Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 3

6 September 2017

CASE I: 10N0979 (JPC 4019127).

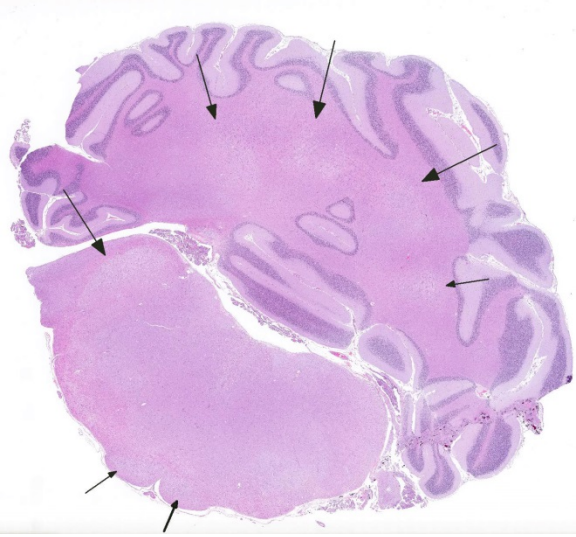
Signalment: 1 year-old, male, Domestic longhaired cat (*Felis catus)*.

History: This tissue was from a stray cat hit by a car 3 weeks prior to presentation to the VMTH. On presentation the cat was non-ambulatory and obtunded, had a fractured right pelvic limb, absent voluntary movement of both pelvic limbs and the left thoracic limb, and no pain perception. Due to a grave prognosis, the cat was humanely euthanized.

Gross Pathology: This cat was thin and had a healing transverse fracture of the right distal tibia. The bladder was distended and had a severely compromised thickened wall with large irregular areas of full thickness necrosis (considered the result of trauma). The brain and spinal cord were grossly normal.

Laboratory results: None provided.

Microscopic Description: Brain: There is severe bilateral, regional and symmetrical demyelination of the white matter (WM) in the brain and spinal cord of varying severity. There are widespread angiocentric accumulations of large mononuclear cells. These cells are CD18 immunoreactive (macrophages) that have abundant, distended pale finely vacuolated eosinophilic cytoplasm, eccentric nuclei, and are occasionally multinucleated. In areas of demyelination in the WM, there is scattered axonal necrosis with axonal spheroids and reactive astrogliosis. The large globoid mononuclear cells are PAS positive, but stain non-metachromatically. The gray

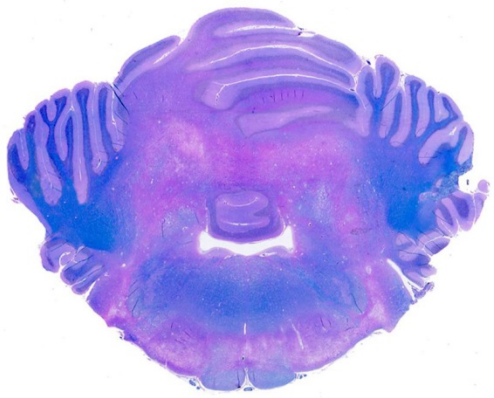


Cerebellum and brainstem, cat. There are bilaterally symmetrical areas of pallor and hypercellularity within the deep cerebellar white matter and the spinocerebellar tracts of the underlying brainstem. (HE, 6X)

matter appears unaffected.

Contributor's Morphologic Diagnosis: Brain and spinal cord: Severe bilaterally symmetrical perivascular histiocytosis and demyelination (consistent with globoid cell-like leukodystrophy).

Contributor's Comment: Globoid cell leukodystrophy is a rare lysosomal storage disease (sphingolipidosis) that involves the white matter of the central and peripheral nervous system. The disease has been described in humans (Krabbe's disease), mutant twitcher mice, rhesus monkeys, polled Dorset sheep, and domestic cats. In all affected animals except the domestic cat there is a demonstrated autosomal recessive mode of inheritance. [1,2,4,5,7] One report documents globoid leukodystrophy in 2 female kittens that resulted from inbreeding of common ancestors, which suggests a hereditary basis in cats as well.[1] Clinical signs appear at a young age (1-3 months of age) and initially consist of ataxia, hypermetria, tremors, and proprioceptive deficits which progress to blindness, anorexia, muscle atrophy, and paraplegia.

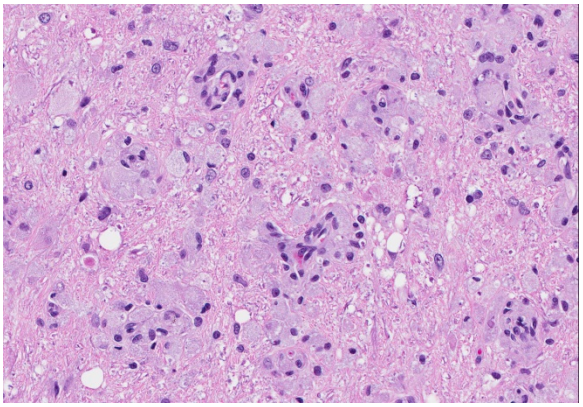


Cerebellum and brainstem, cat. Areas of pallor are also demonstrated on a Luxol fast blue stain, suggesting focal areas of demyelination. (LFB, 6X) (Photo courtesy of: University of California, Davis, Veterinary Medical Teaching Hospital, Anatomic Pathology Service.)

Death occurs by 1-2 years of age.[6]

The disease occurs due to a deficiency in galactocerebrosidase enzyme activity resulting in an accumulation of galactosylsphingosine (psychosine) within Schwann cells and oligodendrocytes.[1] It remains undetermined if accumulation of this intracellular material is a result of abnormal myelin synthesis or abnormal breakdown. Psychosine is cytotoxic thus resulting in degeneration and necrosis of oligodendrocytes and Schwann cells (hence the extensive demyelination) and extracellular release. Macrophage phagocytose and accumulate psychosine and are the hallmark 'globoid cells' seen on histopathology and typically aggregate perivascularly in the white matter.[3] Typical histological findings consist of bilaterally symmetrical white matter demyelination and striking perivascular hypercellularity characterized by numerous plump globoid macrophages.[6] The centrum semiovale, corona radiate, corpus callosum, optic tract, and cerebellar medullare are most severely affected.[6] Peripheral subpial areas are the most commonly affected portion of the spinal cord.[6] The intracytoplasmic material within macrophages is typically PAS positive, non-metachromatic, and non-sudanophilic.[1] Leukocyte immunohistochemical markers such as CD18 or MAC1 can be used to confirm globoid cells are of macrophage/microglial origin.

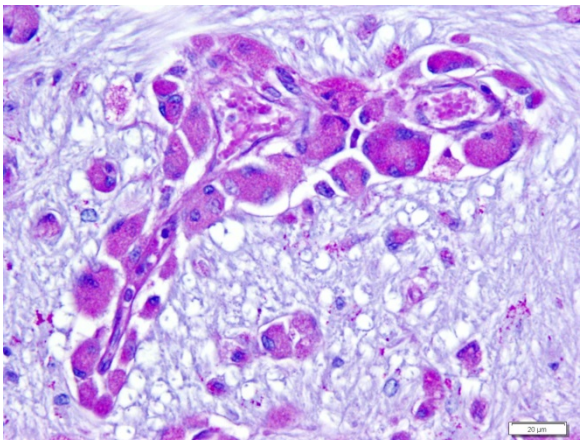
JPC Diagnosis: Cerebellum and brain stem: Histiocytosis, perivascular, multifocal, moderate with demyelination and gliosis, Domestic longhair, feline.



Cerebellum white matter, cat. Blood vessels are surrounded by histiocytes with abundant foamy cytoplasm, which also are present in lesser numbers within the intervening neuropil. (HE, 400X)

Conference Comment: This case nicely demonstrates lesions attributable to globoid-cell leukodystrophy (Krabbe's disease) in a young cat. Participants discussed the bilaterally symmetrical areas of pallor within deep spinocerebellar and brain stem pyramidal tracts corresponding to white matter vacuolation and histiocytosis. Histiocytes are described as having an amphophilic granular cytoplasm. There is evidence of demyelination characterized by axonal swelling (spheroids) and dilated myelin sheaths with macrophages phagocytizing myelin (Gitter cells in digestion chambers). Participants reviewed the immunohistochemical stains submitted by the contributor including Luxol fast blue that further emphasizes the severity of the demyelination and CD18 which classifies perivascular engorged cells as histiocytic in origin. Participants were reminded that the affected histiocytes are clustered around vessels in an attempt to leave the tissue but are too large to pass between endothelial cells and enter circulation.

The contributor provides a complete summary of the pathogenesis of globoid cell leukodystrophy which was also discussed by participants. The conference moderator lead participants in reviewing the broad categories of storage diseases: induced and inherited. In general, storage diseases are characterized by accumulation of a substance that exceeds the capacity of that cell to digest or dispose of that substance. Inherited storage diseases are almost always proved to be due to lysosomal deficits, and commonly occur in young animals owing to the fact that they are caused by genetic defects. Inherited storage disease categories were mentioned: sphingolipidoses, glycoproteinoses, mucopolysaccharidoses, glycogenoses, mucolipidoses, ceroid-lipofuscinoses, and Lafora disease. Induced storage diseases have been produced by ingestion of plants containing swainsonine



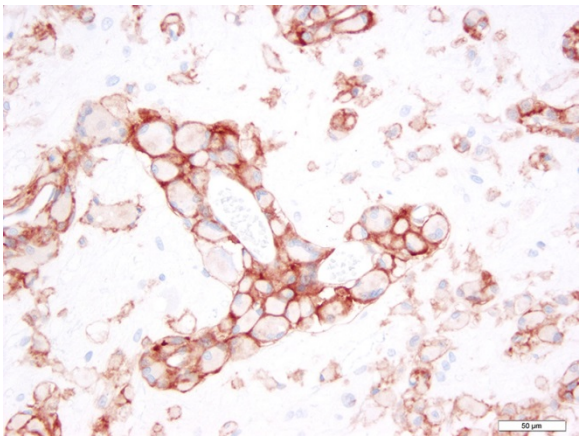
Cerebellum white matter, cat. Perivascular histiocytes stain strongly positive with a PAS stain. (periodic acid-Schiff, 400X) (Photo courtesy of: University of California, Davis, Veterinary Medical Teaching Hospital, Anatomic Pathology Service.)

(an indolizidine alkaloid) and plants of the *Trachyandra* spp. and *Phalaris* spp. genera.[1]

Finally, of the inherited storage diseases, the moderator reviewed sphingolipidoses:

Sphingolipidosis category	Common name	Deficiency	Accumulation	Cellular vacuolation	Animals affected
GM1 Gangliosidosis		β-galactosidase	GM1 ganglioside, some oligosaccharides	Neurons & macrophages	Cats, dogs, cattle, sheep
GM2 Gangliosidosis	Tay Sachs; Sandoff disease	β-Hexosaminidase	GM2 ganglioside, +/- globoside	Neurons & macrophages	Cats, dogs, Yorkshire pigs
Glucocerebrosidosis	Gaucher's disease	Glucocerebrosidase	Glucocerebroside	Neurons & macrophages (not in cerebellar Purkinje cells or spinal cord)	Sydney Silky Terrier
Sphingomyelinosis	Niemann-Pick type A	Sphingomyelinase	Sphingomyelin, cholesterol and ganglioside	Neurons & macrophages	Cats, miniature poodles
Sphingomyelinosis	Niemann-Pick type C	NPC 1 or NPC 2 proteins	NPC 1 or NPC 2 proteins	Neurons & macrophages	Cats, Boxer dogs
Galactosialidosis		β-galactosidase & α-neuraminidase	Gangliosides and oligosaccharides	Neurons & macrophages	Schipperke dogs
Galactocerebrosidosis	Globoid cell leukodystrophy; Krabbe's disease	Galactocerebrosidase (GALC)	Galactocerebroside and galactosylsphingosine (psychosine)	Macrophages only (globoid cells)	Cats, mutant Twitcher mice[4], polled Dorset sheep

Chart adapted from: Cantile C, Youssef S. Nervous system. In: Maxie MG ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol. 1. 6th ed. St. Louis, MO: Elsevier; 2016:284-293.



Cerebellum white matter, cat. Perivascular histiocytes also stain dense cytoplasmic positivity for CD-18. (periodic acid-Schiff, 400X) (Photo courtesy of: University of California, Davis, Veterinary Medical Teaching Hospital, Anatomic Pathology Service.)

Several attendees expressed concern with the diagnosis in this case, noting the previous history severe trauma, the lack of any mention of lesions elsewhere in the central nervous system, as well as the lack of any specific identification of material contained within macrophages in the examined sections. Moveover, as is the case with strays, there is a lack of a familial history. There was general concern that the lesion may be a long-term sequel to the severe neurologic trauma noted weeks prior to this case.

Contributing Institution:
University of California, Davis
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Anatomic Pathology Service

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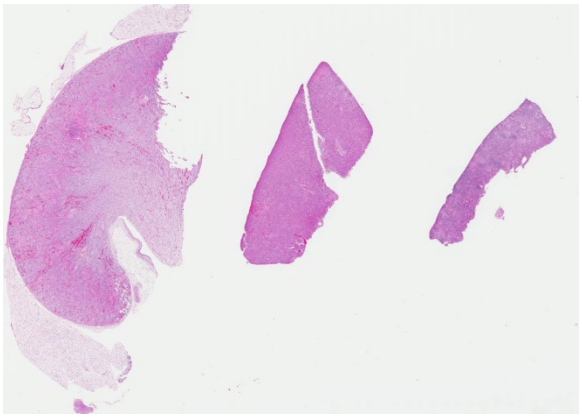
CASE II: 11-30435 (JPC 4020992).

Signalment: 2-week-old male and female intact, terrier mixed breed dogs *(Canis lupus familiaris)*.

History: An adult terrier mixed breed dog was vaccinated with canine distemper virus during pregnancy, and 7 out of 8 puppies died after birth. Upon initial presentation, 6 puppies had normal physical exams and normal temperatures, while the 7th puppy was vocalizing, dyspneic, mildly cyanotic, and had a mildly elevated lymphocyte count. This puppy was euthanized a short time later. The rest of the puppies had intermittent twitching, one puppy was hypoglycemic, and one puppy had moderate amounts of clear nasal discharge. The rest of the puppies died within the next few days.

Gross Pathology: The kidney, liver, spleen, and to a lesser extent within the lungs and small intestines were multifocal pinpoint to 1 mm, dark red foci (petechia). The thoracic cavity contained approximately 5-10 ml of yellow to red-tinged, translucent fluid. The



Kidney, liver, spleen, puppy. A section of each organ is submitted for examination (HE, 5X).

lungs were diffusely red and rubbery.

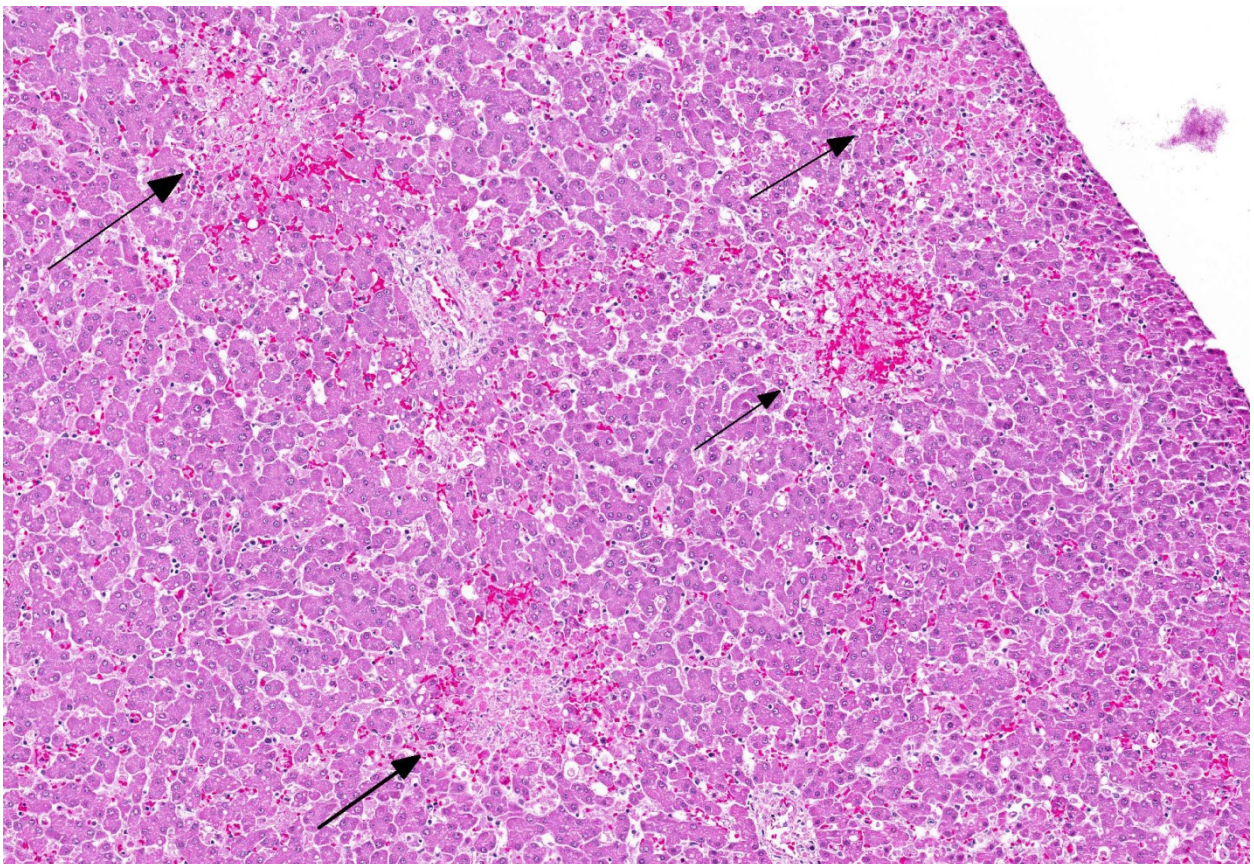
Laboratory results: FA was positive for canine herpesvirus.
FA was negative for canine distemper virus.
Bacteriology showed no growth in the lung and kidney.

Microscopic Description: Multifocally within sections of kidney, liver, and spleen, the parenchyma has lost differential nuclear staining and contains necrotic cellular debris and hemorrhage. The surrounding cells often have karyorrhectic nuclei with hyper-eosinophilic cytoplasm. Few nuclei contain a single, round, eosinophilic intranuclear inclusion bodies with marginated chromatin.

Additionally, tissue from the lungs and jejunum (slides not submitted) have similar multifocal regions of necrosis with few single, round, eosinophilic intranuclear inclusion bodies with marginated chromatin. The alveolar septa are also diffusely expanded by macrophages, lymphocytes, and neutrophils with type II pneumocyte hyperplasia. The grey and white matter of the cerebrum, cerebellum, and brainstem (slides not submitted) are multifocally disrupted by glial cells, macrophages, few neutrophils, and degenerating neurons. The meninges are diffusely thickened with neutrophils, lymphocytes, and macrophages.

Immunohistochemistry: The small intestine was negative for canine parvovirus, and the brain was negative for canine distemper virus.

Contributor's Morphologic Diagnosis: Kidney, liver, and spleen: Nephritis, hepatitis, and splenitis, necrotizing,



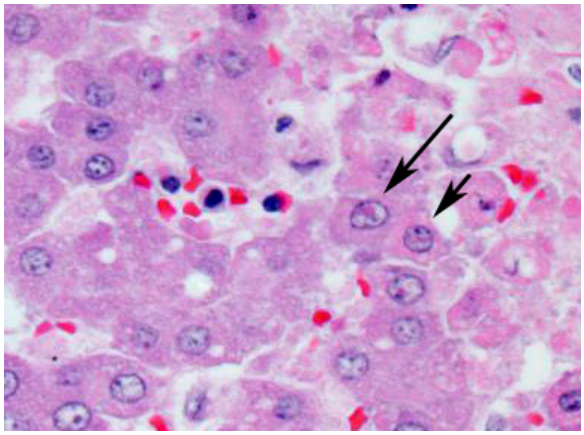
Liver, puppy. The liver contains randomly scattered areas of coagulative necrosis and hemorrhage. (arrow) (HE, 140X).

multifocal, marked, with few eosinophilic intranuclear inclusion bodies.

Etiology: Canine herpesvirus-1 (CaHV-1)

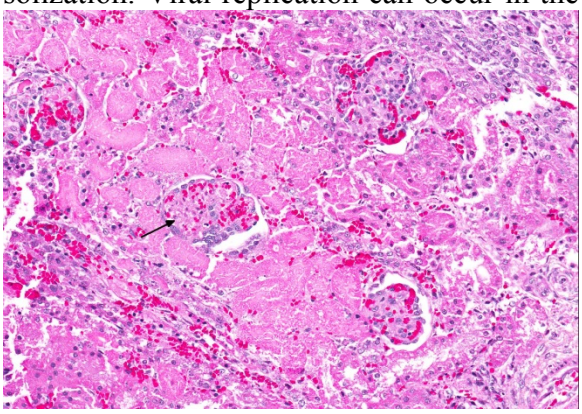
Contributor's Comment: Canine herpesvirus type 1 is a well-known entity first recognized in the 1960s and is a double stranded DNA virus in the alphaherpesvirus subfamily. The virus is deactivated in temperatures greater than 40°C and flourishes in temperatures between 34°C and 36°C; thus, by raising the body temperature, chances of survival are increased.[2, 5, 7]

The age of the dog can determine the clinical presentation of the herpesvirus infection. Dogs less than 3 weeks old generally have fatal systemic disease due to acute neonatal viremia. Dogs older than 3 weeks can have ocular and mucosal disease (either respiratory or vaginal). Naïve pregnant bitches are susceptible to systemic infection that can cause abortions or acute neonatal viremia. Latent infection is a concern for any age group if they survive the original infection and can occur following steroid administration or stress, such as pregnancy.[1, 2, 3]



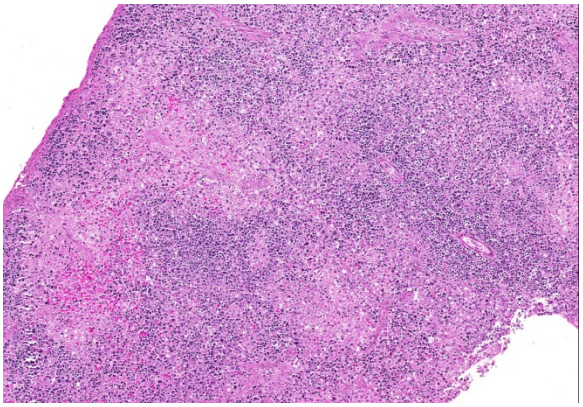
Liver, puppy. Hepatocytes at the periphery of areas of the necrosis rarely contain intranuclear viral inclusions. (arrows) (HE, 400X).

Typically, dogs less than 3 weeks of age acquire the disease in a variety of methods: *in utero*, passage through the birth canal, direct contact with oronasal secretions of the bitch, direct contact with other dogs that are shedding, and humans acting as fomites. Older dogs with ocular and/or mucosal disease are generally infected via aerosolization. Viral replication can occur in the nasopharynx, tonsils, and/or retropharyngeal and tracheobronchial lymph nodes. The virus then spreads throughout the bloodstream via macrophages to infect the liver, kidneys, lymph nodes, lungs, and centralnervous system.[1, 2, 3, 7] The incubation period is approximately 6 to 10 days and litter mortality can reach 100% over the course of a week.[2] Common clinical signs include vocalization, anorexia, hypothermia, abdominal pain, and/or incoordination.[2, 4]



Kidney, puppy. Scattered throughout the cortex are randomly scattered areas of infarction affected glomeruli (arrow) and surrounding tubules. (HE, 324X)

Characteristic gross and histologic lesions in affected dogs less than 3 weeks of age include systemic hemorrhage and necrosis in the kidney, liver, spleen, and intestine with little associated inflammation, enlarged lymph nodes, and non-suppurative meningoencephalitis, all of which were present within this case.[2, 4, 5, 6, 7]



Spleen, puppy. Extensive lymphoid and splenic necrosis is characteristic of CHV-infection in puppies. (HE, 324X)

Eosinophilic intranuclear inclusion bodies were also present. Classic lesions in addition to a positive fluorescent antibody test verified canine herpesvirus. Fluorescent antibody testing and immunohistochemistry ruled out canine distemper virus and canine parvovirus as possible contributors.

JPC Diagnoses: 1. Liver: Hepatitis, necrotizing, random, multifocal to coalescing with few eosinophilic intranuclear inclusion bodies, terrier mix, canine.

2. Kidney, tubules and glomeruli: Nephritis, necrotizing, multifocal, mild.
3. Spleen: Splenitis, necrotizing, diffuse, severe.

Conference Comment: This case serves as a classic example of canine herpesvirus-1 (CaHV-1) in a puppy, causing random necrotizing hepatitis, nephritis, and splenitis. Participants described multifocal areas of coagulative necrosis in the liver and kidney, as well as diffuse necrosis in the spleen, with few eosinophilic intranuclear inclusion bodies, mild vasculitis, and fibrin thrombi (especially in the liver and glomeruli). Participants also noted that inclusions were most prominent in the liver adjacent to areas of necrosis, but were difficult to see in the kidney and spleen due to the extensive necrosis in those tissues.

In addition, participants reviewed select alphaherpesviruses from other species (cats, farm animals, and horses), which are summarized in the following chart[1, 3, 7, 8, 11]:

Alphaherpesvirus	**Common name**	**Primary lesions**
Bovine herpesvirus 1	Infectious bovine rhinotracheitis	Fibrinonecrotic membrane along trachea/larynx, bronchointerstitial pneumonia; Systemic form: Focal areas of necrosis within the alimentary tract
	Infectious pustular vulvovaginitis	Necrotizing vulvovaginitis with intranuclear inclusion bodies in epithelial cells

Bovine herpesvirus 2	Pseudo-lumpy skin disease	Eruption of superficial cutaneous nodules with a depressed center; no scar formation or deep necrotic sequestra (differentiates from true lumpy skin disease)
	Bovine mammillitis	Trauma initiates infection; swollen teats with cutaneous plaques; epithelial syncytia with Cowdry type A intranuclear viral inclusions
Porcine herpesvirus 1	Pseudorabies/Aujeszky's Disease	Abortion; non-suppurative meningoencephalitis; rhinitis, tonsillitis, pneumonia; coagulative necrosis of placenta, liver, spleen, adrenal gland
Equine herpesvirus 3	Equine coital exanthema	Papules, vesicles, pustules on genitalia or muzzles; ballooning degeneration of keratinocytes with intranuclear viral inclusions
Equine herpesvirus 5	Multinodular pulmonary fibrosis	Nodules with thick interstitial collagen with irregular alveoli lined by cuboidal cells; intranuclear viral inclusions in alveolar macrophages
Gallid herpesvirus 1	Avian infectious laryngotracheitis	Mucohemorrhagic fibrinonecrotic exudates (+/- tracheal casts) within nasal turbinates, sinuses, conjunctiva, larynx, and trachea; epithelial ulceration with formation of syncytial cells containing intranuclear viral inclusions
Gallid herpesvirus 2	Marek's disease	Herpesviral induced T-cell lymphoma; four different gross lesion patterns: (1) thickening and yellowing of peripheral nerves (2) discoloration of the iris (3) enlargement of feather follicles and (4) visceral tumors

Anatid herpesvirus 1	Duck Plague	Multifocal hemorrhages in visceral organs; severe enteritis with lymphoid tissue necrosis; foci of necrosis in liver
Feline herpesvirus 1	Feline viral rhinotracheitis	Focal ulcerative (and often eosinophilic) lesions on face or nasal planum (usually without clinical respiratory signs); ulcerative and necrotizing with large glassy intranuclear viral inclusions in epithelial cells

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CASE III: E 1246-14 (JPC 4102122).

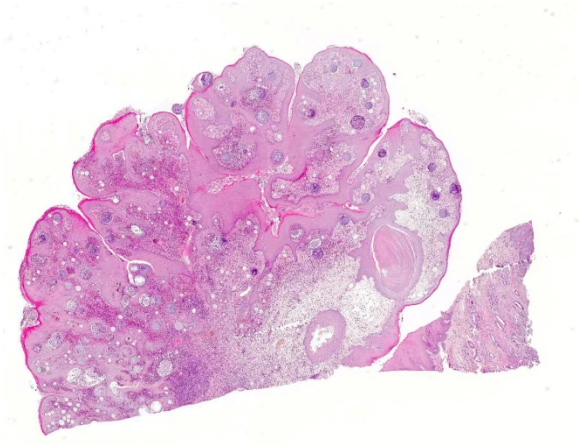
Signalment: 24-year-old, intact female, polo pony (*Equus ferus caballus*).

History: Only little clinical history was provided. Recurring polypoid structures had been observed in the left nostril for seven years, especially growing during the spring and summer period. The polypoid structures had formerly been diagnosed as rhino-sporidiosis seven years ago.

Gross Pathology: A tissue sample from the nasal mucosa fixed in formalin was submitted measuring 2.8 x 1.9 x 1.4 cm, containing two separate masses connected by a tissue bridge. The first mass measured 2.3 x 1.4 x 1.1 cm and the second mass was 1.9 x 1.9 x 1.2 cm in size. Both masses were partially ulcerated.

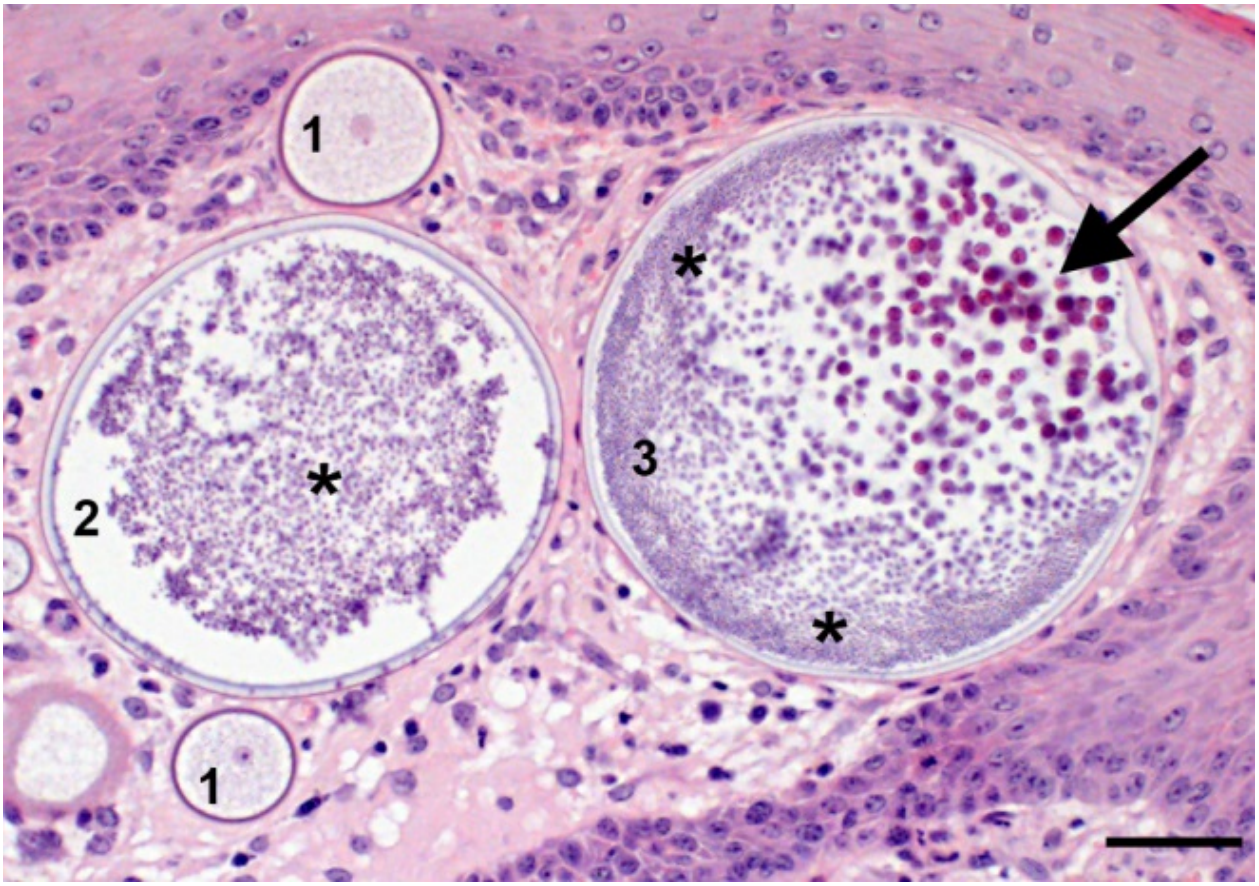
Laboratory results: None provided.

Microscopic Description: Nasal mucosa: There is a polypoid, multilobulated mass expanding the submucosa, formed by loosely fibrovascular tissue and covered by



Nasal polyp, horse. At subgross magnification, a polyp composed of an edemaout collagenous core covered with hyperplastic squamous epithelium contain numerous large basophilic endosporulating sporangia in proximity to the epithelium. (HE, 15X)

hyperplastic stratified squamous epithelium. Within the fibrovascular tissue, there is marked infiltration with lymphocytes, moderate numbers of viable and degenerated neutrophils and macrophages and fewer plasma cells. Randomly distributed throughout the mass are immature to mature, partially ruptured fungal sporangia ranging from 10 to 300 µm in diameter. The smaller round immature sporangia measure 10 to 60 µm in diameter, have a central nucleus and a prominent nucleolus surrounded by loose, basophilic granular material. Their unilamellar wall is up to 2 µm thick. The intermediate sporangia measuring 60 to 200 µm in diameter show a bilamellar wall and an accumulation of ovoid, immature endospores of up to 1 µm in diameter. Large mature sporangia also have a hyaline, bilamellar wall and the immature endospores are located closer to the sporangial wall whereas the 12 µm large, eosinophilic, globular mature endospores are located more centrally. Few of the sporangia are ruptured, showing a discharge of endospores into the surrounding tissue and a marked infiltration by neutrophils. The overlying hyperplastic epithelium shows



Nasal polyp, horse. Nasal mucosa with various maturation stages: 1, immature sporangium with eosinophilic, unilamellar wall, central nucleus with a prominent nucleolus surrounded by basophilic, granular material; 2, intermediate sporangium with a bilamellar wall enclosing abundant immature endospores (asterisk); 3, mature sporangium with a bilamellar wall containing immature endospores located closer to the sporangial wall (asterisks) and eosinophilic, mature endospores arranged more centrally (arrow); note the hyperplastic epithelium; Hematoxylin & Eosin, bar = 50 µm (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

multifocal keratinization and multifocal erosions with formation of serocellular crusts.

Adjacent to the mass the mucosa is lined by multifocally pseudostratified columnar epithelium and prominent seromucous glands.

Contributor's Morphologic Diagnosis: Nasal mucosa: Rhinitis, proliferative, lymphoplasmacytic and pyogranulomatous, chronic-active, focal-expansive, severe with epithelial hyperplasia and fungal sporangia consistent with *Rhinosporidium seeberi.*

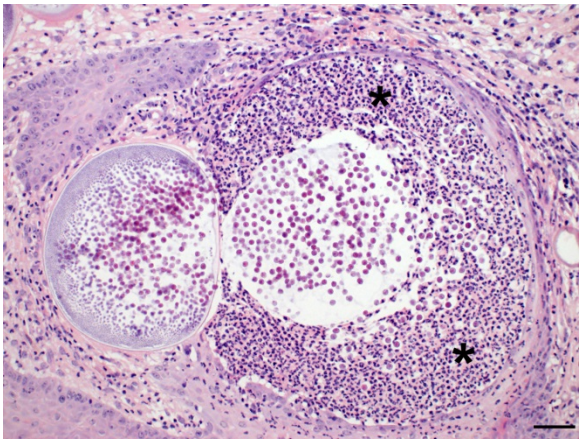
Contributor's Comment: *Rhinosporidum seeberi* is a fungal-like organism causing the mucocutaneous disease Rhinosporidiosis in humans and many different animals including mammalians and birds.[3] Previously classified as a fungus, currently the agent is assigned to a new class of parasites the DRIP clade respectively the clade of the Mesomycetozoea.[6] Rhino-sporidiosis is endemic in different countries in Asia and Africa with high incidence in India, Sri Lanka and Argentina. However, the disease has been reported in about 70 different countries worldwide.

Rhinosporidium seeberi's natural habitat is most likely the ground water. Infection occurs primarily via the nasal cavity through penetration of infectious spores via superficial trauma in the epithelium. The disease seems to be infective but not infectious, although the life cycle and pathogenesis are not yet fully understood. In vitro cultivation is very challenging; so far, the agent's isolation from its habitat was not successful. [1]

The typical clinical appearance of nasal or laryngeal rhinosporidiosis is single to multiple, granular, pink to red, pedunculated or sessile, polypoid growths (strawberry-like appearance).[1,6] The masses are non-infiltrating, slow-growing and painless.[5] Burgess et al.[2] hypothesized that the organism has the ability to persist subclinically for long a time.

The histopathological appearance of rhinosporidiosis seems to be the same in animals as in humans. Histological differentials include *Coccidioides immitis* and _ *(Emmonsia- new name) parvum* because of the size of the sporangia[3]; nevertheless, the identification of all different stages allows a reliable, histopathological diagnosis.[1] A periodic acid-Schiff (PAS) reaction clarifies the thick, unilamellar, eosinophilic and PAS-positive wall of immature sporangia whereas the bilamellar wall of intermediate sporangia stains partially positive for PAS, the inner layer shows positive staining for Gomori methenamine silver.[2] Endospores additionally stain positive with toluidine blue.

The treatment of choice is the complete surgical excision of the polypoid masses using electro-cautery. Recurrence is common, in which sessile polyps seem to recur more often than pedunculated polyps.



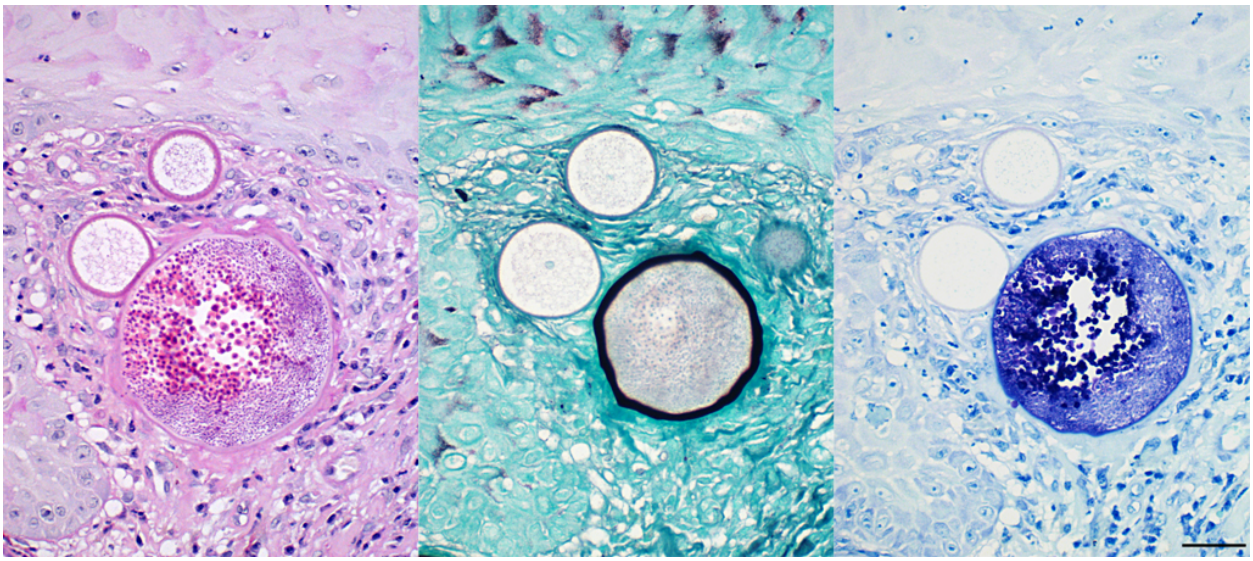
Nasal polyp, horse. Nasal mucosa with mature sporangia, the one on the right ruptured and infiltrated by high numbers of neutrophils (asterisks); Hematoxylin & Eosin, bar = 50 µm (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

Interestingly, rare cases of spontaneous regression have been reported.[1]

Rhinosporidiosis in animals and humans is an extremely rare disease in Europe[6]; the observed infections are mostly linked to former stays in endemic countries.[4] Due to globalization and the growth of international trade with animals, Rhinosporidiosis could become an emerging disease in European countries.

JPC Diagnosis: Nasal mucosa: Rhinitis, proliferative, chronic, diffuse, moderate with numerous sporangia and endospores, polo pony, equine.

Conference Comment: This case demonstrates a classic example of nasal rhinosporidiosis in a horse. Participants described nodular lesions with overlying hyperplastic mucosa that contains variably sized sporangia and endospores in different stages of maturation surrounded by inflammation and fibrous connective tissue.



Nasal polyp, horse. Nasal mucosa with sporangia after PAS reaction (left panel), Grocott methenamine silver (middle panel) and toluidine blue staining (right panel). Immature sporangia are characterized by an eosinophilic, PAS-positive, unilamellar wall while only the outer layers of mature sporangia are fairly PAS-positive (left panel). The inner part of the bilamellar wall of the mature sporangia stains intensely positive with silver (middle panel). Mature endospores stain positive with toluidine blue (right panel); bar = 40 µm (Photo courtesy of: Department of Veterinary Pathology, Freie Universität Berlin http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

The conference moderator presented the images submitted by the contributor and attendees discussed the staining patterns of sporangia and endospores. Periodic acid-Schiff (PAS) highlights endospores, unilamellar walls of juvenile sporangia, and outer walls of mature sporangia and Gomori methenamine silver (GMS) indicates the inner wall of mature sporangia. [2, 4]

Rhinosporidium seeberi exists in three forms in infective tissue. It develops as spherical sporangia (6-300 µm) which mature to develop a thick bilamellar wall. The nuclei undergo changes as well, maturing from uninucleate immature sporangia, dividing their nuclei, and forming numerous uninucleate endospores (6-7 µm) characteristic of mature sporangia. Once the sporangium matures, the endospores are released through a break in the cell wall. Free spores consequently enlarge to form sporangia and continue the tissue cycle.

Grossly, rhinosporidiosis has a nodular, polypoid appearance with numerous pinpoint white foci on the surface representing larger spherules filled with sporangiospores. Systemic dissemination is rare and the disease is seldom fatal, but can cause complications due to obstruction of airways or bleeding. Sporangia generally incite a chronic granulomatous response, whereas free endospores incite an acute neutrophilic response. Eosinophils are rare. Multinucleated giant cells are often prominent around and within empty mature sporangia having entered through the break in the cyst wall. [3, 4, 8]

There are very few differentials for *Rhinosporidium seeberi* due to their large size; they are the largest endosporulators, and characteristic location in the upper respiratory tract. Sporangia of *Coccidiodes immitis,* for example, are rarely larger than 80-200 µm. In addition, their endospores lack internal globular bodies and only their walls stain with fungal stains. Empty

sporangia may resemble *Blastomyces dermatitidis;* however, there is no broad-based budding present, and the presence of mature sporangia with endospores is not characteristic of simple yeast. Another differential is *(Emmonsia parvum- new name?)* which has much thicker trilaminar adiaspore walls.[4] Finally, a rarely seen condition that may be confused with *Rhinosporidium seeberi* is myospherulosis. Myospherulosis is a type of foreign-body reaction in which erythrocytes interact with an exogenous substance, usually ointments, or endogenous fat and form subcutaneous nodules composed of macrophages that have intracytoplasmic homogenous eosinophilic spherules. These structures stain negatively with PAS, but the intracytoplasmic spherules are positive for endogenous peroxidase identifying them as phagocytized erythrocytes.[4, 9]

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http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html

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CASE IV: B17-798 (JPC 4101575).

Signalment: 14-year-old, spayed, female, Domestic shorthair cat (*Felis catus*).

History: The owner identified an approximately 1cm nodule associated with the right mammary chain in December. By the following May, it had grown to approximately 4cm and became ulcerated. The right mammary chain and left inguinal mammary gland were removed surgically, and the tissue was submitted for histopathology. At the time of surgery, a single, approximately 4mm pulmonary nodule was identified on thoracic radiographs. On follow-up radiographs one month later, this nodule had enlarged and additional pulmonary nodules were identified.

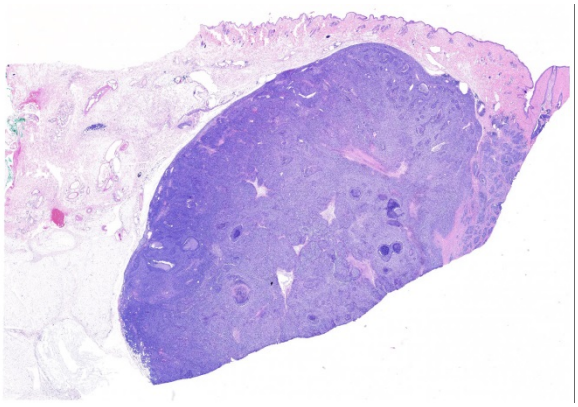
Gross Pathology: The right inguinal mammary gland was expanded by a 3.7 x 3.2 x 2.8 cm firm, ulcerated mass. The right superficial inguinal lymph node was included in the sample and was also enlarged and firm.

Laboratory results: Immunohistochemistry for cytokeratin, vimentin, and smooth muscle actin was performed to characterize the neoplastic population.

IHC for cytokeratin (AE1/AE3) revealed diffuse, strong, cytoplasmic labeling of the polygonal neoplastic population. Rarely, patchy areas of spindle cells exhibit weak to moderate cytoplasmic labeling (<1% overall).

IHC for vimentin strongly stained the cytoplasm of the majority (approximately 75%) of the neoplastic spindle cells and occasional polygonal cells (<5% overall).

IHC for smooth muscle actin highlights vascular smooth muscle within and around



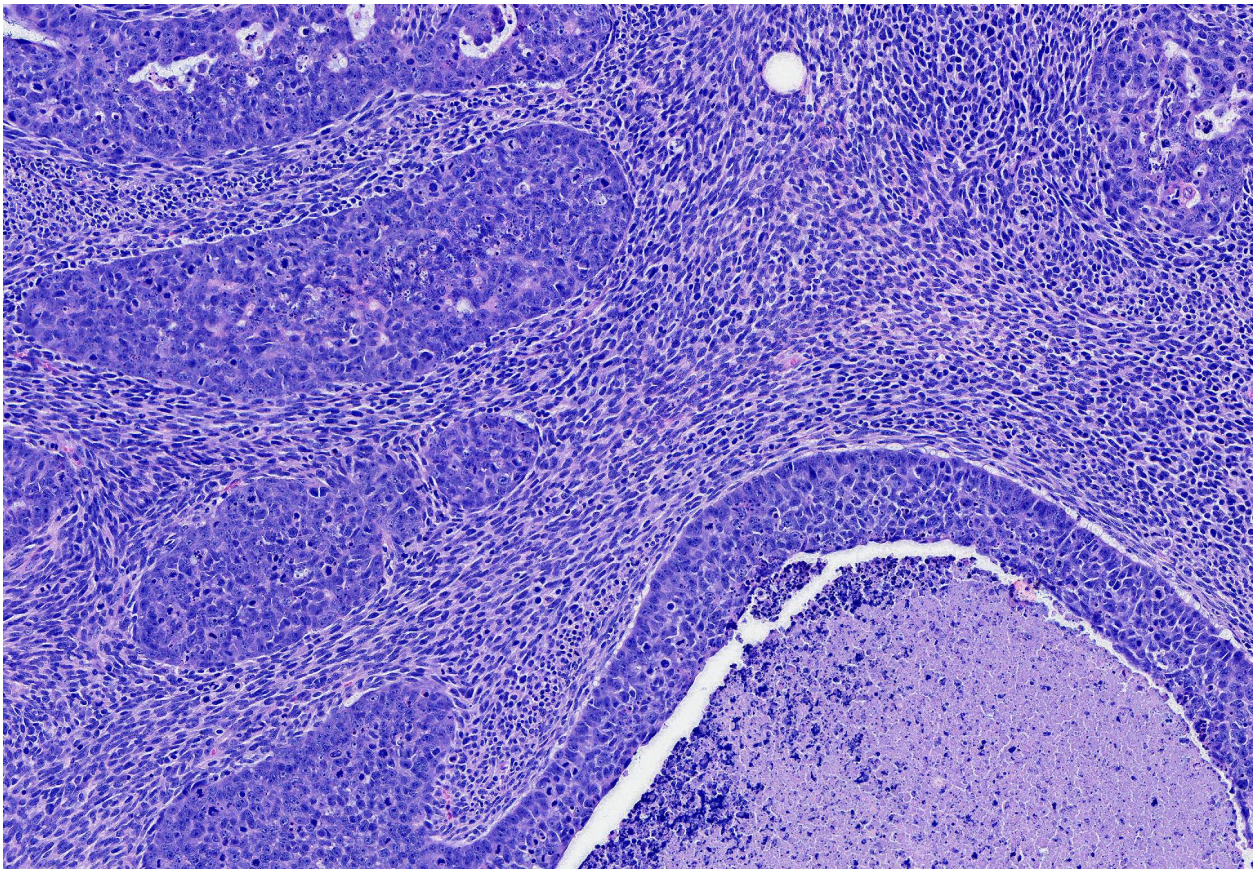
Mammary gland, cat. At subgross magnification, adjacent to the nipple, there is a well-demarcated densely cellular neoplasm. (HE, 10X)

the neoplasm and rarely individual or small groups of neoplastic cells in the epithelial or spindle cell populations (overall <1% of either population).

Microscopic Description: Mammary gland with nipple: Expanding and infiltrating the dermis and subcutis is a neoplasm composed of malignant epithelial and spindle cell populations that are typically but not always closely associated with each other.

The epithelial population is composed of polygonal cells in irregular tubules and islands with frequent central necrosis. The cells have round to oval to irregular nuclei with large amounts of vesicular to coarsely stippled chromatin and a large central nucleolus. They have moderate amounts of eosinophilic cytoplasm. They exhibit marked anisocytosis and anisokaryosis with 29 mitotic figures in 10 HPF. Near the nipple, neoplastic epithelial cells are present without the neoplastic spindle population and are instead inciting a prominent scirrhous reaction. The neoplastic epithelial cells extend along the epithelium and fill the lumen of the teat sinus, where they are mixed with corpora amylacea.

The neoplastic spindle cell population typically surrounds the epithelial population and is composed of streams of spindle cells



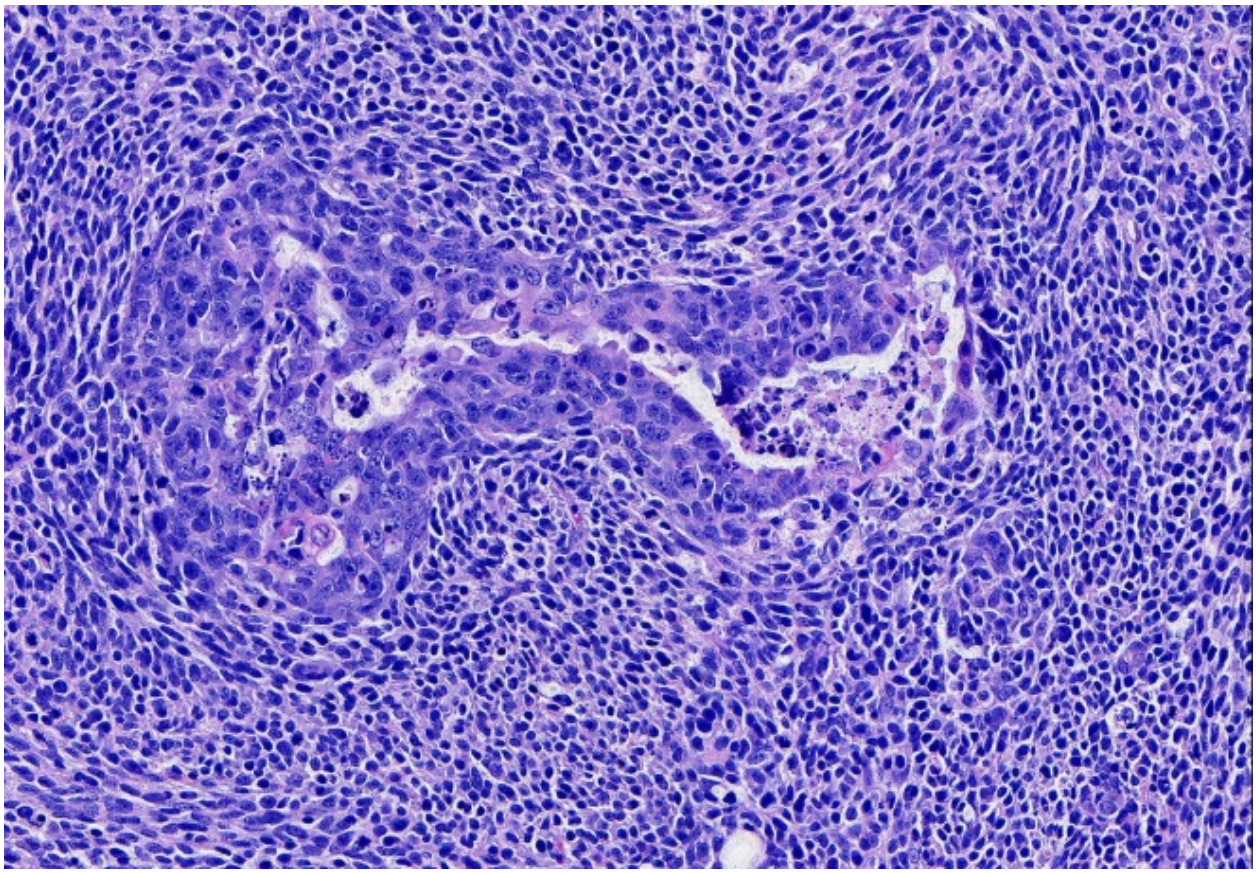
Mammary gland, cat. The neoplasm is composed of two distinct populations of neoplastic cells. Polygonal glandular epithelial cells form trabecular and islands, often with a central area of necrosis. A second population of robust spindle cells form elongate streams and bundles. (HE, 160X)

that have elongate nuclei with large amounts of coarse chromatin and a small nucleolus. They have small amounts of indistinct, eosinophilic cytoplasm. They exhibit moderate anisocytosis and anisokaryosis with 42 mitotic figures in 10 HPF. Narrow fingers of neoplastic spindle cells extend several millimeters from the main mass into the surrounding subcutaneous tissue.

The right superficial inguinal lymph node (not included) is extensively effaced by the neoplastic epithelial population and an associated scirrhous response.

Contributor's Morphologic Diagnosis: Mammary gland: Carcinosarcoma.

Contributor's Comment: Mammary neoplasia in cats occurs less commonly than in dogs but when it occurs, it is more likely to be malignant.[4] The classification of mammary neoplasia is complex and neoplasms may be composed of elements of epithelial, basal/myoepithelial, or mesenchymal origin individually or in combinations. Subtyping of feline mammary neoplasia has been based on the classification scheme published in 1999 by the World Health Organization with some publications proposing updates that would bring the feline classification scheme closer to the recently updated guidelines for canine mammary neoplasia.[4,5,9,17] Immuno-histochemistry can be helpful (or critical) in some cases to help distinguish the cell types involved in a particular neoplasm. Cells of luminal epithelial origin are expected to express so-called luminal cytokeratins (CK7, CK8, CK18, CK19). Cells of basal or

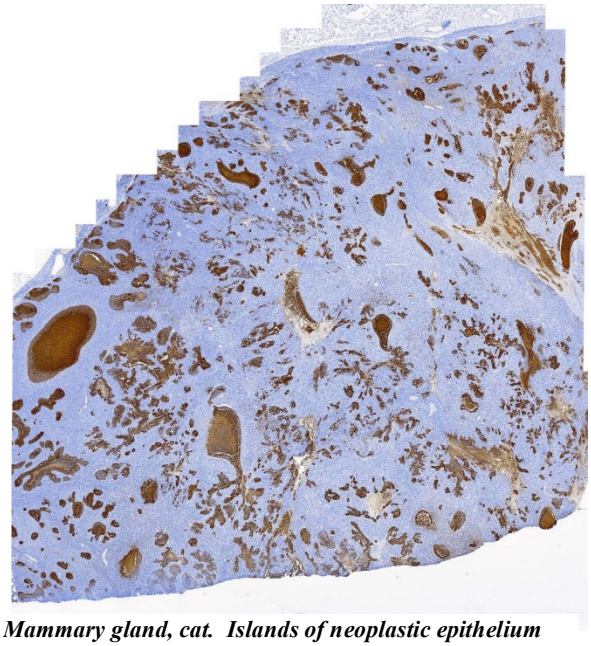


Mammary gland, cat. Higher magnification of the neoplasm. (HE, 300X)

myoepithelial origin are expected to express so-called basal cytokeratins (CK5, CK6, CK14, CK17) as well as smooth muscle actin, vimentin, calponin, and p63.[14]

In this case, proliferative epithelial and spindle cell populations were identified. Both populations had high mitotic rates, atypia, and infiltrative growth to support malignancy. Furthermore, the epithelial population was found within sections of a draining lymph node. Based on the hematoxylin and eosin stained sections, the primary differentials considered were carcinosarcoma (malignant mixed mammary tumor), carcinoma and malignant myoepithelioma, or spindle cell carcinoma arising from tubular carcinoma. The cytokeratin cocktail (AE1/AE3) used for IHC in this case recognizes cytokeratins 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15, 16, 19, so both luminal and basal/myoepithelial cells would be expected to stain. The lack of significant cytokeratin or smooth muscle actin expression in the spindle cell population made it unlikely that this population was of myoepithelial origin and would not be expected with a diagnosis of carcinoma and malignant myoepithelioma. The lack of cytokeratin expression in the vast majority of the spindle cells would also be unexpected in a spindle cell carcinoma.[5] Given the presence of a distinct cytokeratin-expressing epithelial population and a distinct vimentin-expressing spindle cell population, a diagnosis of carcinosarcoma (malignant mixed mammary tumor) was made. Additional IHC for other markers of myoepithelium (calponin and p63) were not performed in our lab to further rule out a myoepithelial component.

Although feline mammary carcinosarcomas have appeared in several reports, they are rare and do not appear in more recent publications discussing prognostic evaluation of feline mammary neoplasia.



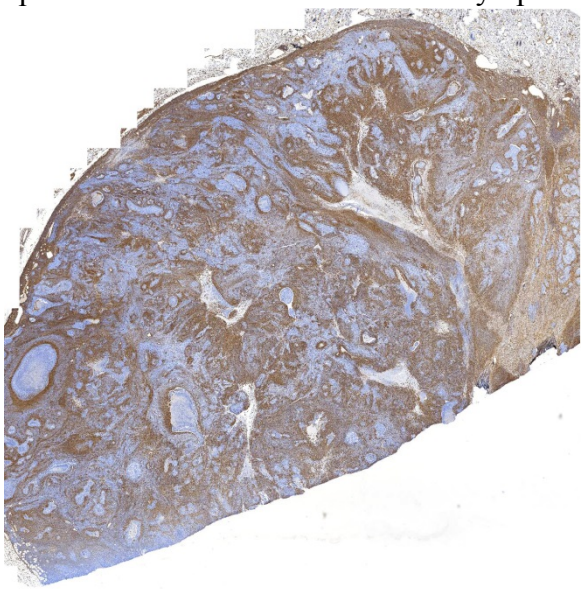
Mammary gland, cat. Islands of neoplastic epithelium stain intensively for cytokeratins. (anti-AE1/AE3, 10X) (Photo courtesy of: Cummings School of Veterinary Medicine, Tufts University http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/)

[2,8,10,13,17] In dogs, carcinosarcomas of the mammary gland are also rare and appear to have an aggressive course with all dogs with carcinosarcoma in one prognostic study (8 cases) exhibiting metastasis and having a median survival of only 4.2 months.[12] This particular cat had evidence of pulmonary (radiographic evidence) and lymph node (histologically confirmed) metastasis at the time of biopsy with progression of pulmonary nodules seen radiographically a month later. Mammary carcinosarcomas in dogs frequently have an osteosarcoma component,[4,5] but this was not seen in any sections from this particular case.

In cats, tumors with malignant epithelial and mesenchymal components (variably referred to as carcinosarcomas, malignant mixed tumors, or sarcomatoid carcinoma) have been rarely described in other organs, including uterus[11], salivary gland[7], prostate[16], lung[3], digital apocrine glands[6], pancreas[15], and biliary system[1].

JPC Diagnosis: Mammary gland: Carcinosarcoma, Domestic shorthair, feline.

Conference Comment: This case provided the rare opportunity to discuss the intricacies of feline mammary neoplasia, as well as the difficulties in precisely classifying the more rare variants.. Participants described a well-demarcated, multinodular neoplasm that expands the dermis composed of two types of cells: polygonal cells arranged in islands and trabeculae with central areas of comedonecrosis and spindle cells arranged in long interlacing streams separating islands of polygonal cells. Near the teat canal, there are areas of desmoplasia and large areas of necrosis with neoplastic cells present within the teat canal and lymphatic



Mammary gland, cat. Spindle cells stain strongly positive for vimentin. (anti-vimentin, 10X) (Photo courtesy of: Cummings School of Veterinary Medicine, Tufts University http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/)

vessels.

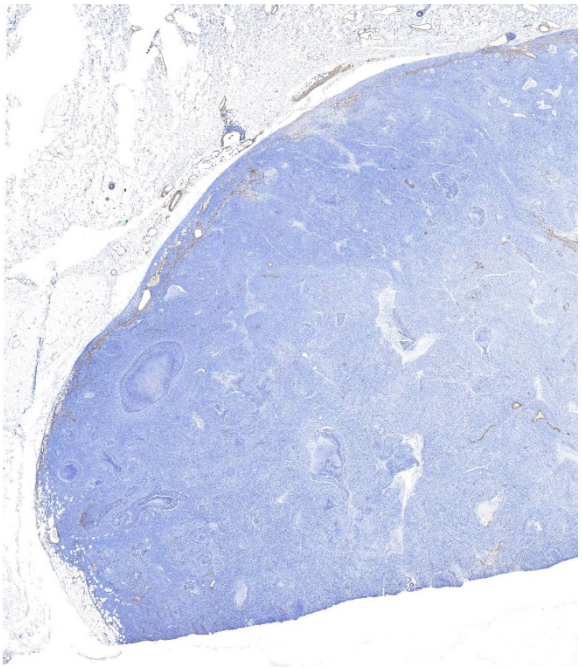
Feline mammary tumors are much less common than their canine counterparts and are more often malignant. Consequently grading and staging are important in regards to prognosis. The Elston and Ellis grading system (used in women) has been adapted for cats as well as a more recent numeric grading system that bases tumor grade on the following criteria: lymphovascular invasion, nuclear form, and mitotic count.

Table 1: Feline-specific malignant mammary gland tumors[4]

Prognostic factors include: epidemiologic factors (age, breed, reproductive status), clinical factors (staging, tumor size, lymph node invasion and metastasis, aggressiveness of surgery), and histological factors (type of tumor, grade).[4]

The conference moderator led a discussion on the published classification of feline mammary tumors. Hyperplasia and benign neoplasms are rare but are similar to what's found in bitches. Malignant mammary neoplasms are split into three groups: malignant epithelial neoplasms, malignant epithelial neoplasms – special types, and malignant mesenchymal neoplasms – sarcomas.

Malignant epithelial neoplasms	
Cribiform carcinoma	Lumina lined by neoplastic cells that are very inconspicuous (compared to tubular carcinomas).
Micropapillary invasive carcinoma	In queens and males, more than 50% have a micropapillary pattern.
Comedocarcinoma	Multiple small areas of well-defined necrosis located at the center of nodules of neoplastic cells. Neutrophil infiltration is attributed to mastitis in addition to the neoplastic process.
Anaplastic carcinoma	Rare in cats.
Intraductal papillary carcinoma	Rare in cats, identical to what is seen in dogs. Malignant variant of intraductal papillary adenoma.
Ductal carcinoma	AKA "feline complex carcinoma", malignant variant of the ductal adenoma which is composed of doubled layered neoplastic cells (luminal epithelial and basal cell components) forming cords, tubules, and solid areas around slit-like lumina. Ductal carcinomas are more solid with increased basal cells and less organized structure, more mitotically active with cellular atypia, and potentially areas of squamous differentiation and keratinization.
Malignant epithelial neoplasms – special types	
Adenosquamous carcinoma	Areas of carcinoma (any type) and areas of squamous differentiation.
Mucinous carcinoma	Rare, contains an abundant mucoid matrix surrounding neoplastic epithelial cells.
Lipid-rich carcinoma	Rare in cats, identical to what is seen in dogs.
Malignant mesenchymal neoplasms – sarcomas	
Inflammatory carcinoma	Clinical diagnosis characterized by sudden onset of subcutaneous edema, erythema, and pain in the ventral abdomen. The common histologic feature is large emboli of neoplastic cells in dermal lymphatics.



Mammary gland, cat. Neither population stain for smooth muscle actin. (anti-SMA, 10X) (Photo courtesy of: Cummings School of Veterinary Medicine, Tufts University http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/)

The contributor's morphologic diagnosis was discussed and debated at length. For expert evaluation, the case was sent to Dr. Michael Goldschmidt, Professor Emeritus at University of Pennsylvania, who commented that based on the morphology of the original HE slide (which was the only slide avaible to participants), a diagnosis of carcinosarcoma was perfectly acceptable. Several additional immunohistochemical stains were run to better categorize the neoplastic cell population. Both calponin and p63 were equivocal, staining some of the mesenchymal cell population but not all. Cytokeratin 5, 6, and 7 were negative for the mesenchymal cell population. Based on these results, we agree with the contributor's diagnosis of carcinosarcoma.

Contributing Institution:
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