Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 2

5 September 2018

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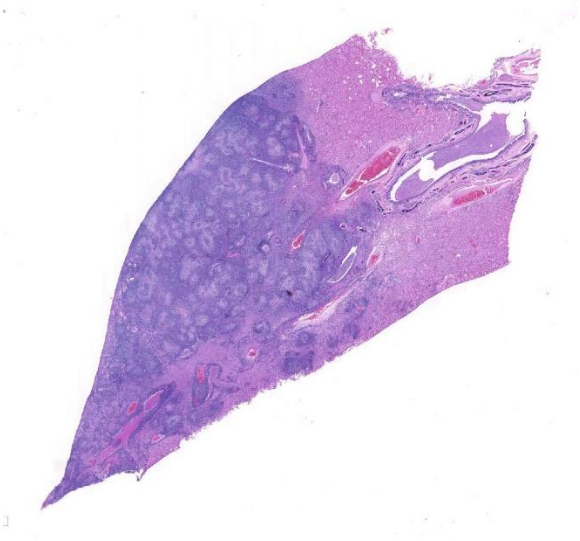
CASE I: 21-168-14/12-168-17 (JPC 4019882).

Signalment: 18-month-old male ferret (*Mustela putorius furo*)

History: This ferret was first initially diagnosed with ferret granulomatous coronavirus-associated disease at about six months of age at the private veterinary referral clinic. Initial clinical findings at that time included diarrhea, lethargy, anorexia, and a 4 cm mesenteric mass. Biopsy of this mass confirmed a diagnosis of granulomatous coronavirus-associated disease. The ferret was maintained by supportive care including occasional antimicrobial therapy to treat diarrhea and lethargy, sucralfate, potassium supplements, and hand feedings of highly palatable soft foods. After continued decline over many months, the ferret was euthanized one year after the initial diagnosis and the carcass submitted for gross and histopathological examination.

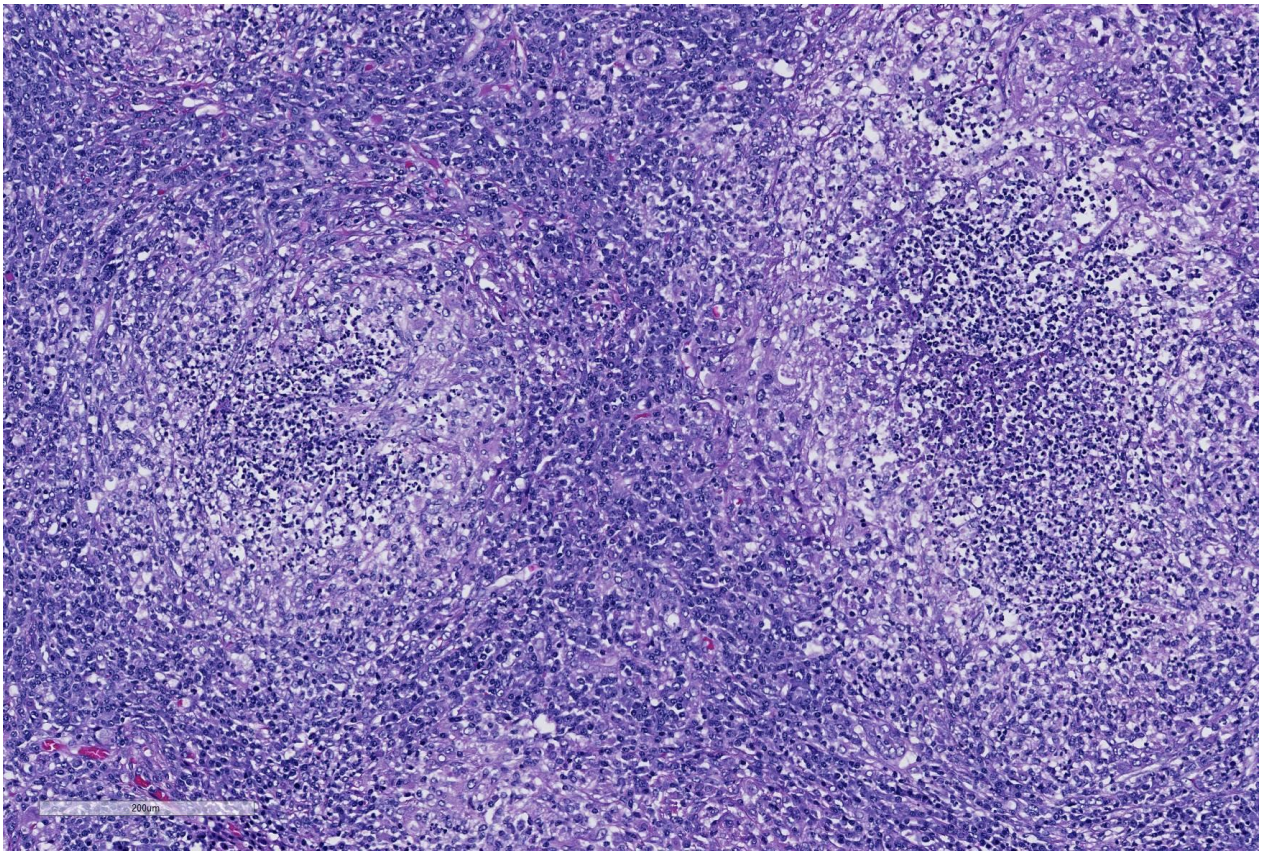


Lung, ferret. Approximately 50% of the section is effaced by multifocal to coalescing granulomas. (HE 5X)

Gross Pathology: Gross postmortem findings included multiple firm tan 1-5 m diameter nodules throughout the mesentery, bilateral pale cortical pitting lesions on the kidneys, multifocal coalescing raised white nodules on the lungs with complete consolidation of the accessory lobe, and splenomegaly with pinpoint pitted lesions.

Laboratory results: CBC and serum chemistry analysis performed at a commercial diagnostic laboratory revealed anemia (PCV 23%, reference, 43-55), leukocytosis ($14x10^3$/um/L) with 89% lymphocytes, mild hypoalbuminemia (1.6 g/dL; reference, 2.6 to 3.8 g/dL) and mild hyperglobulinemia (3.6 g/dL; reference, 1.8 to 3.1 g/dL). Abdominal radiographs revealed a poorly defined area of increased opacity in the midabdomen apparent on the lateral but not ventrodorsal views.[2]

Microscopic Description: Lung**:** Depending upon the section examined, there is either marked diffuse or severe large segmental effacement and expansion of the normal pulmonary parenchymal architecture by variably sized discrete and fairly well organized nodular foci (granulomas). The granulomas/granulomatous foci are comprised of a central variable sized core of necrotic cellular debris surrounded by numerous large epithelioid macrophages with indistinct cell borders, abundant granular to vacuolated to clear cytoplasm, and large oval indented to marginated nucleus. The macrophages are in turn encircled by numerous plasma cells, lymphocytes and fewer neutrophils, which are in turn encircled by fibroblasts and variably thick band dissecting fibrous connective tissue. Variably within the sections, sparse to abundant non–staining clear needle like acicular crystals (cholesterol clefts) are often associated closely with multinucleated (macrophages) giant cells (Langhans' and foreign body type) within the granulomatous foci. The remaining



Lung, ferret. The inflammatory process is characterized by confluent, poorly formed pyogranulomas or granulomas with a central core of degenerate neutrophils and cellular debris, surrounded by layers of epithelioid macrophages, in turn surrounded by lymphocytes and plasma cells. HE, 200X)

discernible alveolar septa and lumen are expanded by numerous type II pneumocytes and large epithelioid macrophages, variable numbers of plasma cells/lymphocytes, degenerate neutrophils and necrotic cellular debris and low to abundant intraluminal proteinaceous material (edema). The bronchial and bronchiolar lumen when discernible are filled with eosinophilic proteinaceous material, mucous, epithelial and inflammatory cellular debris, associated variable bronchial epithelial hyperplasia, dense peribronchial aggregates of lymphocytes and plasma cells. In many regions, the bronchioles are obliterated by fibrosis or are severely necrotic and replaced by inflammatory cellular debris. There is also prominent lymphoplasmacytic and histiocytic perivasculitis (mostly perivenular), and in some instances, vasculitis characterized by adventitial and medial expansion by edema and inflammatory cells and/ or mild mural/degeneration/necrosis. Multifocal moderate alveolar septal necrosis and interstitial and pleural fibrosis is also noticed. Rarely, dystrophic calcification and bone formation is noticed within the granulomatous foci.

Special stains:

PAS/ GMS:

- Lung: Negative for fungal organisms

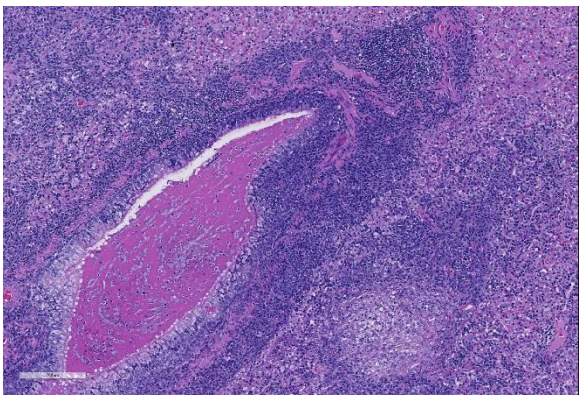
Ziehl-Neelson Acid fast stain

- Lung, mesenteric lymph node, stomach, intestine: Negative for acid fast bacilli.

Electron Microscopy:

Mesenteric lymph node:

Ultrastructurally, coronavirus-like particles were identified in examined tissues inside macrophages, either within cytoplasmic vacuoles or free in the cytosol (Representative images provided).

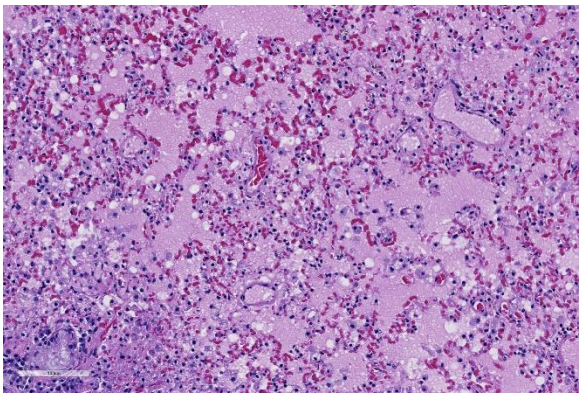


Lung, ferret. Airways contain abundant proteinaceous material, moderate numbers of degenerate neutrophis and are surrounded by profound BALT hyperplasia, consistent with the clinical history of aspiration. (HE, 100X)

Contributor's Morphologic Diagnoses: Lung: Pneumonia, severe, coalescing to diffuse, chronic, organizing, necro-granulomatous with multinucleated giant cells, cholesterol clefts, pulmonary edema and fibrosis.

Contributor's Comment: This case is an interesting example of the recently recognized ferret systemic coronavirus-associated disease resembling feline infectious peritonitis (FIP)-like disease syndrome in ferrets. This relatively new disease entity in ferrets was first recognized in Europe in late 2005-early 2006 [5,8] and since then has been systemically characterized in the US [5,12,17] and more recently identified in Japan[9]. The clinical disease and the lesions in all these described cases are similar to those observed with the "dry" form of FIP.[1] The differentials considered for this case include myco-bacteriosis and systemic fungal infections, however no acid–fast bacilli nor fungal organisms were identified.

Coronaviruses are enveloped, pleomorphic, positive-strand RNA viruses with a diameter or 60-220 nm and classified under the genus *Coronavirus* within the family Coronaviridae, order Nidovirales.[1] The viral particles typically develop in the



Lung, ferret. Alveoli in less affected areas are filled with abundant foamy edema fluid. (HE 211X)

cytoplasm of affected epithelial cells and macrophages inside vacuoles and form characteristic radiating peplomer spikes on the surface of the envelope, a diagnostic feature in EM,[5,8-9,16]

Ferret coronaviruses can cause two distinct clinical conditions, namely epizootic catarrhal enteritis (ECE) in association with ferret enteric coronavirus (FECV)[8,12,15-16] and the more recent ferret systemic coronal viral disease caused by ferret systemic corona virus (FRSCV) with a clinical picture resembling the "dry" form of feline Infectious peritonitis (FIP)[5,12,16]. ECE was first observed in the spring of 1993 on the East Coast of United States and a detailed description of the disease and its association with corona virus was first published in 2000.[15] Unlike FRSCV associated FIP-like disease in ferrets, ECE affects all age groups and is more severe in aged ferrets.[15] ECE is primarily an enteric disease with lesions consistent with intestinal coronaviral infection such as vacuolar degeneration and necrosis of villous enterocytes, villous atrophy, blunting and fusion, and lymphocytic enteritis.[8,12,15-16]

Feline coronavirus (FCoV) typically infects domestic and exotic felids. Two biotypes of FCoV include feline enteric coronavirus (FECV) and feline Infectious peritonitis virus (FIPV). FECV replicates in enterocytes causing clinical disease resulting in diarrhea or an asymptomatic infection, whereas FIPV replicates in macrophages resulting in a systemic infection. Both "wet" and "dry" forms of feline infectious peritonitis (FIP) occur depending upon the immune status of the animal. Histologically, pyogranulomatous inflammation involves multiple tissues predominantly with a vasculocentric distribution targeting small arterioles and venules are hallmarks of this disease.[1] In a similar fashion, ferret systemic coronaviral disease also causes pyo-granulomatous to granulomatous lesions and /or vasculitis in multiple organs including the spleen, mesenteric lymph nodes, intestine, kidneys, brain etc. The common clinical signs associated with the systemic form include anorexia, weight loss, diarrhea and large palpable intraabdominal masses. Less frequent findings included hind limb paresis and central nervous system signs. Immunoreactivity with FIP feline coronaviral antigen (FCoV) on paraffin sections and/or serum samples has been demonstrated by immunohistochemistry in all these cases within the granulomas.[5,9,12,15] It is thought that the positive immunoreactivity is due to cross-reactivity from common antigenic determinants within different host specific corona viruses.

In this case, in addition to the lung lesions, discrete granulomas or coalescing granulomatous inflammatory foci were also observed in the mesenteric nodes, mesentery, pancreas, intestinal serosa and the spleen. Cholesterol clefts were not observed within these tissues and multinucleated cells were also sparse. It is of interest to note that cholesterol clefts and the associated presence of numerous multinucleated cells are not typical of earlier descriptions of this disease and even in these cases it was primarily a feature of the lung lesions only and the underlying pathophysiological basis is

unclear. Immunohistochemistry for feline coronaviral antigen (FCoV) was not performed on this case for confirmation time of submission. However, on electron microscopic evaluation of the mesenteric lymph node granulomas, enveloped coronavirus-like particles, some with radiating spikes were identified within macrophages, either inside cytoplasmic vacuoles or free in the cytosol.

In this individual, the kidneys exhibited predominant plasmacytic interstitial nephritis with occasional granulomatous foci and severe chronic glomerulonephritis and mild perivasculitis. The distribution and nature of the lesions can mimic those seen with chronic Aleutian disease (AD) caused by distinct parvoviruses in minks and ferrets. AD is characterized by progressive wasting, weight loss, hypergammaglobulinemia, plasmacytosis, interstitial pneumonia in young kits, chronic glomerulonephritis, lymphoplasmacytic interstitial nephritis and prominent arteritis involving kidneys and other organs.[13-14] However, in this case we did not have information on the tests for immunoglobulin levels nor additional serology or viral diagnostic tests to rule out a concomitant chronic Aleutian disease.

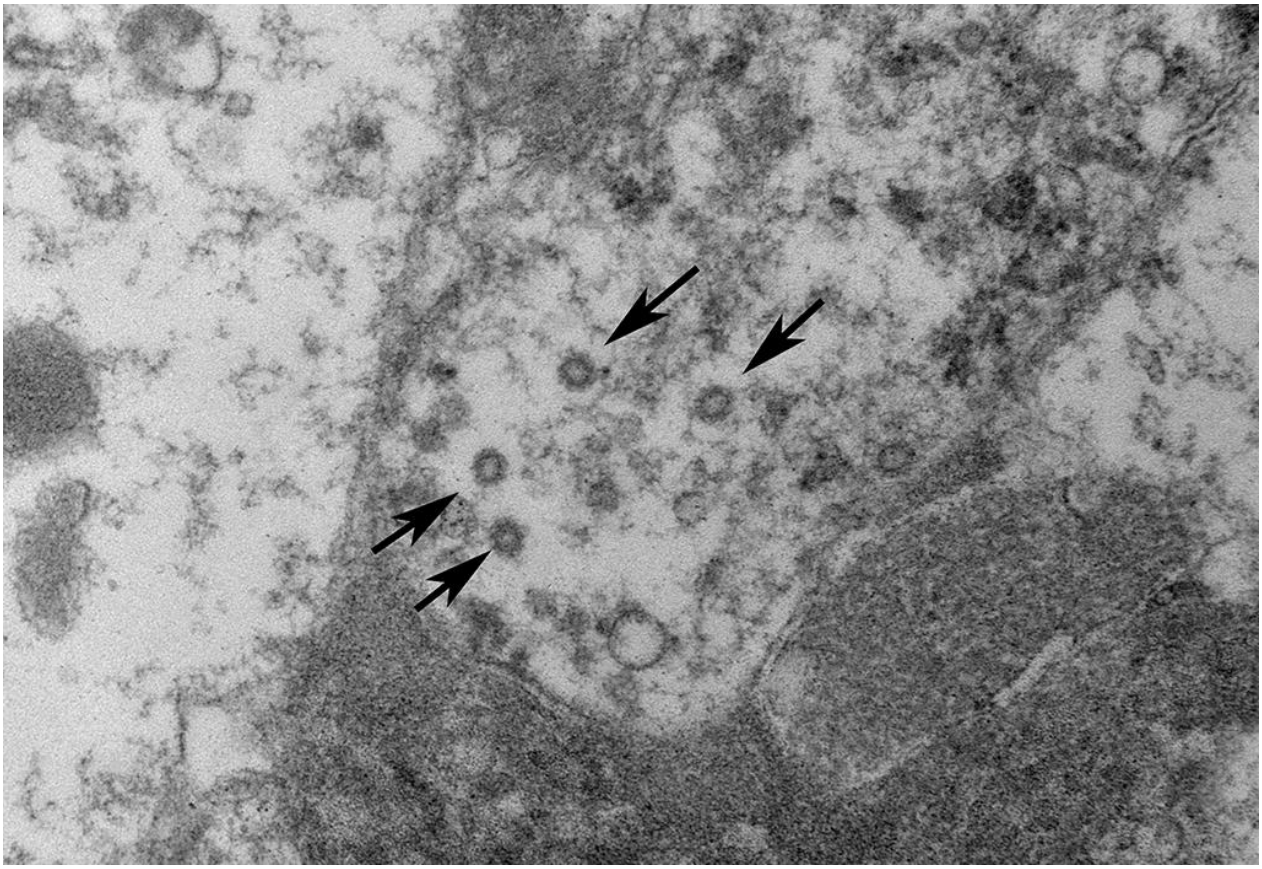
JPC Diagnosis:

1. Lung: Pneumonia, interstitial, pyogranulomatous, multifocal to coalescing, severe.
2. Lung: Bronchopneumonia, neutrophilic and necrotizing, multifocal, mild, with marked BALT hyperplasia and intrabronchiolar proteinaceous material.
3. Lung: Pneumonia, interstitial, histiocytic, multifocal, mild, with intraalveolar *Pneumocystis* trophozoites and cysts.
4. Lung, subpleural alveoli: Histiocytosis, focally extensive, mild (endogenous lipid pneumonia.)

Conference Comment:

Since the submission of this case and 2012 for consideration in the Wednesday Slide Conference, there has been extensive additional work in the investigation of this important disease of both patent and laboratory ferrets. This particular case itself was published as part of a 5 case study by Autieri et al. in 2015.[2] This review was composed of animals from multiple sources including private pets as well as laboratory animals from accredited facility. In addition to identifying cases from seven countries on four continents. The literature review for this publication also identified an interesting report from Denmark in 1951[11] of a syndrome in 142 ferrets, many less than a year of age, with strikingly similar clinical signs and pathologic findings. The syndrome, known as ends are wanted malignant granulomatosis, demonstrated granulomatous inflammatory changes in lymph nodes, spleen, and liver and histochemical stains failed to disclose the presence of bacteria, fungi, and protozoa as well as negative cultures for aerobic, anaerobic, and mycobacterial bacilli.[11]

Continuing investigation of ferret coronavirus-associated disease, which has no definitive treatment and is considered invariably fatal in affected individuals, has resulted in the sequencing of the viral genome of two different FECRV strains in Japan.[6] This work has determined that the virus shares 50-69% nucleotide sequencing with known coronaviruses suggest doing that the ferret enteric coronavirus (FRCoV) might be classified as a new species in the genus *Alphacoronavirus*.[6] additionally, and enzyme-linked him enzyme linked immunosorbent assay (ELISA) using recombinant partial nucleocapsid proteins of the FRCoV Yamaguchi-1 strain was developed to establish a method for detection of FRCoV from blood sampling.[10] Not



Lung, ferret. Ultrastructural examination of macrophages from the center of one of the granulomas reveals coronaviral particles free within the cytoplasm. (Photo courtesy of: Massachusetts Institute of Technology, 16- 849, Division of Comparative Medicine, 77 Massachusetts Ave, Cambridge, MA 02139. https://web.mit.edu/comp-med/)

surprisingly, as anyone who was worked with this virus can understand, 89% of tested ferrets in Japan have been infected with ferret coronavirus.[10]

In Spain, Doria-Torr et al[3] investigated the inflammatory response and antigen distribution of FRCoV infection and compared it to similar studies of FIP in cats. The group identified four distinct types of granulomas in affected animals including those with necrosis, without necrosis, with neutrophils as a central core, and diffuse granulomatous inflammation, very similar to the inflammatory response demonstrated by cats infected with mutated coronavirus. Close inspection of the slide submitted for this case will demonstrate most, if not all of the various types of granulomas discussed in this paper. The authors[3] postulate that the various morphologies might be a consequence of different episodes of viremia, as described in cats with FIP. The authors also noted that vasculitis was an uncommon finding, which has been noted in other reviews of this disease. Other FIP-like syndromes that have recently been described in single case reports since the submission of this case include pyogranulomatous pano-phthalmitis as well as membrano-proliferative glomerulonephritis.

Additional clinicopathologic features of ferret coronavirus infection that bear mention in review includes the progressive nature of this disease of juvenile and young adult ferrets which has an average survival time following diagnosis of 69 days[5]. The most

common clinical signs of affected ferrets include weight loss, a palpable intra-abdominal mass or masses, lethargy and anorexia.[5] Hematologic findings are nonspecific for this disease and many may be within normal reference levels; the most common hematologic abnormality in affected animals is a polyclonal gammopathy.[5] Histologically, Garner at al review many of the same lesions noted in this particular case, however their study identified inflammation involving the adventitial and medial tissue next of small veins and venules[5], which was not particularly prominent in this slide. The EM findings in this study[5] are similar to those noted by the contributor of this case, with intracytoplasmic virus particles found free within the cytoplasm of macrophages within the center of granulomas.

Careful inspection of the regions of the section in which granulomatous inflammation is not present (and in which the primary lesion is marked alveolar edema, will disclose the presence of numerous vacuolated histiocytes admixed with fewer lymphocytes in the alveoli immediately (<1mm) subjacent to the pleura. This is a characteristic incidental finding in ferrets, resulting from endogenous lipid pneumonia.

The composition of the JPC morphologic diagnosis above, due to multiple simultaneous pathogeneses in the submitted tissues, was a subject of spirited debate. The attribution of secondary inflammatory changes to a particular etiology was further complicated due to the history of prolonged therapeutic immunosuppression. The JPC tradition of assigning a separate morphologic diagnosis to each distinct process was ultimately upheld in the post-conference signout session.

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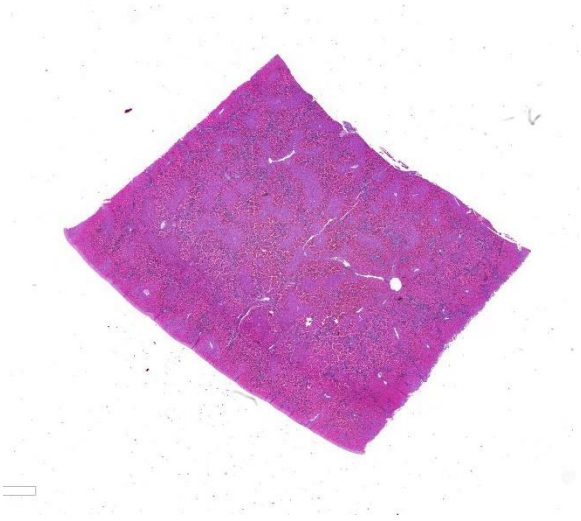
CASE II: 13V1542 (JPC 4034956).

Signalment: 1 year old, female, Hereford heifer

History: Tissue samples sent in formalin from a post-mortem done by the referring veterinarian from Victoria, Australia, in March 2013 with the following history. 14 months old Hereford heifers in good condition in high country on lush green pasture. Vaccination program and management good and properly done. 1 x died 6 days ago. Lost 6 last year from this



Liver, ox. A section of liver with a retiform pattern of periportal hemorrhage is submitted. (HE, 5X)

paddock. Dead on arrival. Showed ataxia before died.

Gross Pathology: Submitted by referring veterinarian. Petechiae through omentum. Abomasum petechiae and filled with brownish blood-tinged fluid. Petechiae and ecchimoses on pericardium, epicardium and endocardium.

Laboratory results: None.

Microscopic Description: Liver; severe periportal hepatocellular necrosis extended throughout all liver sections examined and involved up to 50% of the hepatocytes in each lobe. The degenerate hepatocytes had been replaced with blood and neutrophils. Portal areas contained moderate biliary hyperplasia and mild neutrophil infiltration.

An unidentified artifact similar to acid hematin was present throughout all sections.

Contributor's Morphologic Diagnoses: Liver; severe acute disseminated periportal hepatocellular necrosis, moderate biliary hyperplasia and moderate neutrophilic hepatitis.

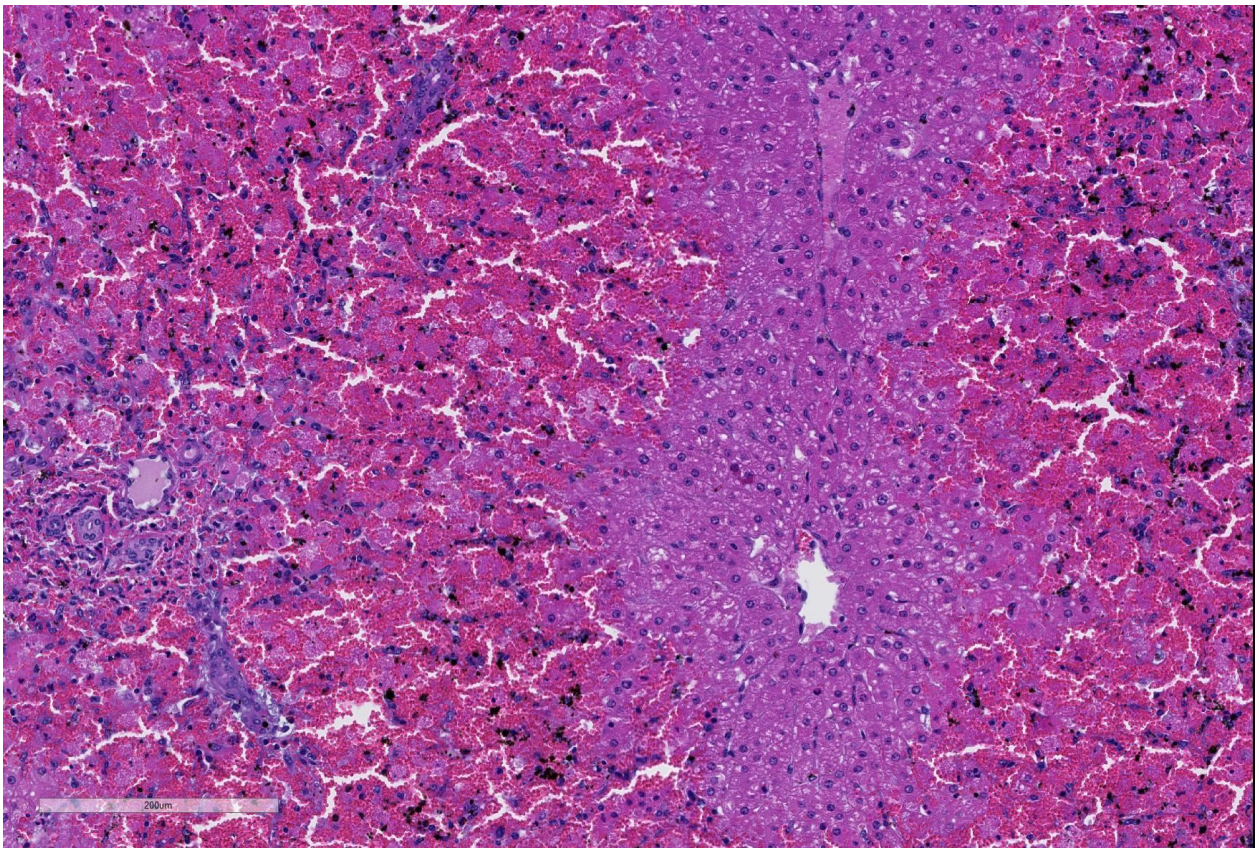
Contributor's Comment: The hepatic lesion is very severe and sufficient to explain the sudden death of the heifer. Periportal hepatocellular necrosis is a relatively rare lesion in cattle and is typical of ABLD (acute bovine liver disease). ABLD typically strikes in autumn in the south eastern areas of Victoria, Australia and affects dairy and beef cattle. Clinical signs of the disease include decreased milk yield and sudden death with photosensitization developing in survivors. The cause is presumed toxic but the agent has never been defined.

It has been associated with rough dog's tail grass (*Cynosurus echinatus*), but no toxic agent has been found in this grass. The known phytopathogenic fungus *Drechslera biseptata* has been isolated from *C. echinatus* collected at the time of, and from the sites of, outbreaks of ABLD and is tentatively associated with its pathogenesis[1]. Whether there are other hepatotoxins involved is not known. An attempt to reproduce the disease in cattle by feeding *Cynosurus echinatus* mixed with an inoculum of *Drechslera biseptata* grown from an isolate taken from a previous outbreak of ABLD was not successful[2].

The petechiation described in the history is also a common PM change of this condition. The slides contained an artifact similar to acid hematin but could not be removed with the usual laboratory treatment for acid hematin. The artifact was not identified.

JPC Diagnosis: Liver: Hepatocellular necrosis, periportal to midzonal, diffuse, with hemorrhage.

Conference Comment: Although there is current heightened sensitivity among cattle



Liver, ox. A portal area is at left, and centrilobular vein at right. Periportal hepatocytes are individualized, many lost, and largely replaced by hemorrhage. Kupffer cells and sinusoidal architecture remains. Hemorrhage extends into the portal areas as well. At right, midzonal and centrilobular hepatocytes are mildly swollen by numerous distinct small lipid vacuoles, occluding sinusoids. There is abundant acid hematin. (HE, 167X)

producers in Australia and New Zealand to the clinical effects and implications of acute bovine liver disease, intensive research into its pathogenesis has not yet been published. An excellent review by Reed et al.[4] was published subsequent to the submission of this case and is summarized below.

Acute bovine liver disease (ABLD) commonly formerly known as phytotoxic hepatitis, affects grazing beef and dairy cattle regardless of age, sex or breed. Clinical signs consistently include an initial drop in milk production, photosensitization, an altered behavior such as seeking shade on overcast days. Depression, pyrexia, appetite loss, and agitation may be observed in some cattle, and acute cases may result in death prior to display of clinical signs. Serum biochemistries are nonspecific, including marked elevation of aspartate transaminase, glutamate dehydrogenase, and moderate increases in gamma-glutamyl transpeptidase activity. Histologic features of acute disease are as seen in this slide, with periportal necrosis as a characteristic feature. The pathology seen in chronic cases has yet to be described.[4]

The nonspecific signs and lesions as well as the sporadic and unpredictable nature of deaths with this condition complicate the investigation of ABLD occurrences. Outbreaks often occur on pastures which contains senescent rough dog's tail grass (*Cynosurus echinatus*), however this plant is found worldwide and does not appear to cause illness on its own. Feeding trials with

this plant have demonstrated no ill effects in several studies.

Because of the similarity of the epidemology of ABLD to other mycotoxicoses, fungal infections of rough dog's tail grass are suspected. The most common fungal species identified in ABLD occurrences include *Colletotrichum graminicola* and *Drechslera* sp. aff *siccans*, with *D. biseptata* and *Colleotrichum* sp. aff. *coccodes* were also identified less commonly. As *Colletotrichum* sp., has not been reported to produce mycotoxins, leaving *Dreschlera* as the more likely candidate. Aslani et al.[1] identified *in vivo* hepatotoxicity from extracts of the spores and mycelia of *D biseptat*a who's mass spectral profile implies a cytochalasin-like activity in rat hepatocytes *in vivo*. This research also demonstrated a lower level of toxicity from spores alone as compared to extracts from mycelia, or mycelia and spores combined.

As most ABLD occurrences occur in the autumn, current thought is that weather change may stimulate production of fungal toxins or toxic spores in infected grasses. Cattle producers are recommended to be vigilant for signs of disease in this time of the year when cattle are grazing high risk pasture. Additionally, sheep may be used to graze out a high risk pasture, as they do not appear to be susceptible, or at least less susceptible to the effects of the putative toxin.

Periportal hepatocellular necrosis is an uncommon pattern of hepatocellular injury caused by a limited subset of toxins in veterinary medicine.[2] The majority of these toxins may not require metabolism by the mixed function oxidases of centrilobular hepatocytes, and simply overwhelm the initial hepatocytes they encounter as they enter the liver from the portal venous circulation. Other toxins may simply require metabolism by the limited amounts of biotransforming enzymes enzymes present within periportal hepatocytes to exert their effect. Additionally, periportal necrosis may be seen in toxins that are excreted in the bile, and there are damage is enhanced by cholestatic disease. Toxins which have been identified in veterinarian medicine as characteristically resulting in periportal necrosis include elemental phosphorus, allyl alcohol, and boobialla (*Myoporum tetandrum*) another toxic plant found in Australia which contains furanoid sesquiterpene oils as the toxic principle.[1]

The lack of traditional descriptors for chronicity and severity is a consequence of the choice of the term "necrosis" as the main feature of this slide. As necrosis is neither acute nor chronic, nor are there levels of severity of necrosis, we generally do not use these terms as modifiers. An estimation of extent of necrosis is probably more appropriate than "severity" in these cases, and diffuse gives an excellent picture of the extent of necrosis seen here, with all periportal hepatocytes in the section being necrotic.

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References:

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CASE III: VPL 1 (JPC 4066919-00).

Signalment: A 12-year-old female spayed Galgo Español, dog, *Canis lupus familiaris*

History: During a routine dental cleaning procedure, a polypoid pedunculated mass on the left palatine tonsil was discovered. The mass has been removed surgically. There have been no other signs of illness during clinical examination.

Gross Pathology: Submitted by referring veterinarian. Petechiation was seen throughout the omentum. There were petechiae in the abomasum which was also filled with brownish blood-tinged fluid. There were petechiae and ecchymoses on pericardium, epicardium and endocardium.

Tonsil, dog: Gross morphology reveals a pedunculated and polypoid appearance and a delicately nodular surface. (Photo courtesy of: Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Germany http://patho.vetmed.uni-leipzig.de)

Laboratory results: None.

Microscopic Description: The mass mainly consists of soft tissue and is covered by a nonkeratinized pluristratified epithelium showing variable degrees of hyperplasia. The epithelium multifocally shows mild to moderate intracellular oedema and there also is a mild to mainly moderate epitheliotropism of mainly lymphocytes but also some neutrophils. Multifocally, the epithelium tends to form crypts which are filled with an eosinophilic material, scaled off epithelial cells, lymphocytes, neutrophils and cellular debris. There are multiple densely-arranged subepithelial areas consisting of mononuclear cells, which can be identified as well-differentiated slightly anisocytotic lymphocytes and a few plasma cells. Partially, the lymphocytes form follicular nodules which show an appearance of primary and secondary lymphoid follicles. Altogether, the histomorphologic structures resemble tonsillar tissue, which is consistent with the localization of the tumor. The center of the mass is formed by collagenous connective tissue, which shows mild to moderate edema. Embedded in that fibrous stroma, numerous dilated and ectatic vascular structures can be seen. Some of them are filled with erythrocytes and resemble arterial and venous blood vessels. Others, mostly thin-walled, are filled with varying amounts of slightly eosinophilic fluid and a few cellular components (lymphocytes and macrophages) or they appear to be empty. These vascular structures may be identified as lymphatic vessels. All of the vessels are lined by a one-layered well-differentiated endothelium. In some slides, there are seromucous glands at the base of the mass, which seem to be salivary glands.

Contributor's Morphologic Diagnoses: Tonsil: lymphangiomatous tonsillar polyp

Contributor's Comment: To characterize the involved cellular components an immunohistological examination was performed. The lymphocytes were immunopositive for CD3 and CD79a and showed the expected distribution patterns for tonsillar tissue. Some of the cells mostly adjacent to small vessels or inside of supposedly lymphatic vessels could be identified as macrophages via expression of MAC387 and lysozyme. While blood vessels often could easily be identified due to the presence of intravascular erythrocytes or thick vascular walls, there were some difficulties in the direct differentiation of lymphatic vessels and thin-walled venous vessels, especially when they appeared to be empty. For that reason, the endothelium was immunolabeled using markers specific for lymphatic endothelium (LYVE-1 and Prox1.[9] While not all lymphatic vessels were immunopositive regarding LYVE-1 (Lymphatic vessel endothelial hyaluronan receptor 1), Prox1 (Prospero homeobox 1) was widely expressed by the lymphatic vessels in the tissue sample, which is consistent with the findings of previously performed studies on immunohistochemical labelling of blood and lymphatic vessels in dogs[9].

The pathogenesis of lymphangiomatous tonsillar polyps is still unclear. There are different theories about the origin of this benign proliferation. Because of the haphazard proliferation of components which are usually found in the tonsil they are discussed to be hamartomatous proliferations[5] and therefore classified as malformations. One author sees them as the result of lymphatic obstruction with consecutive congestion and mucosal prolapse finally forming the polyp[11]. In veterinary medicine those polyps are also considered being a result of chronic recurrent tonsillitis and are regarded as inflammatory polyps which occur infrequently in old dogs[5]. Anyhow, the definite cause remains uncertain.

As in this case such polyps are often undiscovered for a long period of time. They can be asymptomatic or, probably when large enough, they may cause dysphagia[6], non-productive cough[11] or a sore throat[1]. In

Tonsil, dog: The cut surface shows an oedematous fibrous stock and multinodular white foci in the margin areas. (Photo courtesy of: Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Germany http://patho.vetmed.uni-leipzig.de)

human medicine, the complete surgical excision is normally curative and there usually are no recrudescences.[5]

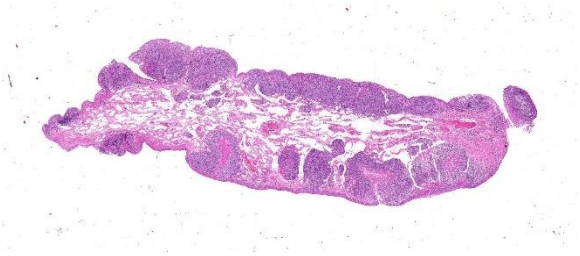
This case shows that such benign proliferations might be considered as differential diagnosis when there is a unilateral mass of the tonsils, especially concerning tonsillar squamous cell carcinoma, even though regional lymph nodes are usually enlarged early in such cases.[7]

JPC Diagnosis: Tonsil: Lymphangiomatous polyp.

Conference Comment: The contributor has provided an excellent review of this uncommon but morphologically unique lesion often seen in association with chronic tonsillitis in older dogs.

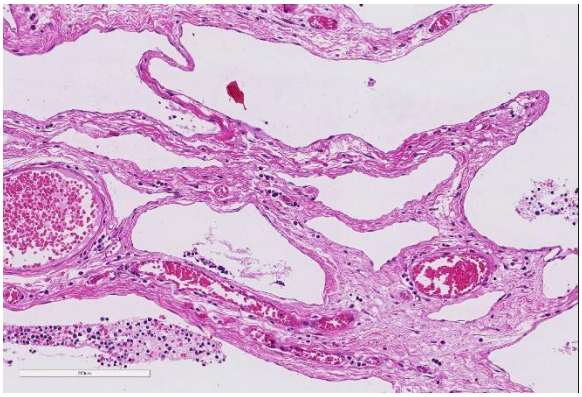
The tonsil samples antigens which are dissolved in the saliva, and develops immune responses akin to other lymphoid organs throughout the body. A layer of non-keratinizing squamous epithelium provide barrier function for the tonsil.[10]

A unique feature of the tonsils is their lack of afferent lymphatic vessels. This prevents them from being effective lymphoid filter for structures in the oral cavity (which drains instead primarily to pharyngeal and submandibular nodes)[3], but also significantly reduces the possibility of finding metastatic tumors within the tonsils. As a general rule, tonsillar neoplasms arise from tissues native to the tonsil itself- squamous cell carcinoma, lymphoma, and rare tumors of the vascular and lymphatic endothelium.



Tonsil, dog: Subgross examination of the submitted polyp reveals a marked expansion of the connective tissue component by abundant clear space. There is moderate follicular hyperplasia. (HE, 7X)

The tonsils also serve as a reservoir or portal vein treated for a variety of viral and bacterial



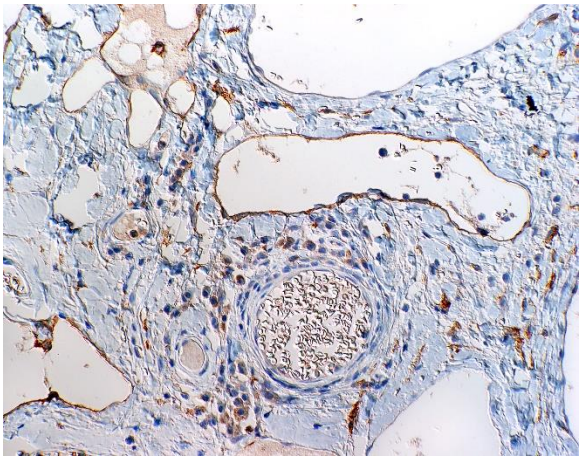
Tonsil, dog. Clear spaces are endothelial lines (presumably lymphatics) and are separated by loosely arranged fibrous connective tissue which contains few lymphocytes and plasma cells. (HE, 198X)

agents.[10] A number of viral agents including pseudorabies in swine and parvovirus in carnivores may utilize the tonsil as an initial site of replication and dissemination. Depending upon the particular virus and stage infection, either involution or hyperplasia of tonsillar follicles may be the result. A significant percentage of swine carry *Erysipelothrix rhusiopathae*, *Streptococcus suis*, and various species of salmonellae within the tonsils as well.[10] Additionally, the scrapie-associated prion protein has been identified in tonsillar tissue as well.[10]

The lymphoid changes seen in the tonsils parallel those seen in reactive lymph nodes with a number of primary follicles surrounding tonsillar crypts. Following antigenic stimulation, development of secondary follicles with mantle zones

extending in the direction of the antigenic stimulus will occur, as well as marked expansion of the interfollicular lymphoid tissue. The majority of histologic changes seen in the biopsy samples of excised tonsils will be chronic in nature; acute tonsillitis is rarely if ever seen. The presence of neutrophils within crypts, crypt epithelium and most importantly with in the parenchyma of the tonsils is required for the diagnosis of acute tonsillitis. The histologic changes of chronic tonsillitis are indistinguishable in most cases from tonsillar hyperplasia.

An appropriate ruleout in this case would be that of a lymphangioma. Lymphangiomas are uncommon benign proliferations of lymphatic derived thin walled cystic spaces often filled with pink, watery proteinaceous fluid.[12] These benign tumors may be found as isolated findings in lymphoid tissues including the lymph node and spleen. The abundant fibrous connective tissue seen in lymphangiomatous polyps is generally not a feature of lymphangiomas, and provides additional support as tonsillar lymphangiomatous polyps are hamartomatous lesions rather than true neoplasms.[5]



Tonsil, dog: Endothelial cells of dilated lymphatic vessels exhibit strong immunopositivity for LYVE-1, while endothelial cells of blood vessels are immunonegative. (Nomarksi Differential Interference Contrast microscopy, 400x) (Photo courtesy of: Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Germany, http://patho.vetmed.uni-leipzig.de)

A very interesting, morphologically distinct lesion of lymphoid tissue that is occasionally seen in the dog and cat is vascular transformation of lymph node sinuses (previously referred to as nodal angiomatosis). This lesion has not yet been reported in the tonsil as of yet. Gelberg and Valentine reported this change in association with thyroid carcinoma in a dog. [4] Vascular transformation of lymph node sinuses is a pressure-induced non-neoplastic conversion of nodal sinuses into anastomosing vascular channels. The inciting pressure is often the result of obstruction of venous or hilar lymphatic drainage and has been reported in humans in association with a variety of neoplasms, as well as various forms of venous obstruction. Vascular transformation may take a variety of appearances believed to be the result of a continual related to the duration and degree of venous or lymphatic obstruction.[4] The plexiform pattern, the most commonly reported in animals, presents as a mass lesion composed of interconnected mature vascular channels, a flat endothelial cell lining, and a low mitotic rate.[10] More chronic cases are associated with increased amounts of collagen separating proliferating vessels. In a second variant, the round vascular type, vascular changes are usually empty or contain amorphous material presumed to be lymph[10]. A recently published review by Xu et al., excellently describes this and other vascular and stromal proliferations in the lymph nodes of humans.[12]

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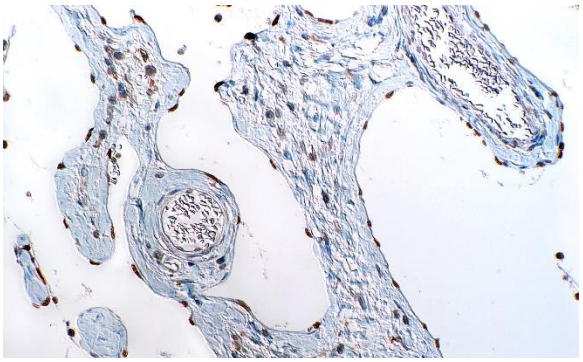

Tonsil, dog: Endothelial cells of dilated lymphatic vessels label intranuclear immunopositive for Prox1, while endothelial cells of blood vessels are immunonegative (Nomarksi Differential Interference Contrast microscopy, 400x) (Photo courtesy of: Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Germany, http://patho.vetmed.uni-leipzig.de)

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CASE IV: 1/12 (JPC 4066919-00).

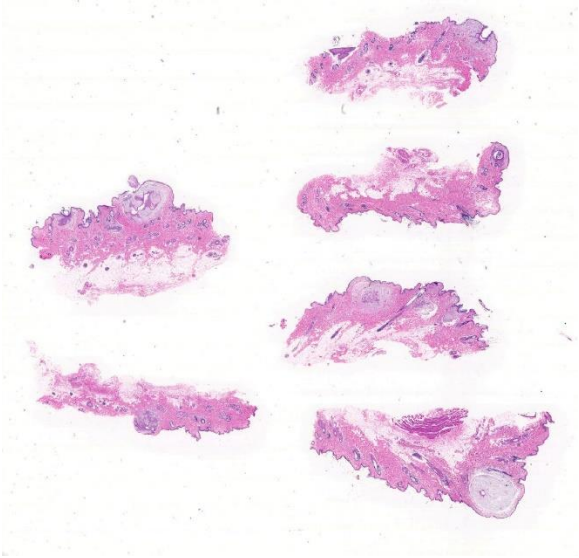
Signalment: Ten-month old, mixed breed, female spayed, canine

History: Multiple diffuse papules. No pruritus.

Gross Pathology: Not available.

Laboratory results: None.

Microscopic Description: Haired skin: All sections have similar histological



Haired skin, dog. Multiple expansile nodules containing proliferative follicular epithelium are present within the superficial dermis. (HE, 5X)

characteristics with variations in intensity. The epidermis is minimally hyperplastic to normal. There is formation of multiple raised dermal nodules, varying from 0.5 to 2mm in diameter. The nodules consist of a large dilated hair follicle with hyperplastic epithelium forming arborizing cords growing into a perifollicular accumulation of myxomatous material. The latter constitutes the bulk of the mass and produces its nodular shape. In several nodules there is a central 20-50µm thick band of deep eosinophilic hyaline material. This hyaline band surrounds a focus of necrotic debris, or, in rare follicles, a degenerated mite larva. There is minimal superficial perivascular dermal mononuclear cell infiltration.

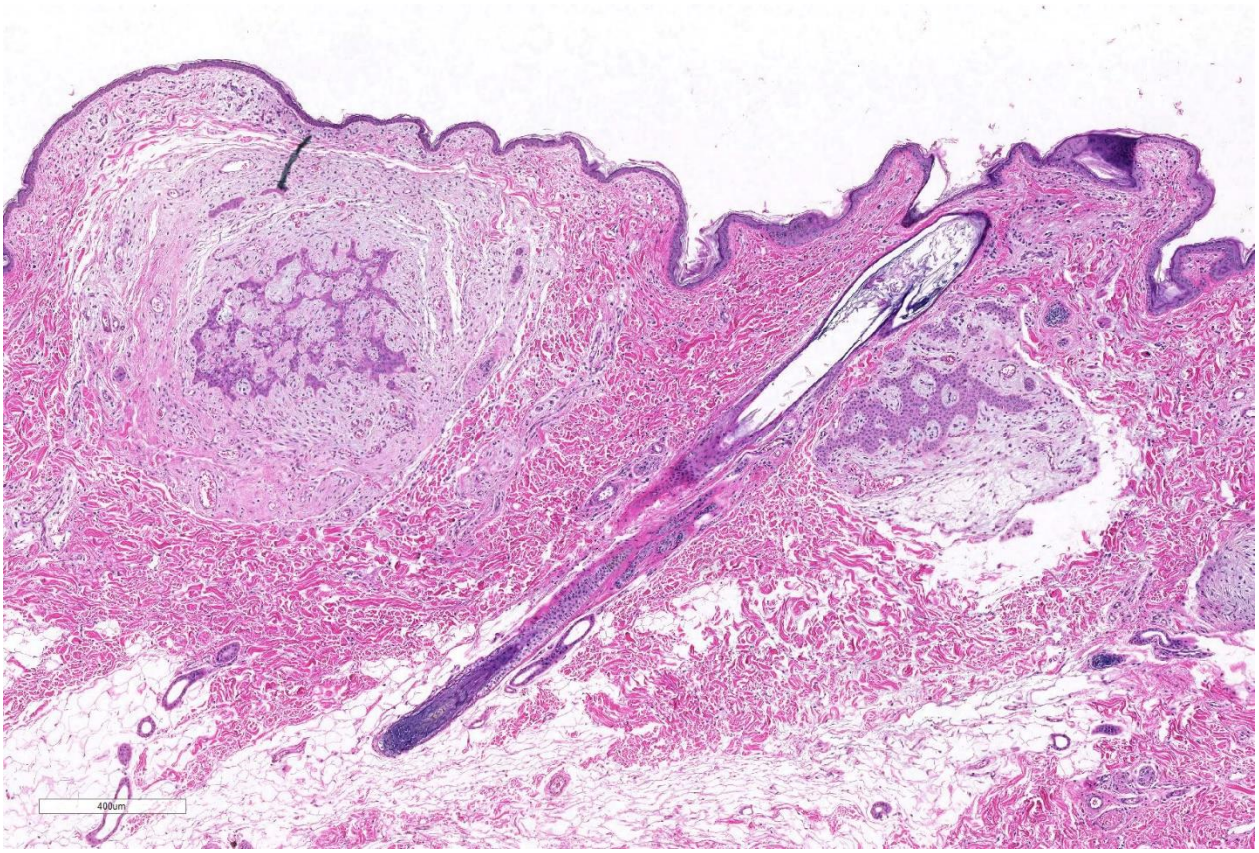
Contributor's Morphologic Diagnoses: Skin, Arborizing follicular hyperplasia with myxomatous degeneration and dilation, intrafollicular hyaline material and arthropod larvae, canine, mixed breed - findings typical of canine straelensiosis

Contributor's Comment: Infection with the trombidioid mite *Straelensia cynotis* has been reported in dogs in France, Portugal and Spain.[2,4,10] The findings described in these reports are virtually identical to several cases we have seen in Israel.

Straelensiosis is a trombidiosis caused by the "chigger" mite *Straelensia cynotis*, and is a relatively newly described etiology for nodular dermatitis in dogs in the Old World.[2,4,10] Nodular trombiculiasis has also been described in white tailed deer and birds.[5,7] The findings are similar to those described for the dog. The first publication of the morphologic characteristics of these mites was by Le Net and Fain who proposed the classification of a new species: *S. cynotis*, superfamily Trombidioid, family Leeuwenhoekiidae. Straelensiosis was first identified as a cause of nodular dermatitis in dogs in France by Le Net et al.[1,4]

In general, the nymphs and adults of trombiculid arthropods are free-living, or parasitize plants or other arthropods. The parasitic stage is the larvae which are known as 'harvest mites', 'chiggers' or 'red bugs'.[3,9] Infected animals may present with accumulation of orange granular material or pin-sized red spider-like foci in the canthi of the eyes.[3,5,9] The larvae attach themselves to areas of the host's skin in contact with the ground e.g. legs, feet, head, ears or ventrum and make a tunnel to the epidermis, called stylosome, through which salivary enzymes are injected and digested tissue fluids are withdrawn. The larvae engorge through a period of 3-5 days after which they drop off to become nymphs and complete their life cycle in the soil. Wild mammals are the usual hosts for trombiculid mite larvae, but pets and people may be accidentally infected.[3,9]

The histologic finding in skin infection with trombiculid larvae is the presence of tunnels within the stratum spinosum or stratum corneum, inducing degenerative and hyperplastic changes in the epidermis. The



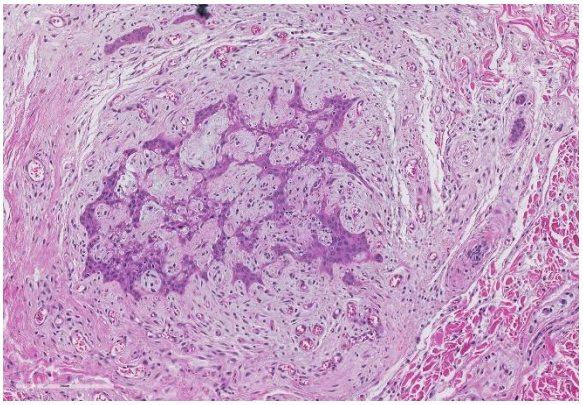
Haired skin, dog. Higher magnification of the perifollicular nodules shows marked pseudoepitheliomatous hyperplasia of follicular epithelium. (HE, 51X)

stylostome is described as a hyalinized tube oriented vertical to the skin surface.[3]

It is thought that the fox is the normal host for *Straelensia* and that hunting and rural dogs are predisposed to aberrant infection.[4] There is a clear geographical pattern of infection in certain areas within the countries reported and an obvious tendency for infection to occur in the fall and spring.[7] The nodules may last 1 to 12 months. On average, they are reported to last 3 months,[4] which is much longer than the course described for chiggers in the US. The follicular reaction to the presence of the larvae is highly characteristic and allows diagnosis to be made in the absence of larvae in the section. The follicle is dilated and contains a ring of hyalinized material identified as the stylosome.[4,8,10] The follicles exhibit epithelial hyperplasia with formation of arborizing epithelial cords within a perifollicular accumulation of myxomatous material. [4,8,10] Associated pyogranulomatous, suppurative and eosinophilic perifollicular infiltration is described. [4,8,10]

In this particular case inflammation is negligible, but we have observed pyo-granulomatous and suppurative perifollicular inflammation in most of the dogs infected in Israel.

Prognosis is variable.[2, 4,8,10] Some dogs may run the course of the infection and have spontaneous regression. Some respond well to antiparasitic shampoos or Ivermectin injections, but some dogs are reported to present with persistent infection and no



Haired skin, dog. Further magnification of a dermal nodule demonstrate pseudoepitheliomatous hyperplasia of follicular epithelum which is bounded by a vascular myxomatous dermis. Inflammation, however, is minimal. (HE, 100X)

apparent response to therapy.[2, 4,8,10] This may represent continuous exposure.

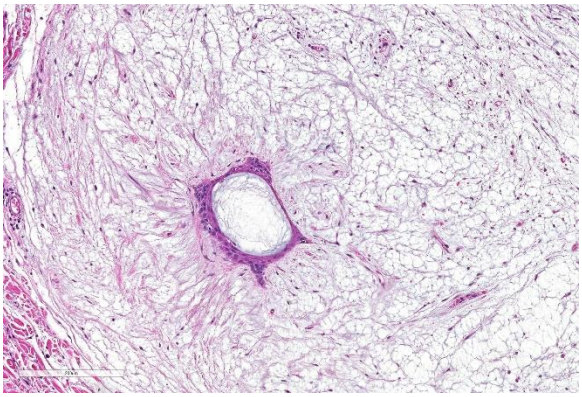
In our experience in Israel, infection is most common in the northern and in the Jerusalem areas, (both of higher altitude), in the spring and winter. Response, as described in the European reports, is variable but most dogs appear to respond well to pesticide shampoos.

JPC Diagnosis: Haired skin, follicles: Pseudoepitheliomatous hyperplasia, multifocal, severe, with marked perifollicular mucin, and occasional intrafollicular trombiculid larvae.

Conference Comment: The contributor provides an excellent description of changes associated with most common trombiculid mite infections, however the histologic findings of *S. cynotis* are quite different than traditional trombiculid mite infections. While other trombiculid mites generally attach to the surface epidermis, the larva of *S. cynotis* live and feed with in follicular ostia. They are separated from the wall of the follicle by the highly characteristic stylosome, a proteinaceous tube secreted by the mouthparts of the larval mite, through which the larva feeds by repeated cycles of extrusion of digestive salivary fluids followed by suction of digested tissue and tissue fluids.[5] The extensive pseudo-epitheliomatous hyperplasia of follicular epithelium and perifollicular mucinosis are additional histologic features which allow for the diagnosis of *S. cynotis* infection even in the absence of the larval arthropods. The larval forms of this parasites may be absent in treated cases or following the completion of the larval stage (as nymphs and adults are free living stages).[8]

Another interesting fact about infection by *S. cynotis* is the almost total lack of dermal inflammation in response to the presence of the parasite. This may be the result of the intra-follicular location of the mites as well as a total enclosure by the presence of the stylosome.[8]

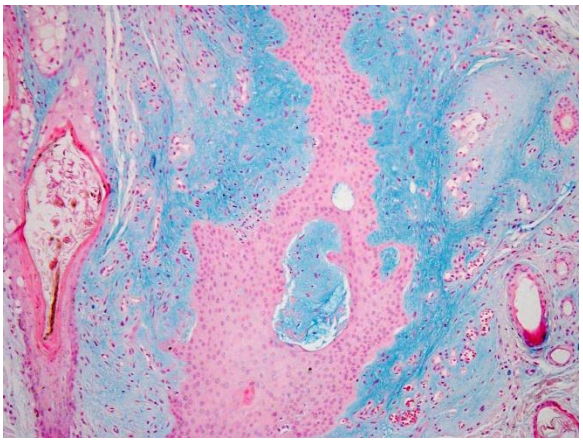
A number of arthropod and helminth parasites inhabit hair follicles. By far the most common genus is that of *Demodex*. which are normal inhabitants of hair follicles and sebaceous glands in most mammals, including humans. Interestingly, their position within the hair follicle is invariably head down.[6] Demodex sp. are obligate parasites which complete their entire life



Haired skin, dog. The adjacent perifollicular dermis is extremely myxomatous with infiltration of few muciphages. (HE, 122X)

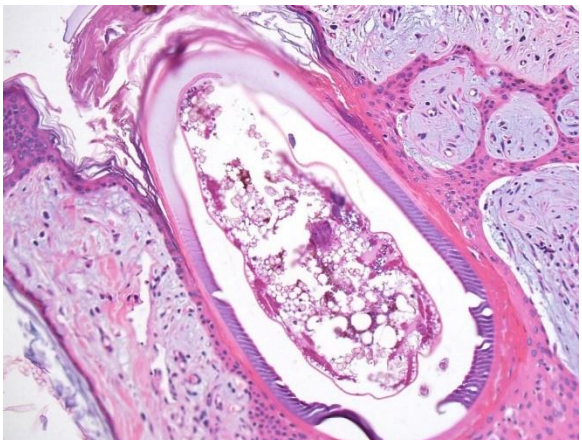
cycle on the host and are usually transmitted from mother to offspring within the first 3 days of life through close physical contact while nursing. *Demodex* mites, unlike trombiculid mites, feed harmlessly on sloughed cells, sebum, and epidermal debris. These mites may move from follicle to follicle, and transmission likely occurs while in transit. Prolonged sojourns on the epidermis may result in death of the mite through desiccation.[6]

Two important helminth species parasitize hair follicles in mammals. Parasitic females of the species *Pelodera (Rhabditis) strongyloides*, normally also free-living indicating plant matter, may indicate the skin and reside within hair follicles.[6] *Pelodera* dermatitis occurs most often the dogs but cases are well documented in cattle, sheet, horses, and humans.[6] Affected dogs are often victim supportive husband 3 and straw embedding is a common finding. *Stephanofilaria* species are uncommon cutaneous parasites of a wide range of ruminant species; with *S. stilesi* occurring in Western and Southwestern North America. While abdominal skin is characteristic for this particular species, other species each



Haired skin, dog. An Alcian blue 2.5 stain demonstrates the mucinous nature of the perifollicular substance. (AB, 200X)

have particular sites where they affect their respective host.[6] Flies are intermediate hosts for these parasites and deposit infective larva on both intact and broken skin of the host species. The adults of *S. stilesi* and similar species live within cystic hair follicles and microfilariae occur free in the dermis or within dermal lymphatics, where they may be



Haired skin, dog. This is a section through the degenerate larva occupying the infundibulum and surrounded by a band of hyaline material (stylosome). (HE, 100X) (Photo courtesy of: Department Vet Resources, Weizmann Institute, Rehovot, Israel http://www.weizmann.ac.il/vet/)

ingested by flies and complete their life cycle. Maturation from microfilaria to infective larva occurs within the intermediate host. A marked inflammatory reaction occurs if the adults exit the hair follicles, resulting in profound lymphoplasmacytic and eosinophilic inflammation, epidermal hyperplasia, and often alopecia. The presence of the adults within the follicle or of microfilariae within the dermis generally results in little inflammation.[6]

Contributing Institution:
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http://www.weizmann.ac.il/vet/

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