some antigen (self or infectious agent) that triggered the lymphocytic infiltration.

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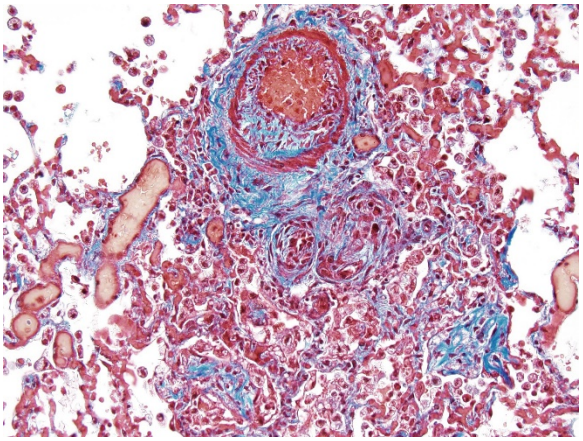
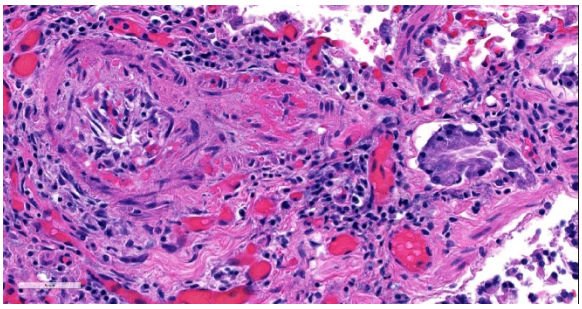
JPC Diagnosis: Lung, small arterioles: Plexiform (plexogenic) arteritis with marked intimal and medial hyperplasia and fibrosis**,** recanalization, and multifocal fibrinoid necrosis and exudative alveolitis.

JPC Comment: Plexiform (plexogenic) arterial lesions are striking histologic lesions which are associated with pulmonary hypertension in man and animals. In general terms, plexiform lesions is a form of "dilatation" lesion developing in late stages of pulmonary hypertension, following a precipitous decrease in pulmonary blood flow and resultant irreversible pulmonary hypertension. While many small arterioles will show evidence of prolonged hypertension, to include intimal hyperplasia, smooth muscle hyperplasia and disarray, and medial and adventitial fibrosis and in some cases fibrinoid necrosis (all present in this section), the plexiform lesion is a unique finding. This lesion is the result of a localized sac-like dilation of a branch of a muscular artery, in which fibrin accumulates from necrosis in the wall of the parent artery.[1] Over time, this fibrin is organized into small vessels populated by cells from the parent artery as well. This pathogenesis also explains why the proliferating vessels are not circumferential but often present eccentrically on only one side of affected arterioles.[1]



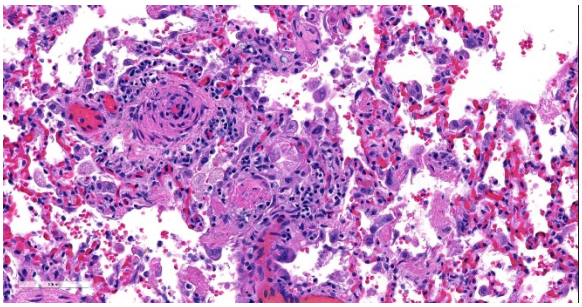
Lung, dog: Plexiform lesion demonstrate marked asymmetric intimal hyperplasia and medical and adventitial fibrosis, as well a proliferation of smaller arterioles (also fibrotic) at the periphery. (Masson's, 400X)

These lesions have been well studied by sequential biopsies taken during the closure of congenital shunts in humans, and many of the associated lesions are seen in this section. The first lesion is increased muscularity of the wall of small arterioles (medial hypertrophy). This is often followed over time by an intimal cellular proliferation, in which smooth muscle cells penetrate the internal elastic lamina and their proliferation at this point may occlude the lumen. They are often accompanied by a proliferation of both collagen and elastin fibers within the intima as well.[4] At this point, dilatation lesions will develop proximally and concurrent fibrinoid necrosis in the wall of the artery begins the final development of the plexiform lesion as described above.[4]



Lung, dog. Affected vessels occasionally contain extruded brightly eosinophilic protein and cellular debris within their walls (fibrinoid necrosis.) (HE, 400X)

In humans, plexogenic arteriopathy may arise



Lung dog – There is often polymerized fibrin, increased numbers of alveolar macrophages, polymerized fibrin, and patchy type II pneumocyte hyperplasia within proximity of plexiform lesions. (HE 282X)

as a result of congenital cardiac shunting, but may also be seen with acquired cardiac shunts, hepatic cirrhosis with portal hypertension, schistosomiasis, and oral ingestion of drugs for anorexia.[4]

The first description of pulmonary plexiform arteriopathy (PPA), also in a Welsh Corgi, was published in 2004, and its etiology was attributed to an untreated patent ductus arteriosus (the animal was 21 months at its death.)[2] PPA was also identified in 4 of 6 animals in another study of dogs with idiopathic pulmonary hypertension. Plexiform lesions were identified primarily at branching points of terminal to alveolar duct arteries.[6]

Plexiform lesions have also been identified in a line of rapidly growing broiler chickens which had a high incidence of idiopathic pulmonary arterial hypertension; however, as opposed to other species, plexiform lesions could be identified immediately post-hatch and did not require a period of pulmonary arterial hypertension.[5] A line of genetically engineered mutant mice that develop plexiform arteriopathy has been developed as a result of decreased expression of intersectin-1s.[3]

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