Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 2

16 September 2015

CASE I: B15-894 (JPC 4050459).

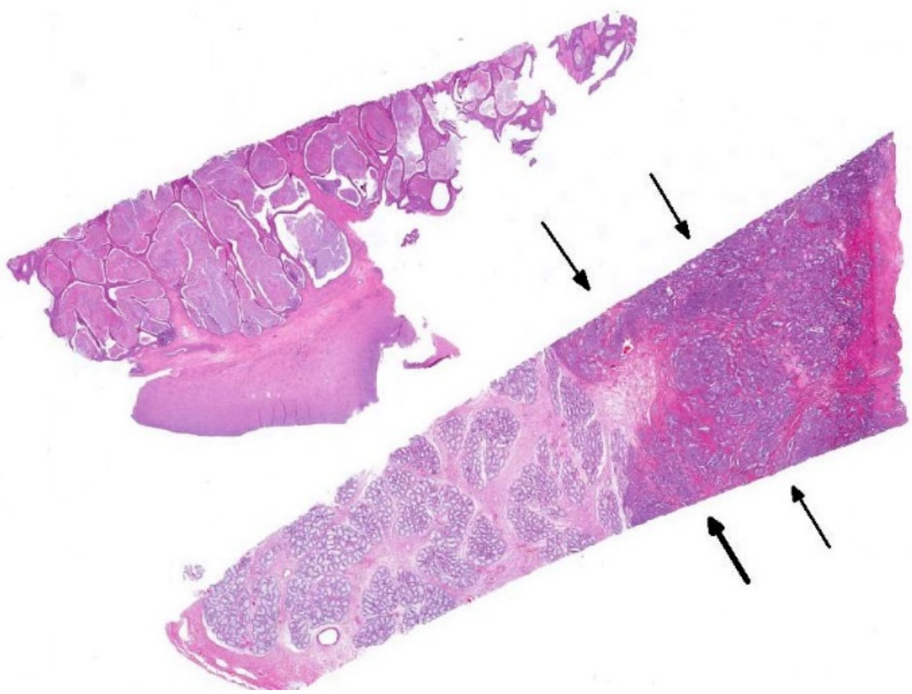
Signalment: 12-year-old, male (intact), Labrador retriever (*Canis familiaris)*

History: Little clinical history was provided with the case. The non-retained testicle was described as "small."

Gross Pathology: Received in two formalin filled jars, labeled prostate and retained testicle, were three pieces of firm, mottled, dark brown to grey tissue, up to 2.0 x 2.0 x 0.5 cm and a 4.0 x 3.5 x 2.0 cm testicle with epididymis, respectively. Near the tail of the epididymis, the testicle contained a well demarcated, ap-proximately 2.5 cm, oval, firm, white to tan mass that compressed the adjacent parenchyma and bulged from the cut surface.

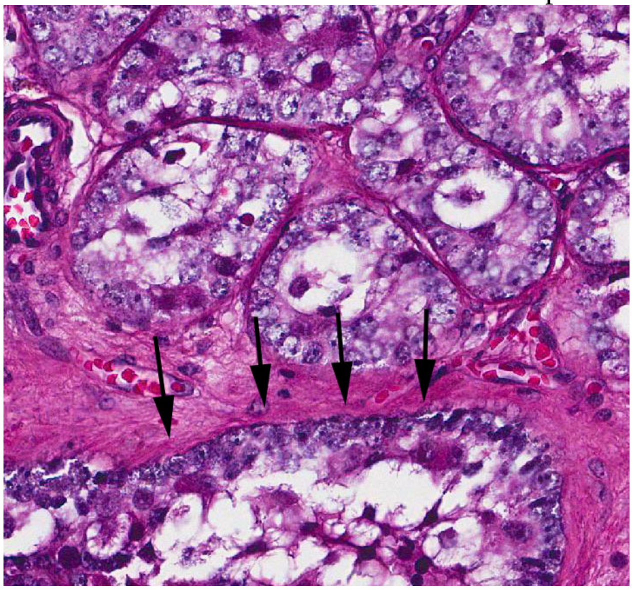
Laboratory Results: None.

Histopathologic Description: Testicle: Com-pressing adjacent seminiferous tubules is a variably encapsulated, poorly demarcated, densely cellular mass. Neoplastic cells are closely packeted into tubule-like nests and cords, within a dense fibrovascular stroma. Neoplastic cells frequently line the fibrous stroma in palisades and occasional pile to form islands. Neoplastic cells are round, to polygonal, to elongate, with indistinct cell borders, and moderate amounts of eosinophilic, flocculent to vacuolated cytoplasm. Nuclei are centrally located and oval to elongate, with vesicular chromatin and 1-3 basophilic nucleoli. The center of packets frequently contain similar cells with hypereosinophilic cytoplasm,



Retained test and prostate, dog: 50% of the testis (arrows) is replaced by a well-demarcated neoplasm composed of tubules. (HE, 4X)

rounded nuclei, and prominent, magenta nucleoli. Small numbers of necrotic neoplastic



Retained testis, dog. The neoplasm is composed of neoplastic Sertoli cells which often fill tubules. Neoplastic cells palisade along the basement membrane of affected tubules (arrows). (HE, 220X)

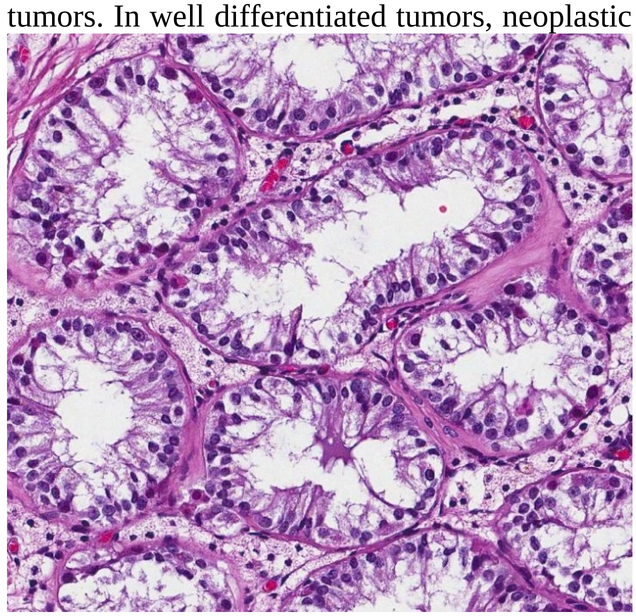
cells are scattered throughout the section. Mitotic figures are less than one per ten 40x HPF. Anisocytosis and anisokaryosis are moderate. Remaining tubules are characterized by complete lack of spermatogenesis and increased prominence of Sertoli cells.

Prostate: Prostatic acini are diffusely and severely distended by abundant mixtures of keratinaceous debris, sloughed epithelial cells, granular debris, and acicular clefts. The normal glandular epithelium is diffusely replaced by a well-differentiated, 3-4 cell thick, stratified squamous epithelium (metaplasia). In some sections, scattered acini are ruptured and infiltrated by numerous foamy macrophages and neutrophils.

Contributor's Morphologic Diagnosis:
Retained testicle: Sertoli cell tumor
Prostate: Squamous metaplasia, diffuse, severe

Contributor's Comment: Microscopic features of the neoplasm in this retained testicle are consistent with Sertoli cell tumor, a neoplasm derived from supporting cells within seminiferous tubules. Additional findings included severe, prostatic squamous metaplasia and tubular atrophy in the non-retained testicle. Sertoli cell tumors have been reported from most domestic species, but are uncommon in all but the dog. Grossly, the tumors are white, irregularly ovoid, lobulated, bulge when cut, and may be cystic. Their abundant fibrous stroma makes then firm to hard, a useful differentiating feature not found in seminomas or interstitial cell tumors. Their presence may cause marked distortion of the testicle, but most remain within the tunica albuginea.

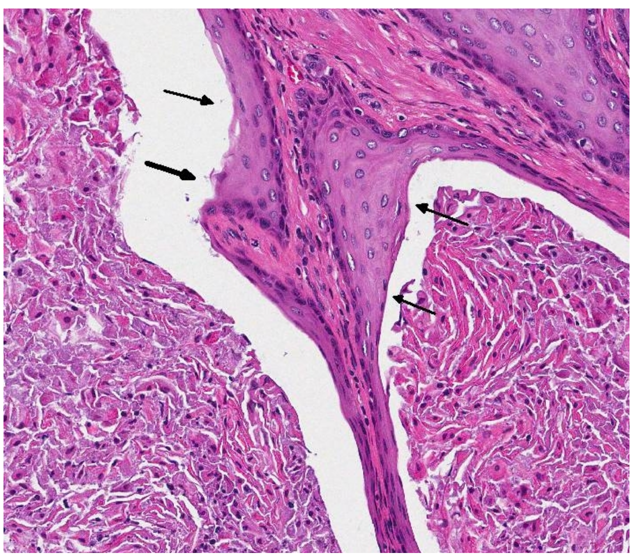
Growth of Sertoli cell tumors may be intratubular, as in this case, or diffuse. Microscopic characteristics of intratubular neoplasms include a dense collagenous stroma surrounding seminiferous tubule-like structures that contain polygonal to elongate cells, with eosinophilic, foamy to vacuolated cytoplasm. In some areas, the neoplastic cells palisade along the stroma. Mitotic figures are few. Cells appear discrete and spherical in diffuse tumors and show little or no tendency to palisade. The dense stroma and palisading cells usually differentiates Sertoli cell tumors from seminomas or interstitial cell tumors. In well differentiated tumors, neoplastic



Retained testis, dog. Seminiferous tubules in the unaffected portion of the section are hypoplastic, lacking spermatogonia and shrunken. Intervening interstitial cells are small and degenerate. (HE, 164X)

cells resemble normal Sertoli cells, with basally located nuclei and frequent cytoplasmic lipid droplets and globules. Cells of less differentiated tumors exhibit disordered growth and increased pleomorphism.

The incidence of Sertoli cell tumors is 20 times higher in cryptorchid dogs and up to 30% of affected dogs manifest signs of hyperestrinism. Especially with larger tumors, production of excessive estrogen and inhibin result in feminization, including attraction of male dogs, reduced libido, testicular and penile atrophy, preputial swelling, perineal hernia, gynecomastia, redistribution of fat, and symmetrical, often ventral, alopecia. Squamous metaplasia of the prostate gland and suppurative prostatitis may lead to dysuria. Estrogenic depression of bone marrow can result in anemia, thrombocytopenia and granulocytopenia, predisposing the dog to hemorrhage and infection. Castration of affected dogs generally results in recovery and regression of associated changes. Most Sertoli cell tumors are benign, but metastasis can occur to regional lymph nodes and distant organs. Metastatic tumors can also be hormonally active.



Prostate gland, dog. Glands are markedly dilated and lined by squamous epithelium (arrows). Glandular lumina contain abundant keratinized epithelium and squamous debris. (HE, 140X)

JPC Diagnosis: Testicle: Sertoli cell tumor.
Testicle, seminiferous tubules and interstitial cells: Hypoplasia, diffuse, severe.
Prostate: Squamous metaplasia, diffuse, severe.

Conference Comment:

Differential diagnosis discussed in this case included the various testicular neoplasms including interstitial cell tumors and seminoma which arise from the interstitial endocrine cells and germ cells respectively. Other less common types of germ cell testicular tumors include teratoma and embryonal carcinoma. Like Sertoli cell tumors, interstitial cell tumors and seminomas are most often considered benign with metastasis being uncommon.[1] With regard to retained testes, Sertoli cell tumors are more common in abdominal testes and seminomas more common in inguinal testes. Interestingly, the contralateral testis is also at increased risk of tumor development.[1] Seminomas have a firm texture with a homogenous appearance and a pink-grey color grossly. Microscopically they are characterized as having round cells with a small amount of cytoplasm, a large nucleus with prominent nucleolus, an elevated mitotic rate, and can be diffuse or intratubular with infiltration of lymphocytes being common.[1] Interstitial cell tumors, being the most common testicular neoplasm in the dog, grossly have a distinctive tan or yellow-orange color due to their high lipid content and frequently contain areas of hemorrhage. Microscopically, they can be solid-diffuse with cells arranged in sheets or cords and separated by fine bands of fibrous connective tissue or cystic in nature with cords of cells surrounding fluid filled areas that may contain erythrocytes. The tumor cells can vary in shape, but the cytoplasm is almost always finely or coarsely vacuolated.[4]

The feminizing effect of Sertoli cell tumors was also discussed during the conference and mentioned above by the contributor. Inhibin inhibits the release of GnRH from the hypothalamus, and ultimately the release of LH and FSH from the anterior pituitary, which affects the production of estrogen and testosterone, resulting in the feminizing effects.[1] In addition to squamous metaplasia of the prostate gland, glandular hyperplasia can also occur. The squamous metaplasia is not considered to be a pre-neoplastic change in this case, unlike in other locations with other causes, such as the lung of smokers. In metaplastic tissue, specialized epithelium is frequently replaced by less specialized epithelium, it is often a reversible change, and the mechanism varies with cause. Another example of squamous metaplasia occurs in the esophageal glands of various avian species in response to vitamin A deficiency, the exact mechanism of which is unclear. [6]

Following discussion of the neoplasm in this section, participants discussed the non-neoplastic portion of the testicle. The lack of developing spermatogonia, small volume of remaining seminiferous tubules and decreased numbers of interstitial cells were considered to be hypoplastic changes due to the abdominal location of the testis, although the hormonal contribution of the Sertoli cell tumor was considered as a contributing factor non-viability of the adjacent seminiferous tubules.

Slide variation was noted with some slides demonstrating more prominent acicular cholesterol clefts and degree of inflammation.

Contributing Institution:

Department of Pathology, University of Georgia (www.vet.uga.edu/VPP)

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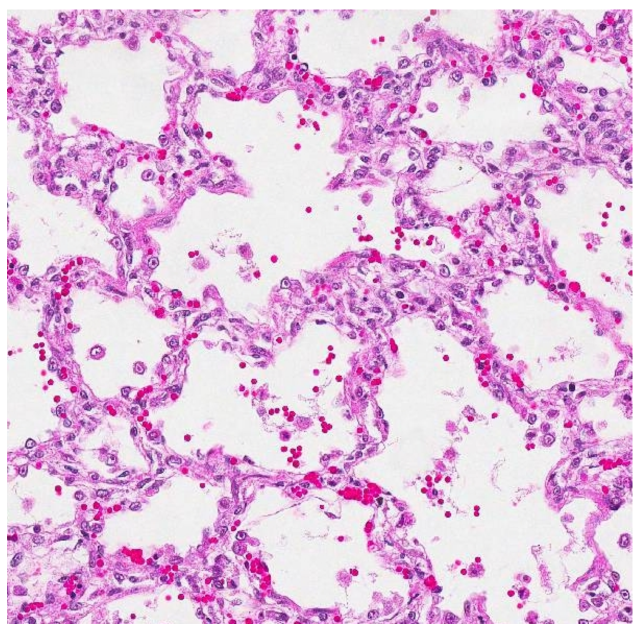
CASE II: S09-1502 (JPC 3164421).

Signalment: Feline, Norwegian forest cat, 2 weeks of age, male (*Felis catus*).

History: All animals from a litter of two week old kittens were treated with infusion and ampicillin, but all kittens died.

Gross Pathology: Lung: pink and whitish mosaic pattern on surface and in the parenchyma, diffusely increased texture.

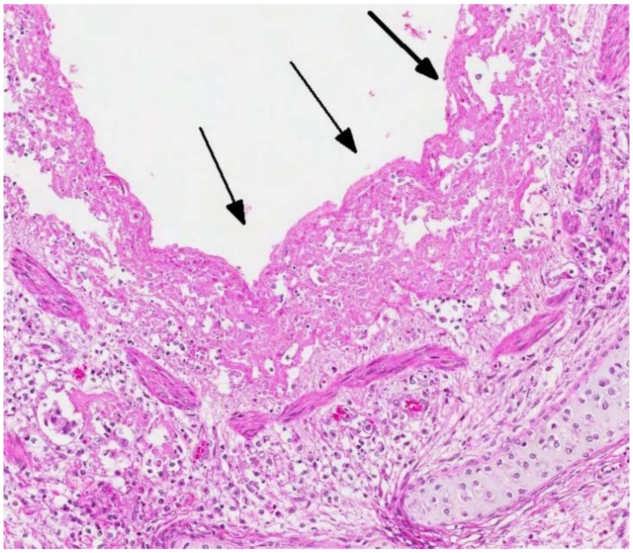


Lung, kitten: Alveolar septa are diffusely and markedly expanded throughout the section by a mix of cellular infiltrate as well as fibrin and edema. (HE 196X)

Laboratory Results: Immunohistochemistry positive for FeHV-antigen.

Histopathologic Description: Lung: Throughout the organ bronchi and bronchioli are necrotic or degenerating. The bronchiolar epithelium is replaced by karyorrhectic and pyknotic cell debris and eosinophilic material (necrosis), infiltrated with numerous degenerating neutrophils and fewer macrophages. In some of the remaining viable epithelial cells eosinophilic intranuclear inclusion bodies are visible, only in some slides syncytia cell formation with eosinophilic intranuclear inclusion bodies can be seen.

In the lumina of the bronchi/bronchioli and in most alveolar spaces cell debris and a slightly fibrillar eosinophilic material as well as numerous degenerating neutrophils showing karyopyknosis and karyorrhexis, macrophages and some erythrocytes can be found. The interstitial walls are still viable but thickened due to a moderate infiltration of the alveolar walls with macrophages and neutrophils and activation of pneumocytes type II. In the lumina of these alveolar spaces moderate amounts of macrophages, few neutrophils and some erythrocytes (acute bleeding) are visible. Peribronchiolar and perivascular tissue is edematous, moderate amount of degenerating neutrophils, few lymphocytes and macrophages are diffusely visible in the edematous tissue. In this region some bronchiolar gland epithelia



Lung, kitten: Airway epithelium in bronchioles and bronchi is diffusely necrotic (arrows.). (HE 144X)

degenerate and in few intranuclear eosinophil inclusion bodies can be seen.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, bronchointerstitial, severe, necrotizing, subacute, multifocal to coalescing, with epithelial intranuclear inclusion bodies (FeHV-1).

Contributor's Comment: Feline herpesvirus 1 (FeHV-1) – feline rhinotracheitis virus, is a double-stranded DNA virus belonging to the subfamily of alpha-herpesvirus (family herpesviridae). FeHV-1 infections are ubiquitous and occur throughout the world, but clinical disease is much less frequent. Carrier cats are either latently or actively infected. Latent carriers do not shed infectious virus unless the genome is activated due to stress or corticoid administration. Clinically apparent disease is more frequent within a population of high density.

Infection can occur in utero, neonatally or in the 2-12 week age range when maternal immunity wanes. Virus is infectious on all mucous membranes but requires contact with nasal or ocular secretions, airborne spread only occurs over short distances.

Infections normally happen intranasal followed by a rapid cytolytic infection of nasal epithelium under an optimal replication-temperature of 37°C and then a secondary spread to conjunctival sac, oropharynx, trachea, bronchi and bronchioli. The typical incubation period is two to four days. Microscopically intranuclear inclusion bodies can be seen in epithelial cells of the described organs named above, but only from the second to seventh day after infection. Transient viremia may occur but is not a prominent feature. Latent infections develop in as many as 80% of infected cats, where the virus is distributed in several tissues of the head. The virus persists in the trigeminal nerve, ganglia of the optic nerve, olfactory bulb and cornea.

Latent carriers have been converted to active virus shedders by giving them corticosteroids for several days or by stress. Also parturition and lactation are stressful events that may trigger activation, which may be an important source of infection for kitten.

The classical rhinotracheitis occurs in kitten within an age range of 6-12 weeks. Severity of clinical signs varies greatly. The most consistent manifestation is rhinitis associated with sneezing and nasal exudation. Low-grade fever occurs but usually clinical signs disappear in 7-14 days. Some kitten can manifest rhinitis, pharyngitis, glossitis, tracheitis, pneumonia, high fever, depression, anorexia and drooling. Mortality occurs among this group of animals.

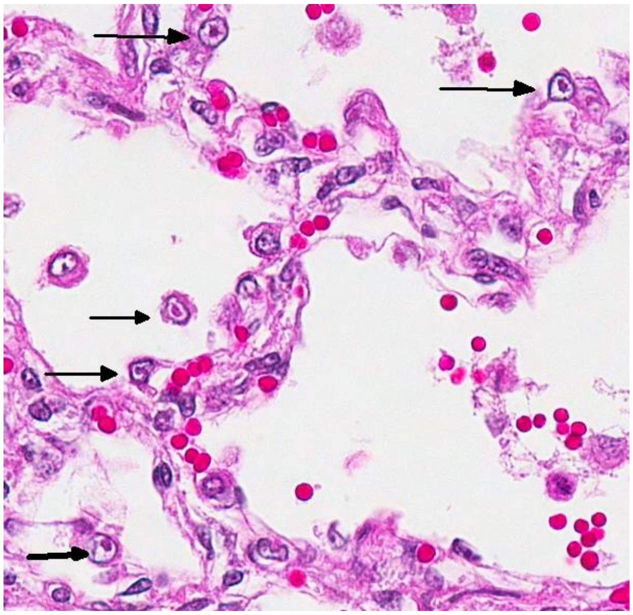
Besides the classical rhinotracheitis other clinical syndromes can be attributed to FeHV-1 infection. Those may belong to the complex of respiratory diseases as recurrent rhinitis in older cats as a result of reinfection or activation of a latent infection. Also a chronic sinusitis with opportunistic bacteria and mycoplasma that follows an acute upper respiratory infection of FeHV-1 with permanent turbinate and mucosal damage can be seen. Another manifestation can be a chronic conjunctivitis and keratitis that can last for months and is usually mild and bilateral but can become copious and purulent with time. Photophobia is particularly characteristic. Acute or chronic herpetic corneal ulcers can be a troublesome complication or herpesvirus ulcerative dermatitis. [1,3,6]

Also problems in context of reproductive events can occur as abortion, which is probably a non-specific phenomenon, and neonatal disease associated with queens that fail to provide maternal immunity or infect their young at birth. There the kitten shows pneumonia without upper respiratory disease after a short period of fading away.[1,6] In our case, the two week old kitten didn’t show pathologic changes in the upper but in the lower respiratory tract.

Alphaherpesvirinae: *focal lesions in skin and mucosa of respiratory and genital tract; abortion; neonates: necrosis in multiple organs, latency in nerves*	Equine herpesvirus 1: Equine herpesviral abortion, rhinopneumonitis, neurologic disease	horse
	Equine herpesvirus 3: Equine coital exanthema	horse
	Equine herpesvirus 4: Equine rhinopneumonitis	horse
	BHV-1: infectious bovine rhinotracheitis/infectious pustular vulvovaginitis/infectious balanoposthitis	cattle
	BHV-2: bovine mammilitis virus (bovine herpes mammilitis)	cattle
	BHV-5: bovine herpesvirus encephalitis	cattle
	SHV-1: Aujezky´s disease, Pseudorabies	pig>others
	Canine herpesvirus 1:	dog
	Feline herpesvirus 1: upper respiratory tract disease (rhinotracheitis) and conjunctivitis (ulcers)	cats
	Feline herpesvirus 1: feline herpesvirus ulcerative dermatitis	cats
	Gallid herpesvirus-1: Infectious laryngotracheitis (ILT)	chicken

	Gallid herpesvirus-2: Marek´s disease	chicken
	Psittacine herpesvirus: Pacheco's disease	psittacines
	Anatid herpesvirus-1: Duck plaque/Duck virus enteritis	ducks, geese, swan
	Simplexvirus: HSV-1, HSV-2, HBV, BHV-2	
	Herpesvirus simplex, type 1/type 2	human & nonhuman primates
	Herpesvirus simiae/Herpes B/Cercopithecine HV	rhesus macaques
	Simian varicella virus	macaques, AGM, Patas monkeys
Betaherpesvirinae: *no cell lysis, karyomegaly, latency in secretory glands, lymphoreticular organs, kidney*	HHV-5, HHV-6, MCMV-1	humans
	Porcine herpesvirus 2: porcine cytomegalovirus disease/Inclusion body rhinitis	porcine
	Cytomegalovirus	humans + nonhuman primates
Gammaherpesvirinae: *primates: lymphoproliferative disease, latency in lymphoid tissue*	EHV-2	
	EHV-5	
	BHV-4: bovine herpes mammary pustular dermatitis	cattle
	OHV-2/AHV-1: malignant catarrhal fever	various ruminants
	Epstein-Barr virus (lymphocryptovirus-gamma 1)	primates
	Kaposi-sarcoma-associated herpesvirus/human herpesvirus-8 (KSHV/HHV8) (Rhadinovirus-gamma 2)	primates
Deltaherpesvirinae	Anatid herpesvirus-1: duck plague	
	SHV-2: Einschlusskörperchenkrankheit	pig
	Karpfenpocken	fish
?	Koi-herpesvirus (KHV, carp nephritis and gill necrosis virus, CNGV, Cyprinid-Herpesvirus-3, CyHv-3)	fish

JPC Diagnosis: Lung: Bronchointerstitial pneumonia, fibrinonecrotic, diffuse, marked with eosinophilic intranuclear inclusion bodies.

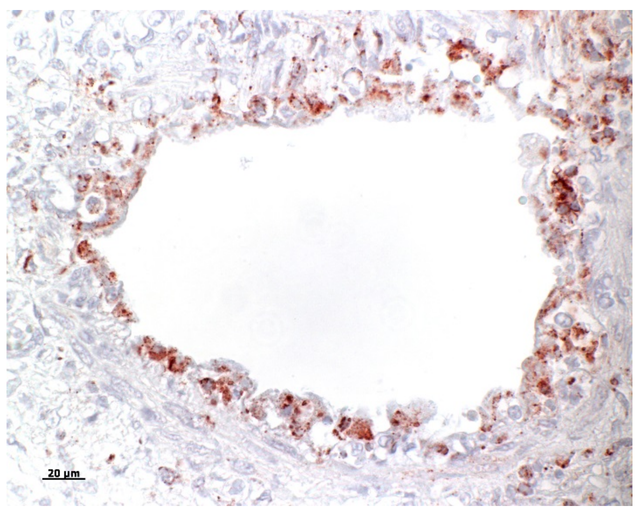


Lung, kitten: Intranuclear viral inclusions consistent with herpesvirus are present in macrophages and pneumocytes (arrows) as well as epithelium of airways and submucosal glands (not shown) (HE 400X)

Conference Comment:

This excellent case of feline herpesviral (FHV) pneumonia clearly demonstrates viral intranuclear inclusion bodies, a histologic finding typically seen in the acute phase of the disease, and not often visualized in most animals that succumb to the disease in the subacute or chronic phase. There was extensive discussion during the conference regarding the location of the intranuclear inclusions. Most agreed viral inclusions were present in macrophages and endothelial cells; but they were also identified in bronchiole glandular epithelium, due in part to the prominence of feline bronchiole glands. Participants also briefly discussed differentiating a prominent activated nucleolus from an intranuclear inclusion. Although both have a similar eosinophilic staining pattern, the herpesviral intranuclear inclusions were generally larger and peripheralized the chromatin. Slide variation was noted with a few slides having a focally extensive area of coagulative necrosis, an uncommon presentation for this entity. Other histologic changes discussed include the discontinuous alveolar septa, alveolar and interstitial edema, and relatively fewer numbers of type II pneumocytes than anticipated.

Differential diagnosis discussed for this lesion included feline calicivirus and high pathogenicity avian influenza, although viral inclusions are not a histologic feature of either entity. Feline calicivirus (FCV), in addition to causing rhinitis, stomatitis and conjunctivitis, can also cause an interstitial pneumonia and necrotizing bronchiolitis with secondary bacterial infection. The clinical and pathologic findings in FHV and FCV are similar in some aspects, and together account for the majority of feline respiratory diseases. FCV can also result in a transient lameness due to a self-limiting arthritic condition, termed "limping kitten syndrome." There is a virulent form of FCV, described as a systemic hemorrhagic syndrome with high mortality[5], in which cats present clinically with diarrhea, pneumonia, edema and hemorrhage. Unlike the more virulent strain where viral antigen is present systemically in both endothelial cells and epithelial cells, in the less virulent more ubiquitous form, viral antigen is generally only present in oral mucosa and respiratory epithelium.[7]



Lung, kitten: Attenuated bronchiolar epithelium is multifocal strongly positive for FHV-1 antigen. (anti-FHV1, 400X)

Cats can also become infected with highly pathogenic avian influenza virus (HPAI) H5N1 from infected wild birds. The primary respiratory lesions in H5N1-infected cats includes bronchointerstitial pneumonia and bronchiolar necrosis, but significant disease outside the respiratory system, including multifocal hepatic necrosis, is also a noted and described finding.[4,8] To date, horizontal transmission between domestic cats has not been documented in the wild [4] but has been demonstrated experimentally with excretion from both the respiratory and digestive tracts.[8] Additionally, cats have also been shown to be susceptible to other forms of influenza virus such as H1N1 from humans as well as low pathogenicity avian influenza viruses.[2]

Contributing Institution:
Institute of Veterinary Pathology, Vetsuisse Faculty, CH-8057 Zurich, Switzerland
www.vetpathology.unizh.ch

References:

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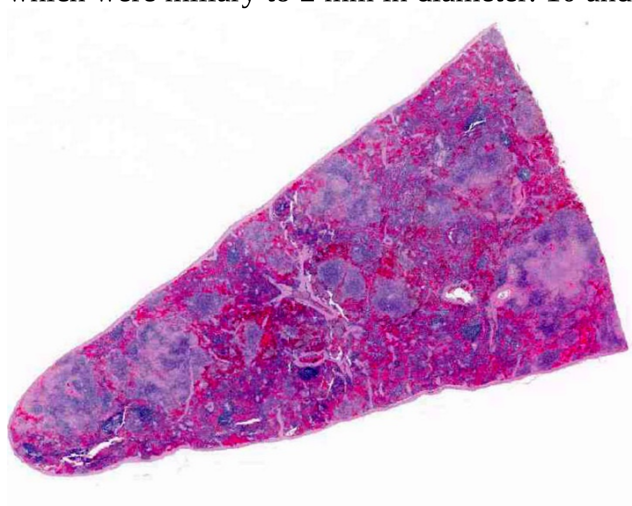
CASE III: 08-2379-7 (JPC 3169265).

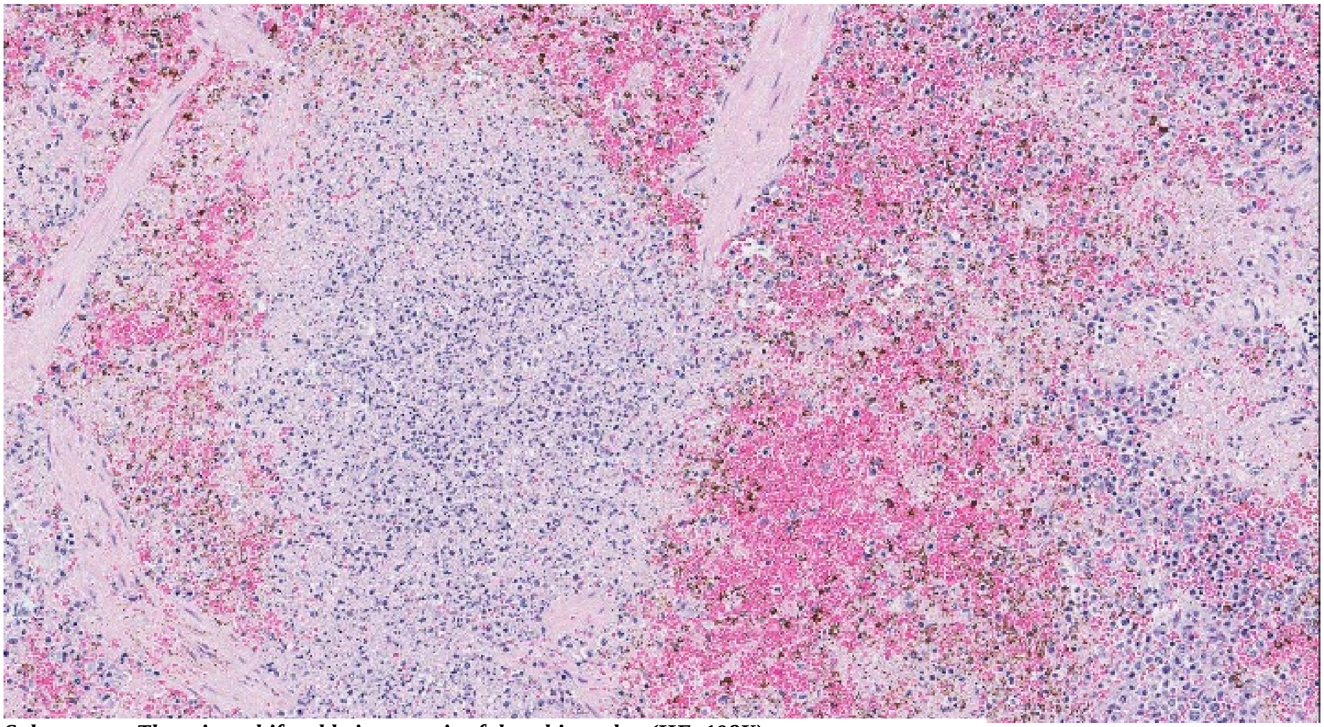
Signalment: 12-year-old male castrated domestic shorthaired cat (*Felis catus).*

History: Presented to the referring veterinarian with a history of anorexia, pyrexia, lethargy, diarrhea and oral ulcers of seven days duration.

Gross Pathology: The mesenteric and mandibular lymph nodes were markedly enlarged, and the corticomedullary architecture was effaced by friable, pinpoint yellow-white raised nodules. The pulmonary parenchyma contained several slightly raised, white nodules which were miliary to 2 mm in diameter. 10 and



Spleen, cat. There is diffuse necrosis focused on white pulp, which extends into the surrounding red pulp. (HE, 6X)



Spleen, cat. There is multifocal lytic necrosis of the white pulp. (HE, 198X)

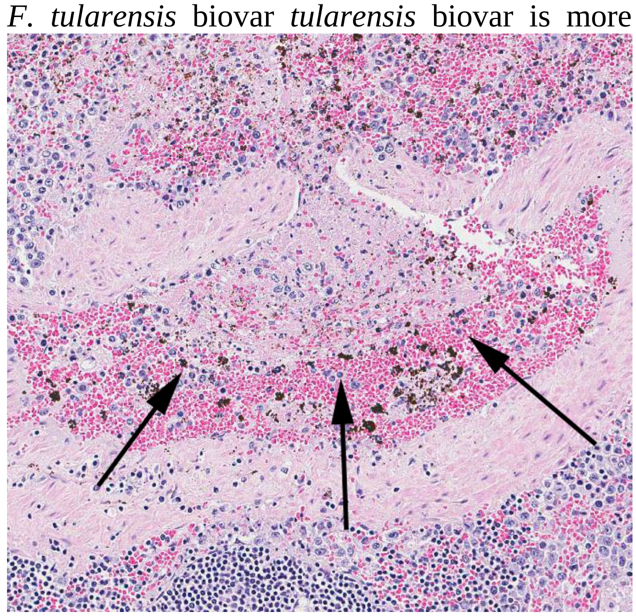
20 ml of clear, yellow-red fluid was found in the abdominal cavity. The livers were yellowish-brown and the hepatic parenchyma contained several white, slightly raised nodules which were miliary to 2 mm in diameter and evenly distributed through the parenchyma. The spleens contained multiple white, round, raised nodules and were approximately 1-2 mm in diameter.

Laboratory Results: Specimens of spleen, liver, and lung submitted for culture, PCR and DNA sequencing were positive for *Francisella tularensis*.

Histopathologic Description: Spleen: The parenchyma is disrupted by multiple nodules composed of degenerate neutrophils, and macrophages and varying amounts of amorphous to slightly fibrillar eosinophilic material (fibrin) and basophilic cellular and nuclear debris (liquefactive necrosis) with loss of lymphoid tissue from the areas surrounding these nodules. Overall, there is a moderate degree of depletion of the periarterial lymphoid sheaths. Erythrocyte and myeloid precursors are scattered throughout the splenic parenchyma with rare mega-karyocytes (extramedullary hematopoesis).

Contributor's Morphologic Diagnosis: Moderate to severe multifocal acute necrosuppurative splenitis

Contributor's Comment: *Francisella tularensis*, the causative agent of tularemia, is an aerobic, gram-negative coccobacillus. The two most common biovars are *F. tularensis* biovar *tularensis* and *F. tularensis* biovar *palaeartica*. *F. tularensis* biovar *tularensis* biovar is more

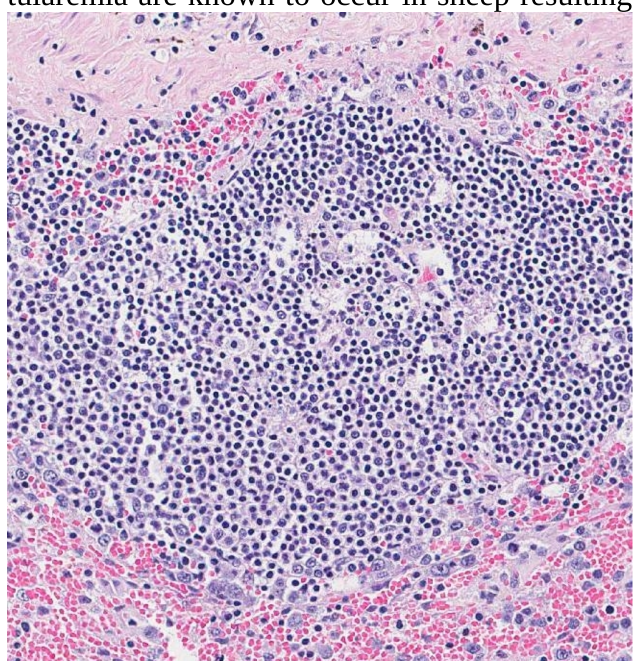


Spleen, cat. Splenic vessels occasionally contain non-occlusive fibrinocellular thrombi. (HE, 220X) common in North America.[1, 10, 6, 2, 8] *F. tularensis* biovar *tularensis* has been the only biovar identified in fatal systemic infections in cats.[2] Infections have been reported in cats, rabbits, rodents, dogs, cattle, sheep, horses, non-human primates, and humans with cats, rabbits, rodents, and sheep appearing to be the most susceptible to natural infection.[1,6,2] Tularemia is transmitted via bites of infected arthropods (most often ticks or flies), ingestion of infected rabbits and or rodents, inhalation, and mucous membrane exposure.[1, 10, 6, 8] Clinical signs commonly associated with tularemia include fever, anorexia, lymphadenopathy, hepatosplenomegaly, dehydration, and neutrophilia or neutropenia with toxic change. Common gross findings with tularemia include multifocal splenic, hepatic, lymphoid, and pulmonary necrosis, oral and gastrointestinal ulceration, and enterocolitis.[1, 10, 6, 2, 8] Microscopic findings typically include necrotizing inflammation in multiple organs (spleen, liver, lungs, lymph nodes, and gastrointestinal tract common) with varying numbers of associated neutrophils and macrophages. Colonies of intralesional gram negative coccobacilli may be visualized as well.[1,6,2] Definitive diagnosis is based on successful isolation of *F. tularensis* from specimens obtained from affected animals,[1, 10, 6, 2, 8] fluorescent antibody reaction and agglutination with *F. tularensis* anti-serum,[1, 10, 6] immunohistochemistry on formalin fixed paraffin embedded tissues,[2,8] or PCR and DNA sequencing.[8]

JPC Diagnosis: Spleen: Splenitis, necrotizing, multifocal, marked with lymphoid depletion, fibrin thrombi and extramedullary hematopoiesis.

Conference Comment: Conference participants agreed both red and white pulp are affected by necrosis, but the lesions are centered on the white pulp, with severe lymphoid depletion. Participants described the abundant fibrin thrombi within the section, but noted they are non-occlusive with no evidence of infarction. Differential diagnoses which were discussed included *Yersinia pseudotuberculosis, Yersinia enterocolitica* and *Salmonella* spp. infection, which share many gross and histologic characteristics with tularemia, as well as *Yersinia pestis*. The history provided is typical for both plague and tularemia infection. However, *Yersinia enterocolitica* and *pseudotuberculosis* are generally associated with large colonies of gram-negative bacilli visible histologically, and *Salmonella* spp. infection is less commonly seen in domestic cats. Special stains that can be used to identify *F. tularensis* include Gram stains as well as both Giemsa and Warthin-Starry. In this case Gram stains did not help identify organisms in the section.

Tularemia has been reported in nearly all 50 states except Hawaii, and in 2015 is on the rise in North and South Dakota, Colorado, Wyoming, and New Mexico. More common modes of infection in humans include cleaning/skinning infected lagomorphs and arthropod bites, but also include infectious from contaminated water supplies as well as consumption of contaminated undercooked meat. Sporadic outbreaks of tularemia are known to occur in sheep resulting



Spleen, cat. The white pulp is hypocellular and contains tangible body macrophages and cellular debris, suggesting lymphocytolysis. (HE, 280X)

in late term abortion and may be associated with abundant tick populations.[5] In some cases of small mammal infection, classic lesions may not be present grossly or histologically. Additionally, in wildlife species, autolysis and freeze artifact may interfere with evaluation of gross lesions. Subclinical infections are known to occur, but the role of subclinically infected animals in disease transmission is unclear, which serves to highlight the importance of taking appropriate safety precautions when working with the carcasses of deceased wildlife in endemic areas.[4]

The agent is highly infectious with as few as 10-50 organisms capable of causing an infection and it is classified as a category A bioterrorism agent. Experimentally induced lesions from inhalation in African green monkeys included necrotizing pyogranulomatous lesions which targeted the lung and lymphoid tissue.[9] The organisms are most commonly located intracellularly in macrophages, in both the inhalation form in primates[9] as well as in small mammals, but may also be present extracellularly in exudates and necrotic debris as well as in other cell types.[3] *F. tularensis* is able to evade the immune system by infecting and replicating in macrophages and disseminating to distal organs in the acute phase of the disease without being detected. It prevents activation of macrophages that would result in an appropriate host innate immune response, which is important in survival in infections with virulent *F. tularensis*.[7]

Contributing Institution:
Department of Veterinary Biosciences
College of Veterinary Medicine
The Ohio State University
1925 Coffey Road
Columbus, OH 43210
http://vet.osu.edu/biosciences.htm

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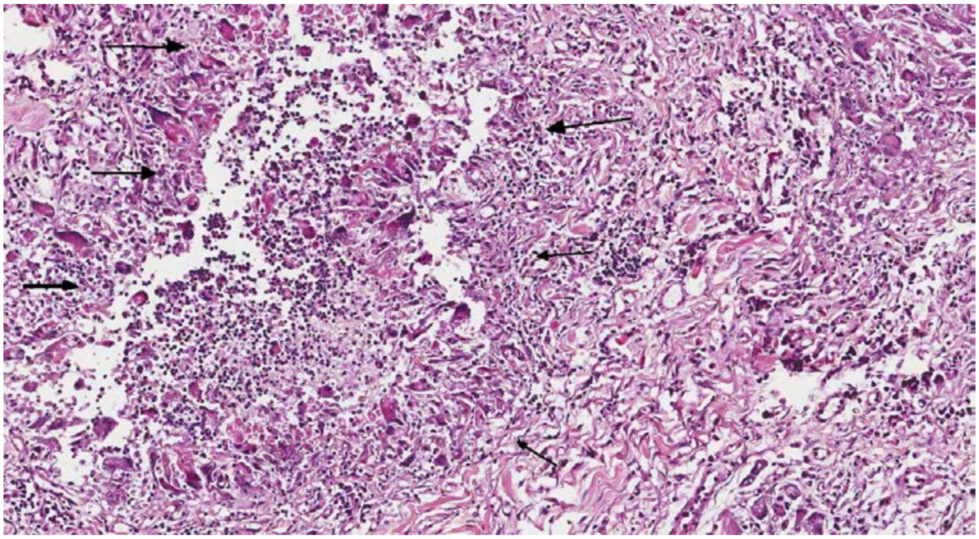
CASE IV: B14-182 (JPC 4066360).

Signalment: 2 years of age, female, neutered, Springer Spaniel canine (*Canis lupus familiaris*).

History: The animal was admitted to the Tuskegee University teaching hospital for a mass in the right mammary chain and a mass in the left mammary chain. The animal swam in a pond less than 1 week after a laparotomy. Both masses were warm to the touch and ulcerated. There was a draining swollen area on the medial surface of the right stifle joint and a swollen area on caudal

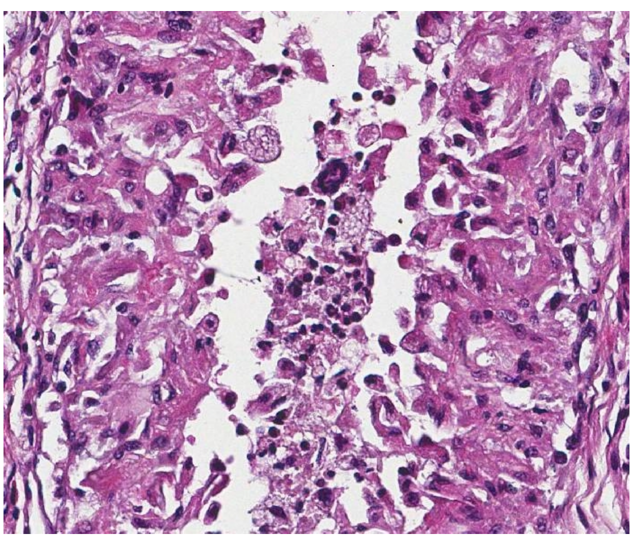


Mammary tissue, dog: The tissue is effaced by multiple poorly formed granulomas surrounding a central necrotic core (arrows).. (HE. 120X)

aspect of the left carpus. The two mammary gland masses were surgically excised and submitted for microscopic evaluation.

Gross Pathology: Both mammary masses were irregularly shaped, firm, ulcerated and contained several draining tracts. The mass in the right mammary gland was 22 cm X 5 cm and the mass in the left mammary gland was 15 cm X 5 cm. The right stifle joint swelling and the swelling on the left carpus were round to oval, 4 – 5 cm in diameter, hard, ulcerated and exuded serosanguinous fluid.

Laboratory Results: None.



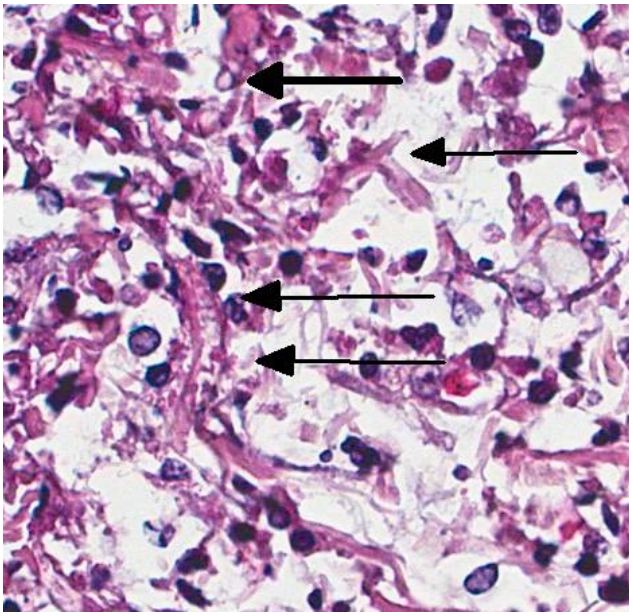
Mammary tissue, dog: Granulomas are lined by a layer of mildly pleomorphic epithelioid macrophages. (HE. 196X)

Histopathologic Description: Several sections from the mammary masses were examined microscopically. Microscopic lesions are similar in all tissues. All sections submitted were from the 2 mammary masses.

In all tissues, normal architecture is obscured by variably-sized coalescing cellular nodules that are often separated by thick bands of fibrous connective tissue. Nodules often have centers comprised of cellular debris, few to moderate neutrophils and few eosinophils. Macrophages, epithelioid cells, multinucleated giant cells, Langhans giant cells, few to moderate lymphocytes, plasma cells, and fibroblasts surround these necrotic centers. Other nodules lack necrotic centers and are comprised of variable combinations and concen-trations of plasma cells, macrophages, epithelioid cells, multinucleated and Langhans giant cells. Giant cells often contain intracytoplasmic round, 2-4 micron structures with clear centers and thick eosinophilic walls. Within cellular masses, the periodic acid-Schiff stain reveals numerous non-parallel hyphae with thick walls. Hyphae are poorly septate, 6-12 micron wide with occasional non-dichotomous branching (Fig. 1).

Contributor's Morphologic Diagnosis: Mammalitis, granulomatous, diffuse, severe with intralesional fungi.

Contributor's Comment: The lesions in both mammary glands were induced by *Zygomycetes* or *Pythium insidiosum*. The location of lesions, morphology, size of the organism, and inflammatory reaction are features consistent with both zygomycosis and pythiosis. Staining of the organism with the periodic acid -Schiff stain (PAS) and not the Gomori's methenamine silver stain (GMS) is more consistent with zygomycosis; however, the history of swimming in a pond several days after a laparotomy is more consistent with a diagnosis of pythiosis.

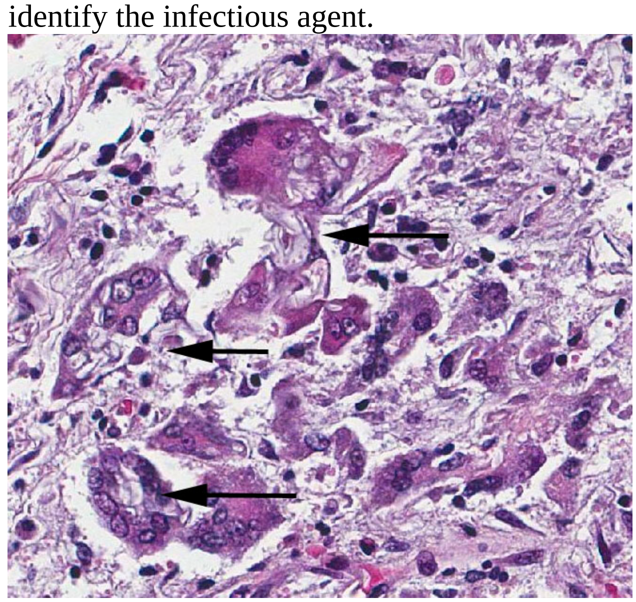


Mammary tissue, dog: Within necrotic cores, cross-and tangential sections of hyphae may be occasionally visualized in negative relief (HE. 280X)

Dogs with compromised immune systems are highly susceptible to both conditions. These infections are usually obtained via inhalation or ingestion with primary sites in the respiratory and gastrointestinal systems. Cutaneous infections in both immunosuppressed and immunocompetent dogs are often secondary to open skin wounds. *Pythium insidiosum* may be present in water, and when animals with open skin wounds enter contaminated bodies of water, they are susceptible to these infections. Skin lesions are typically characterized by nodules that are frequently ulcerated with necrotic centers and granulomatous and or pyogranulomatous inflammation. Hyphae with non-septate or poorly septate, non-parallel walls, and non-dichotomous branches are present in nodule centers. Hyphae are 4-16 micron wide. These features are beneficial in distinguishing *Zycomycetes* and *Pythium* from their primary differential diagnoses of aspergillosis and candidiasis. *Aspergillus* sp. is septate and 2-3 micron wide and *Candida* sp. is 2-3 micron wide with pseudohyphae.[2] Drugs that are effective against fungal infections are ineffective for treatment of *Pythium* species, which is not a fungus but a member of the phylum Oomycota.[3]

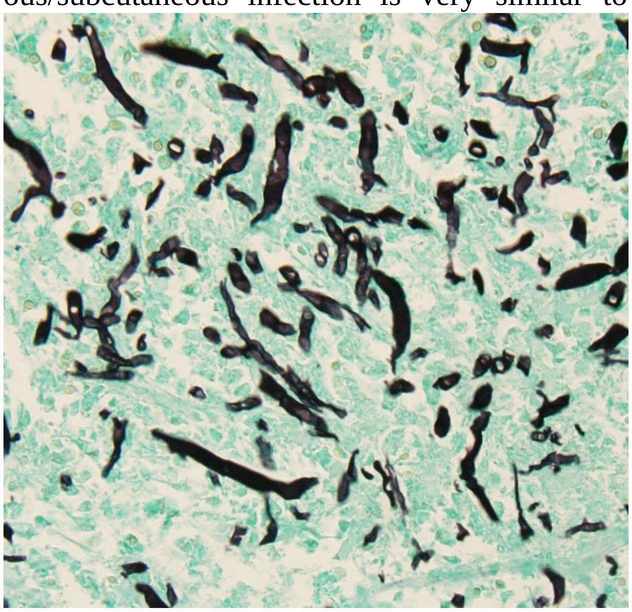
JPC Diagnosis: Mammary gland (per contributor): Panniculitis, granulomatous and eosinophilic, chronic-active, diffuse, marked with numerous hyphae, Springer spaniel, canine.

Conference Comment: Tissue identification was particularly challenging in this case, with a mix of interpretations by conference participants; considerations included glandular tissue, pancreas, intestine, and uterus. Many conference participants commented on the presence of eosinophils in the inflammatory milieu and profound fibrosis, indicating chronicity. Serial tissue sections subjected to silver staining by the Grocott methenamine silver (GMS) method and periodic-acid Schiff (PAS) reaction were viewed and discussed during the conference; while hyphae were demonstrated with both stains, the GMS method proved superior in highlighting the hyphae. In our experience, even with special histochemical stains, the infectious organisms of *Pythium insidiosum*, *Lagenidium* species, Oomycetes, and Zygomycetes are problematic to differentiate histologically, and ancillary laboratory testing, such as microbial culture or PCR assay, is often required to definitively identify the infectious agent.



Mammary tissue, dog. Often, hyphae are surrounded by robust multinucleated foreign body giant cells. (HE, 360X)

Pythium insidiosum and *Lagenidum* species Oomycetes are dimorphic water molds (previously thought to be fungi) found in tropical or subtropical areas such as the southern United States. Infection is primarily cutaneous or subcutaneous, but systemic, gastrointestinal and vascular sites can occur in domestic animals and humans. Infection occurs when the host comes into contact with standing or stagnant water containing the motile aquatic flagellate zoo-spores; this infectious form of the organism is attracted to injured mammalian tissue. Infections in domestic animals are most commonly reported in the limbs, ventral thorax and abdomen of horses. In horses, the organisms cause large granuloma like mass lesions that may contain a purulent discharge and clinically resemble cutaneous habronemiasis, neoplasia, and exuberant granulation tissue.[1] The cutaneous condition in the dog is less common than the gastrointestinal form, where the condition is also often progressive and refractory to medical therapy; the lesion starts as a dermal nodule that may resemble a lick granuloma and expands into the subcutis, spreads peripherally, may ulcerate and develop draining tracts and is often associated with an eosinophilia. Infection in cats and cattle has been reported, but is less common in these species. *Lagenidum* species cutane-ous/subcutaneous infection is very similar to pythiosis clinically, and is equally aggressive, but has only been reported in dogs.[1]



Mammary tissue, dog. Hyphae range from 4-12 um in diameter with non-parallel walls and lack septations. (GMS, 400X)

Zygomycosis is caused by fungi in the class *Zygomycetes* which is divided into the two orders, *Mucorales* and *Entomophthorales*. The hyphae are described as being infrequently septate and their broad nature allows them to be differentiated from other fungal organisms. Like pythiosis, zygomycosis most commonly occurs in horses and the cutaneous lesions resemble those of pythiosis. However, zygomycotic cutaneous lesions in horses are usually found on the lateral neck, trunk or head, are smaller than those of pythiosis, and often have no association with standing water.[1]

Contributing Institution:

Tuskegee University
College of Veterinary Medicine, Nursing and Allied Health
http://www.tuskegee.edu/academics/colleges/cvmnah/school of veterinary medicine.aspx

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Self-Assessment - WSC 2015-2016 Conference 2

1. Which of the following is not seen with Sertoli cells tumors in the dog?
    a. Hyperestrinism
    b. Production of excessive progesterone
    c. Squamous metaplasia of the prostate
    d. Estrogenic depression of the bone marrow

2. Which of the following is not a true statement?
    a. Interstitial cell tumors and seminomas are most often considered benign with metastasis being uncommon.
    b. With regard to retained testes, Sertoli cell tumors are more common in abdominal testes and seminomas more common in inguinal testes.
    c. Sertoli cell tumors, being the most common testicular neoplasm in the dog, grossly have a distinctive tan or yellow-orange color due to their high lipid content and frequently contain areas of hemorrhage.
    d. Contralateral testes are also at increased risk of Sertoli cell tumor development.

3. Which of the following is not a clinical syndrome associated with Feline herpesvirus-1
    a. Necrotizing bronchopneumonia
    b. Necrotizing otitis media-interna
    c. Chronic sinusitis with opportunistic bacteria and mycoplasma infection
    d. Chronic conjunctivitis and keratitis with photophobia

4. In which of the following states has *Francisella tularensis* NOT been reported?
    a. Alaska
    b. Maine
    c. Wyoming
    d. Hawaii

5. Which of the following agents may be differentiated from the others based on morphology in tissue section?
    a. Zygomyces
    b. Pythium
    c. Lagenidum
    d. Aspergillus