WEDNESDAY SLIDE CONFERENCE 2019-2020

Conference 2

CASE I: Case #2 (JPC 4117378).

**Signalment:** 9-year-old, male, African lion (*Panthera leo*).

History: Caesar was born in June 2007. He and his sister, Cleopatra, were purchased from a breeder in Louisiana by a man who lives in Austin and had a large ranch in the northern Hill Country. The owner contacted the Austin Zoo and Animal Sanctuary in spring 2009 wanting to surrender the pair as he could not get a permit from the county to

Cerebrum, lion. A slightly compressed section of cerebrum was submitted. No obvious lesions are present at subgross magnification. (HE, 5X)

keep them. Caesar and Cleopatra arrived at the Austin Zoo and Animal Sanctuary in May 2009. Cleopatra got sick in spring 2011 and was humanely euthanized in July 2011 after she was diagnosed with canine distemper virus (CDV) and developed seizure activity. Caesar had never shown any symptoms at this time. All of the large felids were vaccinated for CDV shortly after Cleopatra died. Caesar had a diminished appetite in December 2013 and started exhibiting unusual behavior (e.g. jumping around the walls of his den). His appetite returned in January 2014, but he became ataxic in March 2014. The ataxia became progressively worse until he was no longer able to get his feet under him to walk up the steps into his den. He was admitted to the Texas A&M CVM for examination. He had a high positive titer for toxoplasmosis and a low positive titer for CDV which was thought to be a result of his vaccination. He was alert with an excellent appetite while being treated for toxoplasmosis. After some instances of apparent improvement, he ultimately started star gazing, stopped eating and became nonresponsive, at which time he was humanely euthanized.

Gross Pathology: Patient is emaciated (BCS 2/9). There are multiple, red, inflamed areas on skin from trauma and pressure sores.

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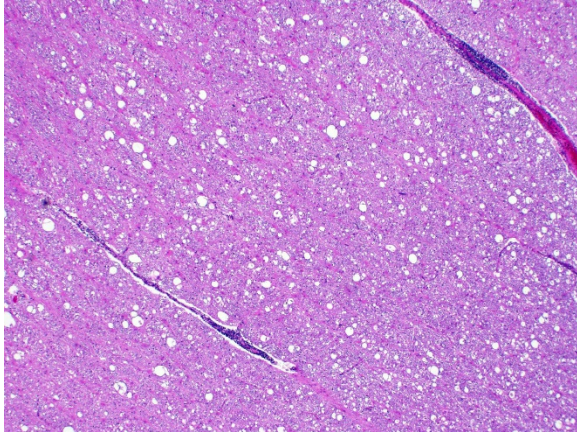
Cerebrum, lion. There is extensive vacuolation of the cortical white matter. In addition, Virchow-Robins space is multifocal expanded by low to moderate numbers of lymphocytes. (HE, 100X)

There is an abscess in the ventral neck associated with the right mandibular lymph node. The brain appears slightly shrunken. No other significant gross lesions are observed.

Laboratory results: Brain, positive for CDV by IHC.

Microscopic Description:
Cerebrum: Approximately 40% of myelin sheaths are lost with replacement by gitter cells (demyelination), or are markedly dilated (spongiosis) and occasionally contain swollen axons with bright eosinophilic axoplasm (spheroids) or gitter cells and cellular debris (Wallerian degeneration). Neurons and astrocytes often contain intranuclear viral inclusion bodies that marginate the chromatin and, rarely, 3-5 um eosinophilic intracytoplasmic viral inclusion bodies. Multifocally, there is vacuolation of the gray matter. Multifocally, there are small foci of glial nodules and numerous astrocytes with abundant eosinophilic cytoplasm and eccentric nuclei (gemistocytes). Occasionally, neuronal cell bodies are swollen and round with central chromatolysis (degeneration) or rarely, shrunken with bright eosinophilic cytoplasm with pyknotic nuclei (necrosis). Numerous neurons contain yellow to light green, granular material (lipofuscin). Multifocally, there is expansion of the Virchow Robin space around the blood vessels in the affected gray and white matter and leptomeninges by lymphocytes and plasma cells and lined by reactive endothelium.

Contributor's Morphologic Diagnoses: Cerebrum: Demyelination, multifocal, marked with spongiosis, spheroids, lymphoplasmacytic perivascular meningoencephalitis and eosinophilic intranuclear and intracytoplasmic viral inclusion bodies, African lion (*Panthera leo*), felid.

Contributor's Comment: Canine distemper virus (CDV) is an important, ubiquitous infectious disease of domesticated dogs[2], wild canids in the family Canidae[2] (e.g., dingo, fox, coyote, jackal, wolf), wild cats in Felidae (e.g., African lion[11], Bengal tiger[3], Amur tiger[18], leopard), Mustelidae[13] (e.g., weasel, ferret, mink, skunk, badger, marten, otter), Procyonidae[10] (e.g., raccoon, coati), Phocidae (e.g., Lake Baikal seal[5]), Rodentia (e.g., Asian marmot[18]) and nonhuman primate (e.g., rhesus macaque[16]) within the
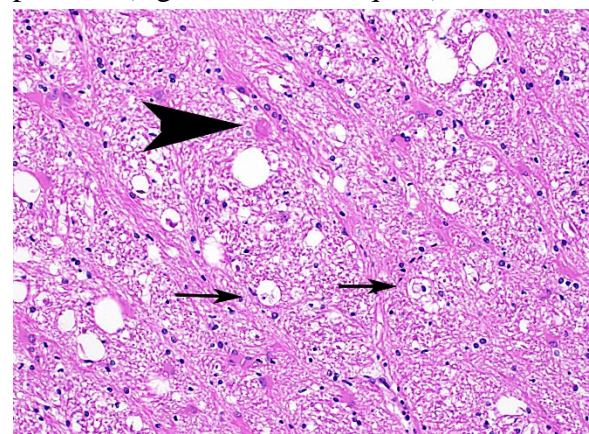

Cerebrum, lion. The vacuolation of the white matter is the result of numerous dilated myelin sheaths, some of which contain myelin debris and Gitter cells (arrows). Occasional spheroids are present (arrowhead). (HE, 400X)

genus *Morbillivirus* in the family *Paramyxoviridae*. Other morbilliviruses include rinderpest, peste des petits ruminants virus, cetacean morbillivirus, phocine distemper virus and the measles virus.

CDV causes systemic disease often with respiratory, gastrointestinal and central nervous system (CNS) involvement. There are four distinct forms of CNS disease: 1) classic CDV encephalitis; 2) multifocal distemper encephalomyelitis in mature dogs; 3) "old dog" encephalitis (ODE)[14]; and 4) post-vaccinal canine distemper encephalitis. In large felids, fatal neurologic disease is due to a distinct CDV variant. Specifically, in African lions, it is thought they contract the virus from feral dogs or hyenas[2]. CNS disease is most common, followed by gastrointestinal and respiratory disease; lesions of the hard pads are rare[10]. Generalized seizure activity, the most common neurologic abnormality, usually culminates in acute death[10].

CDV is a pantropic, negative-sense, single-stranded, enveloped RNA virus 150-300 nm in diameter. There is one recognized serotype with variable strain pathogenicity and tissue tropism. Virulence factors include hemagglutinin glycoproteins on the viral
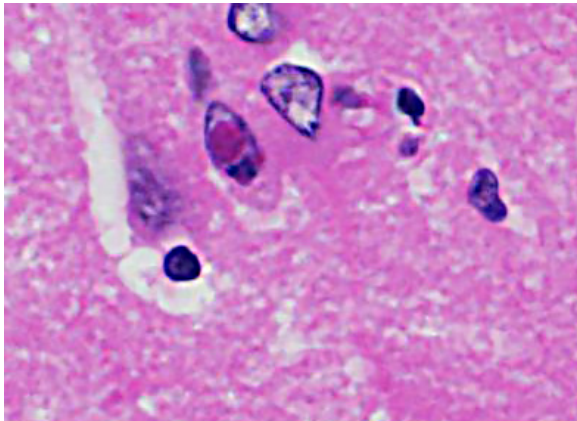
Cerebrum, lion. An astrocyte contains a large intranuclear viral inclusion. (HE, 400X)

envelope, which mediate attachment to host cells and fusion glycoproteins which allow penetration of host cells and fusion of infected cells with uninfected cells[22]. Receptors include signaling lymphocyte activation molecule or SLAM (CD150)[21], which is leukocyte-restricted and mediates entry into cells and CD46 which is widespread.

Infection is via inhalation and the virus infects macrophages in the upper respiratory tract or lungs and replicates in the tonsils and lymph nodes in the first 24 hours. There is cell-associated viremia by two days postinfection with spread to all lymphoid tissues and blood lymphocytes by two to five days postinfection, followed by lymphocytolysis, leukopenia and immunosuppression. Animals with adequate humeral/cellular immunity neutralize the virus by 14 days postinfection and may not shed virus. Animals with delayed intermediate humeral/cellular immunity, have a viral infection/persistence in the mucosal epithelium and brain and may develop neurologic disease and disease associated with epithelial infection. Animals that fail to develop neutralizing antibody by eight or nine days postinfection have virus that disseminates to the respiratory, gastrointestinal, urogenital and central nervous systems. The integumentary, exocrine and endocrine systems may also be affected. CDV is shed in all excretions during the systemic phase of infection which makes secondary infections with Adenovirus, *Bordetella* sp., *Clostridium piliforme*, *Cryptosporidium* sp., *Escherichia coli*, *Encephalitozoon* sp., *Pneumocystis* sp., *Sarcocystis* sp., and *Toxoplasma* sp. common.

CNS lesions develop one to three weeks after systemic signs or may occur after a subclinical infection. The virus is spread

hematogenously to the brain and choroid plexus via macrophages shed into the cerebrospinal fluid and disseminates virus within the ventricles. The virus spreads to ependymal cells and spreads locally to infect astrocytes and microglia which leads to the characteristic white matter vacuolation (intramyelinic edema) thought to result from a direct effect of the virus on oligodendrocytes. ODE may be caused by infection with replication of a defective virus[12]. Post-vaccinal CDV occurs due to vaccination with a modified live vaccine and is often fatal in exotic carnivores (e.g., ferret, mink, lesser panda, grey fox). CDV rarely causes fatal encephalitis in young dogs, possibly due to immune stimulation by other canine viruses (e.g., canine parvovirus) at the time of vaccination. Vaccination of pregnant dogs can cause abortion or disease in puppies.

Disease with classic canine distemper is most common in 12-16 week old puppies. Early clinical signs include fever, conjunctivitis, coughing, vomiting, diarrhea, depression, anorexia and serous to mucopurulent oculonasal discharge. Clinical pathology reveals lymphopenia, thrombocytopenia, regenerative anemia, hypoalbuminemia, hypergamma- and alpha-globulinemia. After one to four weeks, clinical signs include neurologic disease (e.g., seizures, cerebellar or vestibular ataxia, paraparesis, myoclonus) with minimal or no signs of epithelial infection. Hyperkeratosis of the footpads and nose and enamel hypoplasia are late manifestations. Of note, 50-70% of infections are subclinical.

Multifocal distemper encephalomyelitis in mature dogs occurs when CDV infects dogs at four to eight years of age and is a rare, chronic, progressive disease, but is not preceded by signs of classic distemper[6].
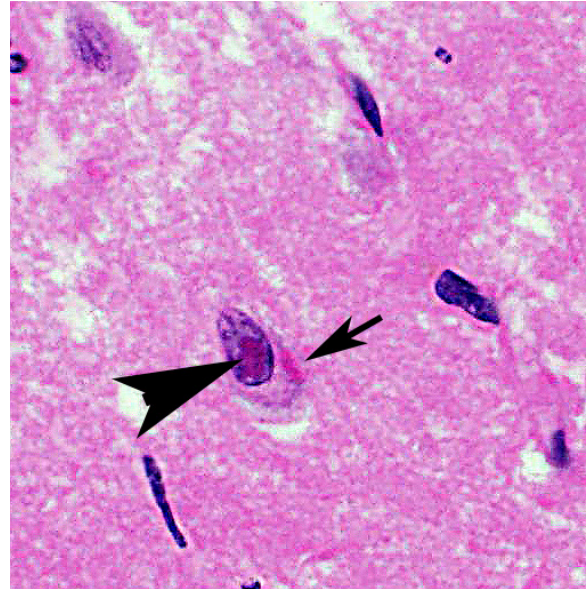
Cerebrum, lion. A small neuron contains both an intracytoplasmic viral inclusions (arrow) and an intranuclear viral inclusion (arrowhead). (HE, 400X)

There is a slow, progressive course of weakness and incoordination, but no seizures. "Old dog" encephalitis (ODE) is extremely rare; most cases occur in dogs past middle age. There is an insidious onset of circling, incoordination, compulsive walking and pushing against fixed objects, but no paralysis or convulsions. The disease progresses over three to four months to coma and death. Post-vaccinal canine distemper encephalitis affects young dogs one to three weeks post-vaccination with a modified live CDV vaccine. There is an acute/subacute clinical course which lasts one to five days. Clinical signs are similar to the furious form of rabies, to include aggressiveness and attack behavior.

Grossly, CNS lesions are uncommon, but white matter softening with brown discoloration, with or without hemorrhage and reduction in brain size with dilated ventricles, can be seen in ODE. Non-CNS lesions include bronchointerstitial pneumonia[7], catarrhal enteritis[17], conjunctivitis[15], hyperkeratosis of the foot
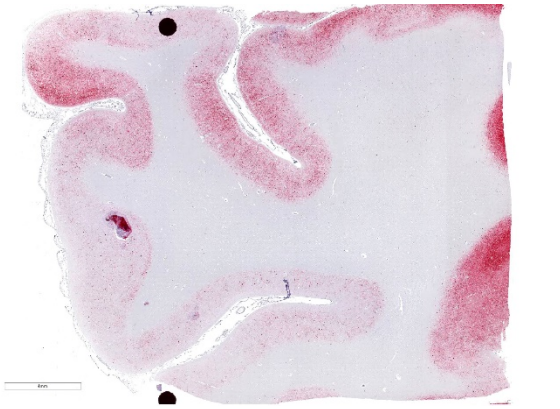
Cerebrum, lion. The cortical gray matter shows extensive immunostaining for canine distemper virus.

pads and nose[17], tonsillar enlargement[4], thymic atrophy[4], enamel hypoplasia[9] and metaphyseal osterosclerosis[9] in young growing dogs.

Histologically, eosinophilic intranuclear and/or intracytoplasmic viral inclusion bodies are most numerous 10-14 days postinfection. Intranuclear viral inclusion bodies are typically most obvious in the brain and intracytoplasmic viral inclusion bodies in the epithelium (especially the urinary bladder), but less obvious in the lymphoid tissues. In classic canine distemper, lesions usually involve both gray and white matter, but predominate in one. In the white matter there is demyelination, with early axon sparing, and status spongiosis with axonal degeneration and necrosis, which is most severe in the cerebellar peduncles, rostral medullary velum, optic tracts, hippocampal fornix, spinal cord and surrounding the fourth ventricle. There is also nonsuppurative encephalitis, gitter cells, astrocytosis and viral syncytia. In the gray matter, there are intranuclear viral inclusion bodies with or without intracytoplasmic viral inclusion bodies in the neurons, neuronal necrosis, mononuclear infiltrate surrounding necrotic neurons and nonsuppurative perivascular encephalitis with or without a mild meningitis.

In multifocal distemper encephalomyelitis in mature dogs, lesions are restricted to the CNS and are most prevalent in the cerebellum and white matter of the spinal cord. In contrast to ODE, the cerebral cortex is often spared. There is multifocal necrotizing non-suppurative encephalitis with rare intranuclear viral inclusion bodies in astrocytes and demyelination in the internal capsule and corona radiate. In ODE, the cerebral cortex and brainstem are consistently affected. The characteristic features are widespread nonsuppurative perivascular encephalitis, intranuclear viral inclusion bodies in neurons and astrocytes and neuronal necrosis. Viral antigen is detectable by immunohistochemistry, however the virus cannot be isolated from the brain. In post-vaccinal canine distemper encephalitis, the lesions are always restricted to the CNS and resemble classic canine distemper, but with relative sparing of white matter.

Ultrastructurally, tubular CDV nucleocapsids are seen in non-membrane bound intracytoplasmic aggregates[8]. Similar tubular structures may be seen in the nucleus despite lack of viral replication in the nucleus[8].There is also destruction of ensheathing myelin envelope[8]. Diagnostic tests used to identify CDV include virus isolation, immunohistochemistry and the fluorescent antibody test. Differential diagnosis for CNS lesions include *Toxoplasma gondii* or *Neospora caninum* (random, multifocal, necrotizing foci in the grey and white matter with protozoa at the margins of the lesions), rabies virus (intracytoplasmic viral inclusion bodies in the hippocampal neurons and Purkinje cells with occasional lymphocytic perivascular cuffing), pseudorabies virus (polioencephalomyelitis with or without ganglionitis and intranuclear viral inclusion bodies in the neurons of the spinal ganglia,

spinal cord, medulla and pons), granulomatous meningoencephalitis (progressive neurological disease of adult dogs with granulomatous inflammation), canine adenovirus type 1 (brainstem hemorrhages with mild vasculitis and intranuclear viral inclusion bodies in endothelial cells) and canine herpesvirus (usually puppies less than 21 days old with multifocal necrotizing lesions in many organs including lung, liver, kidneys and CNS).

Contributing Institution: United States Army Institute of Surgical Research Website: http://www.usaisr.amedd.army.mil/

JPC Diagnosis: Cerebrum: Meningoencephalitis, necrotizing and lymphoplasmacytic, diffuse, moderate, with marked demyelination, gliosis, and moderate numbers of neuronal and astrocytic intra-nuclear and intracytoplasmic viral inclusions.

JPC Comment: The contributor has done an excellent job in the description of the multisystemic pathogenesis of morbillivirus infection in the canine host, and much of this information is applicable to the disease in wild felids. Interestingly, transmission of virulent CDV to domestic cats has not been possible.[1] A report of autopsy findings in large felids in an outbreak in several zoos in North America notes that the histologic findings between the disease in large felids and in dogs are quite similar with the exception of marked Type II pneumocyte hyperplasia in large felids and a more mild and patchy meningoencephalitis than that seen in canine cases.[1]

The disease is not new in large felids; retrospective studies of archived tissues have identified CDV antigens in cases prior to its initial description in this species.[20] Most studies agree that outbreaks in large felids
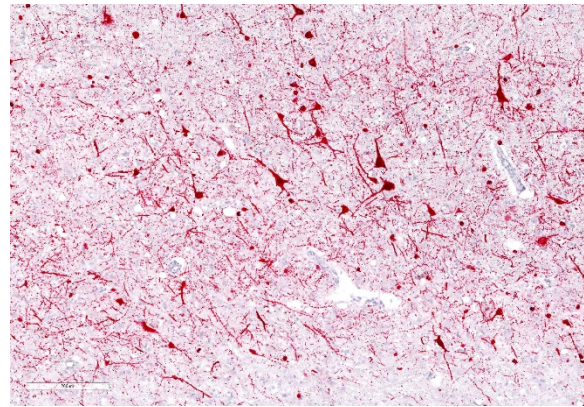
Cerebrum, lion. There is extensive immunostaining for canine distemper virus in cortical neurons.

represent spillover from infected dogs[1,14,20,21] and that this mode of transmission represents a very significant threat to a wide species of animals, many highly endangered, in both wild and captive settings. However, following widespread canine vaccination programs in proximity to the Serengeti ecosystem, the cyclic peaks in lion infections became desynchronized to those of the surrounding canine population, suggesting the possibility of other wildlife species may be maintaining and driving CDV infections in the lion population.[20,21] Widespread vaccination programs (generally aimed at vaccinating 95% of local dog populations) have shown some efficacy in decreasing spillover into wildlife; problems associated with direct vaccination of susceptible wildlife species include mode of vaccine delivery and administration of boosters (often requiring tranquilization and handling of animals), safety and efficacy of distemper vaccines in target species, and the logistics and costs of administering this type of program.[14]

Clinical signs in wildlife species are similar to that of domestic dogs, but may vary as a result of host age, immune status, and viral strain variability. One of the major determinants of strain virulence is genetic variation in the H protein, one of six structural proteins of the CDV virus.[14] The

H protein binds to two cellular receptors in susceptible cells, the signaling lymphocyte adhesion molecular (SLAM, CD150), which is present on the surface of T and B lymphocytes, macrophages, and dendritic cells. A second epithelial receptor, nectin-4, participates in cell entry as well as cell-to-cell spread.

The moderator reviewed various aspects of the pathogenesis and corresponding macroscopic and microscopic lesions of canine distemper virus in big cats as well as other susceptible carnivores. Peculiarities of CDV infection in large cats include prominent alveolar type 2 pneumocyte proliferation and a lack of inclusion bodies within the urinary bladder.[1] She also stated than in addition to being a very illustrative case of a classic lesion, she chose this case to add this entity to the WSC database, where it is the first instance of CDV in a large felid.

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CASE II: 16GR108 (JPC 4118174).

Signalment: 1-year-old, male, Beagle (*Canis familiaris*).

History: Two-week exploratory toxicity study.

Gross Pathology: Unilateral (left) small epididymis was noted macroscopically.

Laboratory results: None

Microscopic Description: Epididymis: Within the epididymis, there is moderate focal arterial inflammation characterized by expansion of tunica intima with prominent endothelial cells, disruption of the internal elastic lamina, and presence of fibrin, cellular
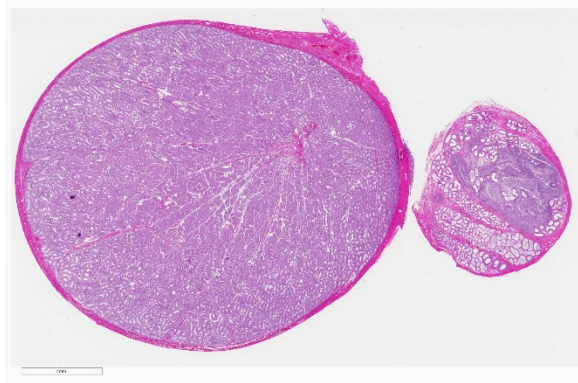
Testis and epididymis, dog. A section of testis (left) and epididymis (right). Approximately 33% of the epididymis is expanded by an inflammatory focus that replaces tubules. (HE, 6X)

debris, and inflammatory cells (lymphocytes, plasma cells and macrophages) in the tunica media and adventitia.

Disrupting ductular and stromal architecture is a focally extensive inflammatory reaction composed of a ring of macrophages, lymphocytes, and plasma cells (granuloma) surrounding a large focus of extravasated spermatozoa.

Contributor's Morphologic Diagnoses:
Epididymis: 1) Arteritis, lymphoplasmacytic, focal, moderate, subacute

2) Sperm granuloma, focal, moderate, subacute

Contributor's Comment:
This finding was unrelated to the test article because it occurred in just one animal without dose relationship and was consistent with idiopathic juvenile polyarteritis of young Beagles, a background finding in dogs.[1,2] Small to medium caliber arteries are most commonly affected by this form of vascular injury. Histologically, vascular changes are characterized by medial necrosis, mononuclear cell inflammatory infiltrates, intimal proliferation, and fibrinoid necrosis.[4,7,8] There is a higher incidence in males with a trend towards the epididymis as the organ most commonly affected. Differentiation from drug-induced vascular injury (DIVI) is important in toxicity studies.

DIVI includes vasoactive arteriopathy, toxic vasculitis, and hypersensitivity vasculitis. Histologic changes induced by vasoactive drugs include necrosis and inflammation in all three vessel layers, most commonly in small vessels of the skin, and an absence of fibrinoid necrosis. Toxic vasculitis affects small to medium-caliber vessels and is characterized morphologically by segmental fibrinoid necrosis and neutrophilic inflammation of the vessel wall, periadventitial tissue, and thrombosis.
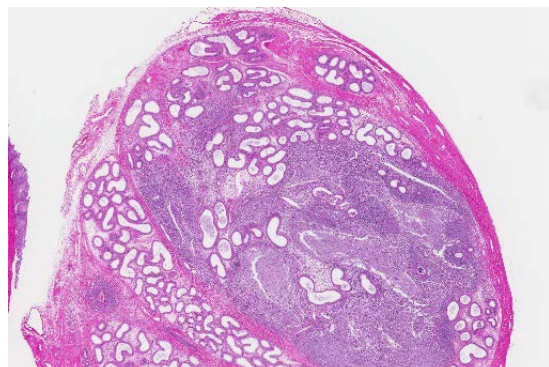
Testis and epididymis, dog. The epididymis is expanded by poorly formed granulomas which replace tubules. (HE, 18X)

Vasoactive arteriopathy represents the most common type of DIVI and primarily affects coronary arteries (vasodilators) and small arteries systemically (vasoconstrictors). Histologically, changes are characterized by medial hemorrhagic necrosis with minimal inflammation (vasodilators) and focal medial fibrinoid necrosis (vasoconstrictors).[1]

Epididymal sperm granulomas are typically an incidental finding and similarly not test article-related in the present case. There are multiple causes of sperm granulomas including, most commonly, trauma and inflammation, congenital ductular abnormalities, adenomyosis of epididymal ducts, parasitic lesions, and toxins.[2]

Contributing Institution:
Pfizer Drug Safety Research and Development
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Groton, CT 06340
https://www.pfizer.com/partners/research-and-development

JPC Diagnosis:
1. Epididymis: Sperm granulomas, multiple.
2. Arteriole, head of the epididymis: Arteritis, necrotizing and granulomatous, chronic, diffuse, moderate.
3. Testis, seminiferous tubules: Atrophy and aspermatogenesis, diffuse, moderate.
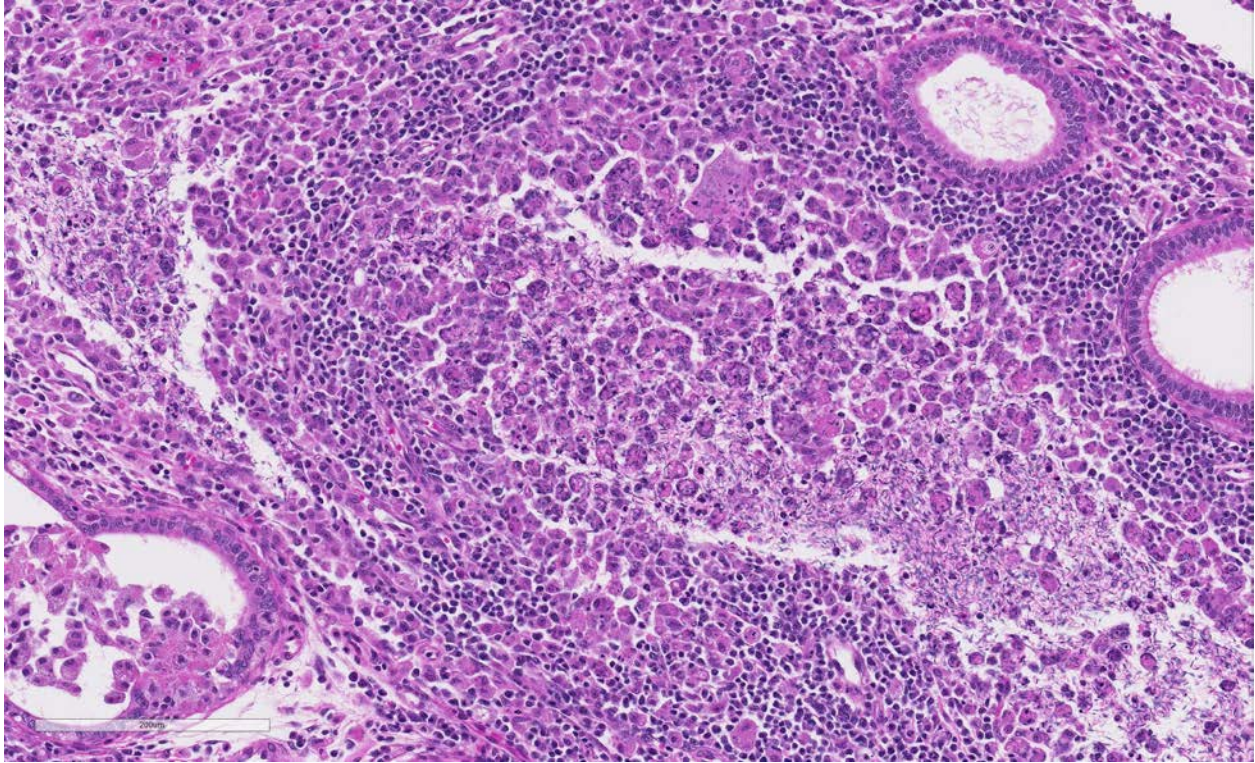
Testis and epididymis, dog. The granulomas are centered on free sperm in the interstitium, which are often engulfed by macrophages. Peripherally, the granuloma contains moderate numbers of lymphocytes and plasma cells and small amounts of collagen with plump fibroblasts. At lower left, macrophages infiltrate an epididymal tubule. (HE, 186X)

JPC Comment: Idiopathic juvenile polyarteritis of beagle dogs, first described in 1992 by Scott-Moncrieff et al. in 1992,[6] is one of the most well-known primary vasculitides in the dog. The term "primary" vasculitis comes from human medicine and refers to a vasculitis whose cause is unknown, versus "secondary" vasculitides, which have defined infectious causes, such as endotheliotropic viruses, or drugs (as described by the contributors). A retrospective study of canine vasculitis by Swann et al. in 2015[9] failed to disclose significant differences between the two groups; primary vasculitides were more common in female dogs (this case notwithstanding), and dogs with elevated serum globulin concentrations were seen with more frequency in dogs with primary vasculitides.

Idiopathic juvenile polyarteritis (IJP), also known as "beagle pain syndrome", is an acute febrile multisystemic vasculitis most often seen in dogs less than 36 months of age.[7] It is characterized by acute onset of fever and pain (often cervical) with animals assuming a characteristic hunched stance.[4] Vascular lesions range from a mild lymphohistiocytic perivasculitis to transmural arterial inflammation with fibrinoid necrosis with loss of elastic laminae, intimal and medial fibrosis, and marked perivascular inflammation.[4,7] Lesions are most commonly seen in the small- and medium-sized arterioles (i.e., arterioles other than the great vessels) of the cervical meninges, heart, and cranial mediastinum.[7] Animals with repeated episodes may develop gross signs of progressive bilateral atrophy of cervical and temporal muscles,[4] and histologic evidence of hepatic, splenic and renal amyloidosis.[4,7]
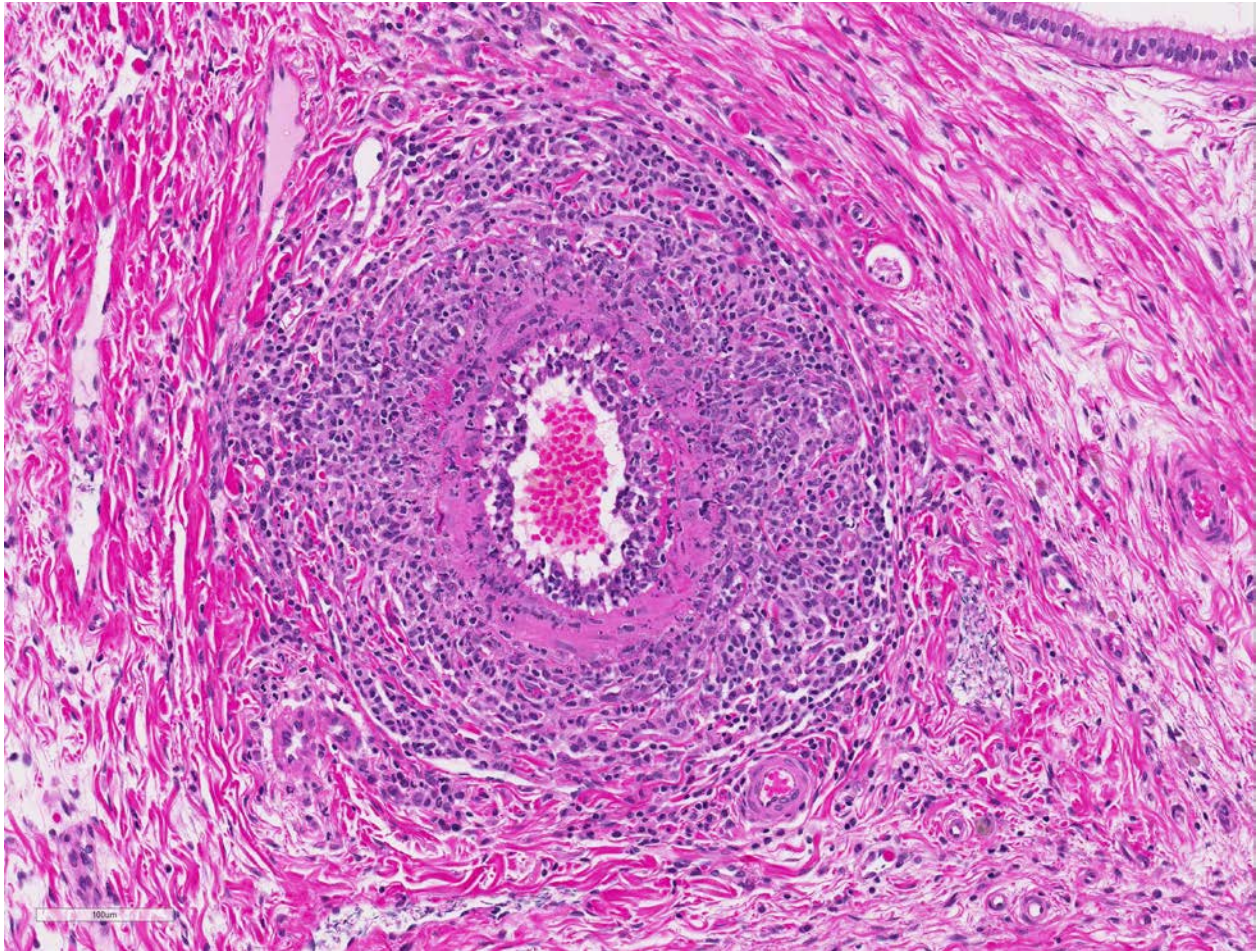
Testis and epididymis, dog. There is fibrinoid necrosis of an epididymal arteriole with marked infiltration of the adventitia and outer media by numerous neutrophils and macrophages. At its periphery, dilated lymphatics contain sperm. (HE, 218X)

Interestingly, a primary vasculitis affecting the testicular artery of raccoons has been reported.[5] Histologic lesions of segmental arteritis of the extratesticular (epididymis and spermatic cord) arterioles similar to that of IJP and other forms of polyarteritis nodosa were described. The lesions were seen in both wild and breeder animals of all ages.[5]

The blood-epididymis barrier (BEB) is an important factor in protecting maturing sperm from the immune system. This barrier protects developing spermatozoa in the testis and mature spermatozoa within the epididymal tubules not only from infiltrating inflammatory cells following injury, but also from cytokines released in local and systemic inflammatory conditions.[3] The breakdown of the BEB may result from traumatic injuries, developmental abnormalities, or the administration of testosterone and various chemicals and drugs. In addition, BEB barrier function diminishes with age. Sperm granulomas (which may be seen not only in the epididymis but also within the testis proper and vas deferens) are cardinal signs of breakdown of the BEB, and arise as a result of the antigenicity of spermatozoa.[3] An excellent review of factors contributing to the breakdown of the BEB and the resulting inflammatory process is provided in the 2014 article by Gregory and Cyr cited below.[3]

The moderator discussed the formation of sperm granuloma and the fact that the inception of spermatozoa formation does not occur until well after the immune system is formed, which is why sperm is so antigenic. She pointed out that the tail of the epididymis is more likely to be affected in infectious disease, while the head (caput) of the epididymis has a higher incidence of granuloma formation as a result of the presence of an increased number of abnormal ductules and reviewed the general pathology behind granuloma formation. She gave an excellent review of "beagle pain" syndrome, pointing out that the disease is not restricted to Beagles, nor is it always a painful condition. She chose this case as an example of a classic lesion as well as a descriptive case which has two concurrent processes.

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CASE III: Case #2 (JPC 4084695).

Signalment: 12-year-old, spayed female, Mix breed (*Canis familiaris*).

History: Patient was referred to a local veterinary hospital because of left exophthalmos. A very large orbital contrast-enhancing mass compressing the left eye was revealed by computerized tomography (CT). The mass along with the left eye was surgically resected.

Gross Pathology: Grossly there was a whitish-gray, firm mass, approximately 5×4.3×3cm in diameter, located in the retrobulbar area. On cut surface, the mass was solid and whitish-marble, and completely involved the optic nerve.

Laboratory results: None

Microscopic Description: The tumor composed of sheets of closely packed large neoplastic cells supported by scant stroma harboring numerous small vessels.

Eye, dog. There is a 5x4x3 cm retrobulbar mass which involves the optic nerve. (Photo courtesy of: Setsunan University)

Neoplastic cells were large, mainly round to polygonal cells, with abundant eosinophilic granular cytoplasm. The nuclei showed minimal pleomorphism and hyperchromasia and tended to be situated towards the periphery of the cell. Mitoses were not observed. There were also few whorl-like structures. Abundant cytoplasmic eosinophilic granules were PAS-positive. Immunohistochemically, the neoplastic cells were diffusely positive for vimentin, but did not express cytokeratin AE1/AE3, neurofilament, GFAP and IBA-1.

Contributor's Morphologic Diagnoses: Eye: Orbital meningioma, granular cell type.

Contributor's Comment: Orbital meningioma can derive from the optic nerve arachnoid cap cells that extend through the dura mater into the connective tissues of the orbit. In the dog, which has the higher incidence among the domestic species, 82% of the meningiomas are intracranial, 15% are intraspinal, and the remaining 3% are retrobulbar or orbital.[5] Primary orbital meningiomas are generally thought to be slow growing and benign, but intraocular invasion and malignant variants with extracranial metastasis have been reported. Poodles, poodle crosses, Samoyeds,

Samoyed crosses, German shepherds, and German shepherd crosses were the only breeds affected.[7] In the same study, the mean age at the time of diagnosis was 9 years (range: 3 to 7 years of age), and a male sex predisposition with a male:female ratio of 2:1 was also evident.[7] Clinically, they are frequently associated with unilateral protrusion of the ocular globe and blindness. Apparently there is no predilection for either side. Although not highly invasive, canine orbital meningiomas are difficult to remove, and local regrowth or extension through the optic foramen leading to blindness is a common complication.[7]

Orbital neoplasms are seen rarely in the dog, almost always present as slowly progressing, painless exophthalmos in older dogs, and are often malignant. A wide variety of tumor types can occur in the orbit, with fibroma, meningioma, osteosarcoma, malignant lymphoma, lipoma, rhabdomyomas, and lachrimal gland tumor.

In this case, histomorphological differentials are thought to be included rhabdomyomas, oncocytomas, and histiocytic sarcoma. Rhabdomyomas are benign skeletal muscle neoplasms that are characterized by glycogen-rich, eosinophilic, striated muscle
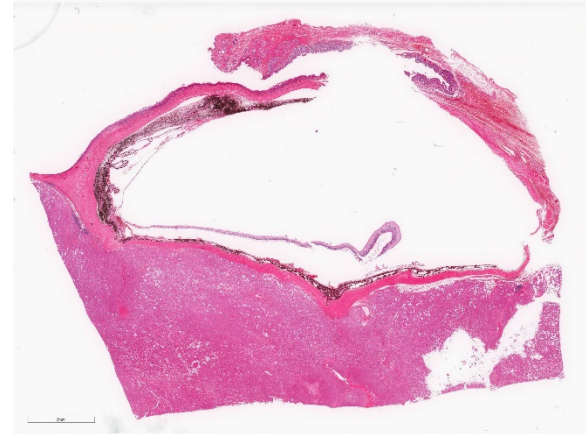

Eye, dog. Subgross magnification of a tangential section of the eye and the associated mass. The optic nerve is not visible in the submitted section. (HE, 8X)

13

cells with numerous mitochondria, and myofilaments. In oncocytomas, the abundant eosinophilic cytoplasmic granules correspond to large numbers of mitochondria. In histiocytic sarcoma, tumor cells sometimes contain many small vacuoles. Multinucleated giant cells with variably sized nuclei are common, and the mitotic rate is high in many areas.

The current classification of meningiomas in domestic animals describes nine histological types: meningothelial, fibrous, transitional, psammomatous, angiomatous, papillary, granular cell, myxoid and anaplastic.[4] Among these types, granular cell meningioma is specific to domestic animals,

and does not exist in the WHO classification of human meningioma.[6] Otherwise, there are some reports of canine granular cell tumors in the meninges.[2,8,10] At present, the histopathological differences between granular cell meningiomas and granular cell tumors in the central nervous systems are obscure. Furthermore, the description of the histological characteristics of granular cell meningioma in the WHO classification for domestic animals is very similar to that of granular cell tumors. In the rat, there is convincing gross, histologic, and ultrastructural evidence suggesting that intracranial granular cell tumors originate from meningeal arachnoid cells and that some of these tumors may also contain
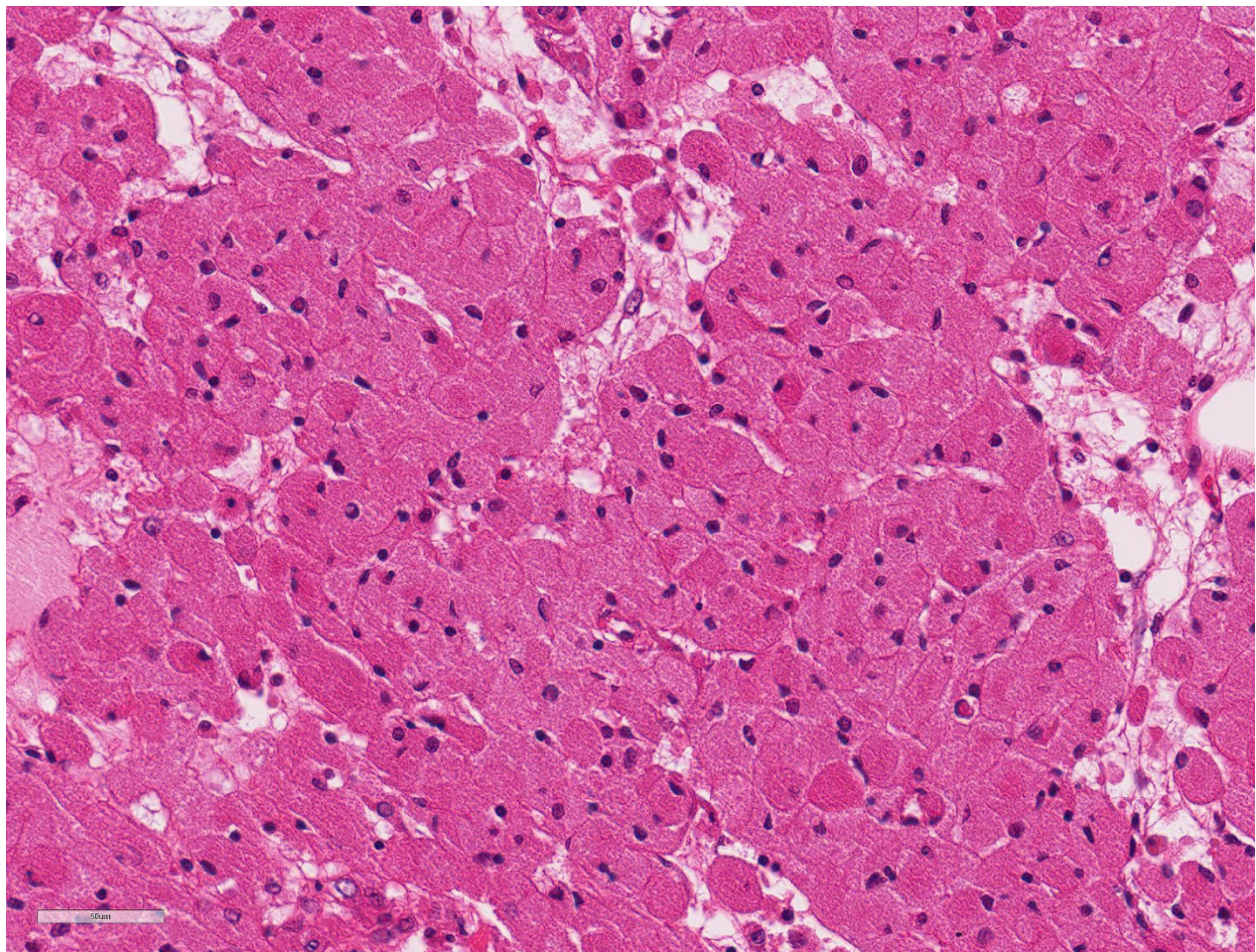

Eye, dog. Neoplastic cells range up to 3-0um in diameter, with abundant eosinophilic granular cytoplasm. (HE, 400X)

mixtures of both cell types.[2,12] The major meningeal involvement in both these canine tumors suggests that, as in the rat, they may be derived from a cell type forming the leptomeninges. Other support for this hypothesis is provided by a canine meningotheliomatous meningioma in which a substantial granular cell component has been described.[2,9]

JPC Diagnosis: 1. Retro-orbital fat: Granular cell tumor.
2. Eye: Retinal detachment and atrophy with diffuse severe ganglion cell loss. .
3. Eyelid: Conjunctivitis, lympho-plasmacytic, chronic, diffuse, mild.

JPC Comment: While careful consideration was given to the contributor's diagnosis of "orbital meningioma, granular cell type", we prefer the diagnosis of granular cell tumor in this case.

Much of the diagnostic confusion in this particular case arises from changes in the current classification of meningiomas. While present in the 1999 WHO Classification of Tumor of Domestic Animals[4] (referenced by the contributor), "granular cell meningioma" has been dropped in more recent classifications.[3] In addition, the images of the "granular cell meningioma" in the WHO
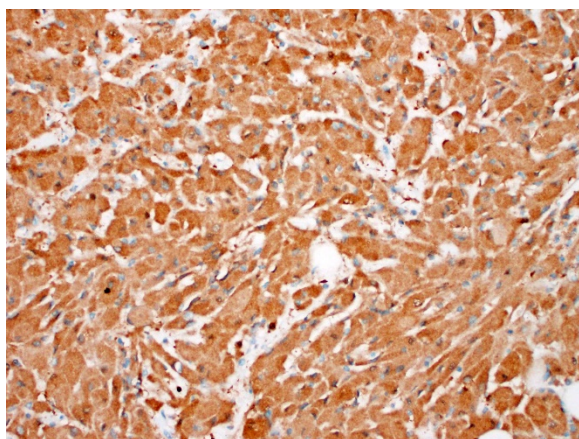
Eye, dog. Neoplastic cells demonstrate strong S-100 immunopositivity. (anti-S-100 400X)

Classification[4] are quite similar to those ascribed to "granular cell tumors" in other publications, including the paper by Higgins et al.[2,3]

A number of WSC cases have dealt with the unique classification of orbital meningioma in previous years, including WSC 2015-2016, Conference 20, Case 4 and WSC 2018-2019, Conference 7, case 4. A common feature of orbital meningiomas is the presence of chondroid or osseous metaplasia,[1,7] which is absent in this case, as are other features of meningioma. While a few granular cell meningiomas are present within the collection of the Comparative Ocular Pathology Lab of Wisconsin (COPLOW) (and one had been published)[11], these tumors all had areas of osseous and cartilaginous metaplasia, as well as areas of neoplastic cells that resemble those seen in more classic meningiomas of the meningotheliomatous type (Personal communication, Leandro Teixeira.) Careful examination of the available literature on canine orbital meningiomas[1,7] failed to disclose mention of a "granular cell type"; in one reference of an orbital meningioma with a "granular cell component",[9] the morphology is significantly different that that seen in this case, with central nucleolus and homogenous eosinophilic cytoplasm as compared to that seen here, in which the cytoplasm is granular and the nuclei are peripheralized and hyperchromatic.

Granular cell tumors have been described in the central nervous system of the rat, ferret, dog, and humans.[2] The etiology of these neoplasm remains in question, although prevailing theory in the rat, dog, and ferret is that they indeed are of meningothelial origin. Granular cell tumors in humans may be of astrocytic origin[2] (A JPC-run GFAP stain was negative). Additionally, they may have a similar appearance to oncocytic meningeal

variants in which the granules are numerous mitochondria rather than lysosomes, as seen in animal species (Personal communication, Jey Koehler, Auburn University).

The second morphologic diagnosis was suggested by Dr. Leandro Teixeira of COPLOW upon slide review: If you look at the retina in the slide it is completely devoid of ganglion cells. "This is due to compression of the optic nerve by the orbital neoplasm causing axonal (wallerian) degeneration and complete ganglion cell loss. This is a very important lesion since it is the reason these dogs go blind and thus I think it deserves a separate morphologic diagnosis." (Personal communication, Leandro Teixeira).

The moderator reviewed differential diagnosis of retro-orbital meningioma, oncocytoma, rhabdomyosarcoma, and granular cell tumor. In addition, in this particular case, a section was floated off the section for ultrastructural analysis and the granules in the cells were demonstrated to be lysosomes further cementing the diagnosis of granular cell tumor. The moderator also presented data supporting her contention that
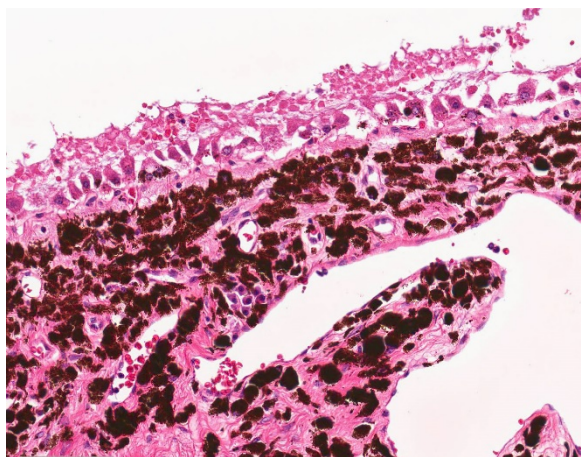
Eye, dog. The retina is detached. The pigmented retinal epithelium is markedly hypertrophic and covered with hemorrhage and fibrin. (HE 256X)

the morphology of granular cell tumors may be the result of widespread cellular degeneration and lysosomal overload, which might explain how various cell types in various organs in various species may attain a similar morphologic appearance.

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CASE IV: 14/1790 (JPC 4117447).

Signalment: 13-year-old spayed female Labrador retriever dog, *Canis familiaris*, canine

History: This dog presented for a mass on the vaginal floor. Complete blood count and chemistry were unremarkable.

Gross Pathology: 3cm diameter, asymmetrical, lobulated and highly vascular mass protruding from the vaginal floor.

Laboratory results: None

Microscopic Description: Vaginal floor mass: This is an expansile, fairly well-demarcated, unencapsulated, multilobulated
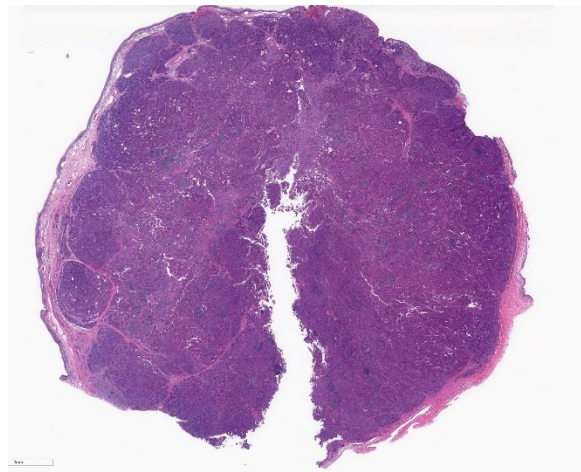

Vagina, dog. A large multilobulated neoplasm expands the submucosa, elevating the overlying ulcerated surface. Aggregates of lymphocytes may be seen at low magnification. (HE, 7X)

and peripherally invasive neoplasm composed of cuboidal to polygonal cells arranged in cords, acini, occasional rosettes (Figure 1) and solid areas with fine fibrovascular stroma. Neoplastic cells have indistinct cell borders, a moderate amount of eosinophilic cytoplasm, and round to oval finely stippled nuclei with prominent nucleoli. There is mild anisocytosis and anisokaryosis and one mitotic figure in 10 high power (400x) fields. Rafts of neoplastic cells are within multiple endothelial lined vessels (vascular invasion) (Figure 2) [varies by slide]. Scattered throughout the mass are perivascular clusters of lymphocytes and foci of hemorrhage with hemosiderin-laden macrophages.

Contributor Morphologic Diagnoses: Clitoral gland adenocarcinoma

Contributor Comment: Vaginal and vulvar tumors are uncommon neoplasms which typically occur in older, sexually intact females.[7] The majority of these neoplasms are benign and of smooth muscle or fibrocyte origin.[7] Few, individual case reports of epithelial neoplasms resembling apocrine gland anal sac adenocarcinomas
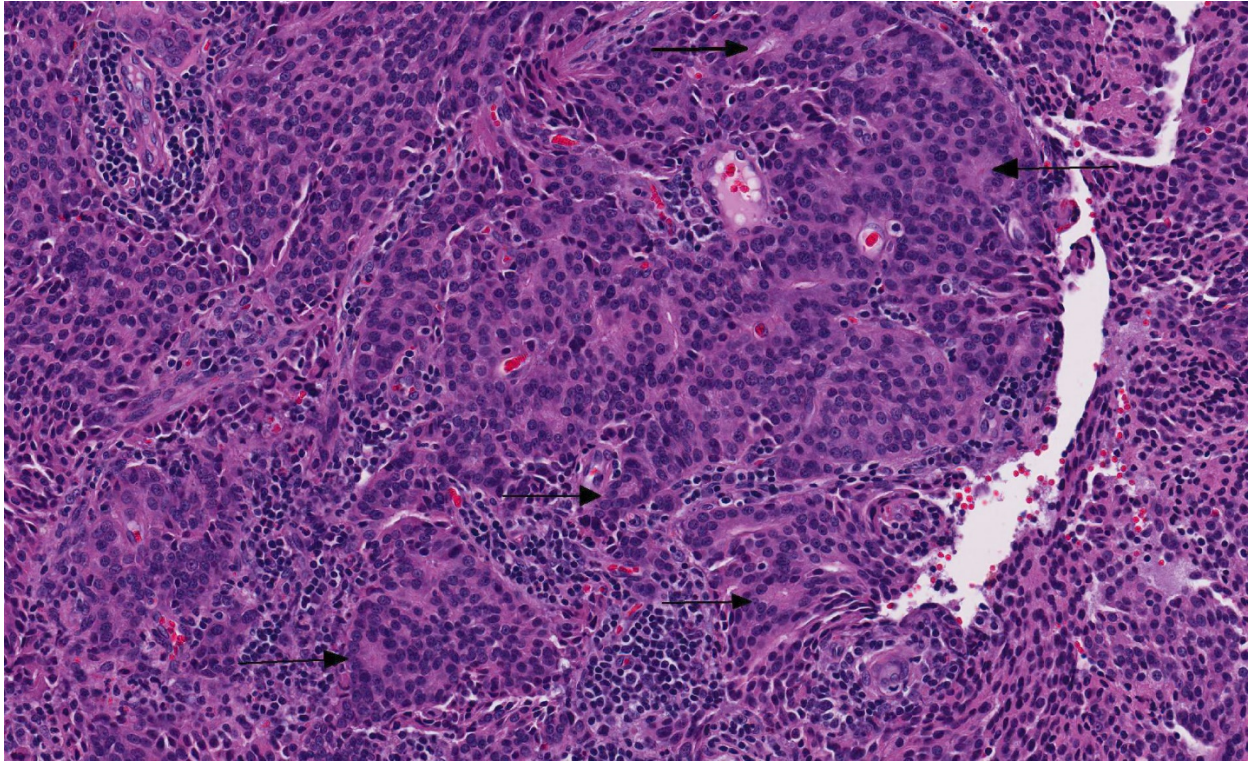
Vagina, dog. Neoplastic cells are arranged in nests and trabeculae, and rosettes are common (arrows). Numerous lymphocytes and fewer plasma cells are present within the stroma. (HE, 246X)

(AGASACA), but arising within the clitoral fossa, have been documented in the literature since 2010.[3,4,5,8] A case series published in 2018 has added 6 more cases to the literature.[8]

Clitoral gland carcinomas are thought to arise from individual apocrine glands within the fibro-fatty tissues of the canine clitoris. They typically present as ulcerated, multilobular masses protruding from the vulva. Histologically, these tumors closely resemble their anal sac counterpart, and it is possible that tumors at this location have been previously reported as AGASACAs. Tubular, rosette, and solid forms are described in the paper by Verin et al.,[8] although most tumors are mixed-type. Only carcinomas have been reported; a majority of cases have vascular invasion at the time of diagnosis.[8] Tumors that have been stained with neuroendocrine markers are typically uniformly positive for neuron-specific enolase, with mixed positivity for chromogranin A and synaptophysin and negative for S-100.[8]

Interestingly, in two earlier reports of clitoral gland adenocarcinoma in the dog, hypercalcemia of malignancy (HM) was a feature.[3,4] However, in the six cases reported recently, only 2 of 6 cases exhibited HM; one case confirmed an elevated parathormone-related hormone (PTHrp) as the likely cause.[8]

In this case, the animal received two doses of chemotherapy before treatment was stopped due to undesirable side effects. Although the neoplasm has recurred at the original site, there was no evidence of metastatic disease or hypercalcemia of malignancy more than 2 years after initial diagnosis and treatment.
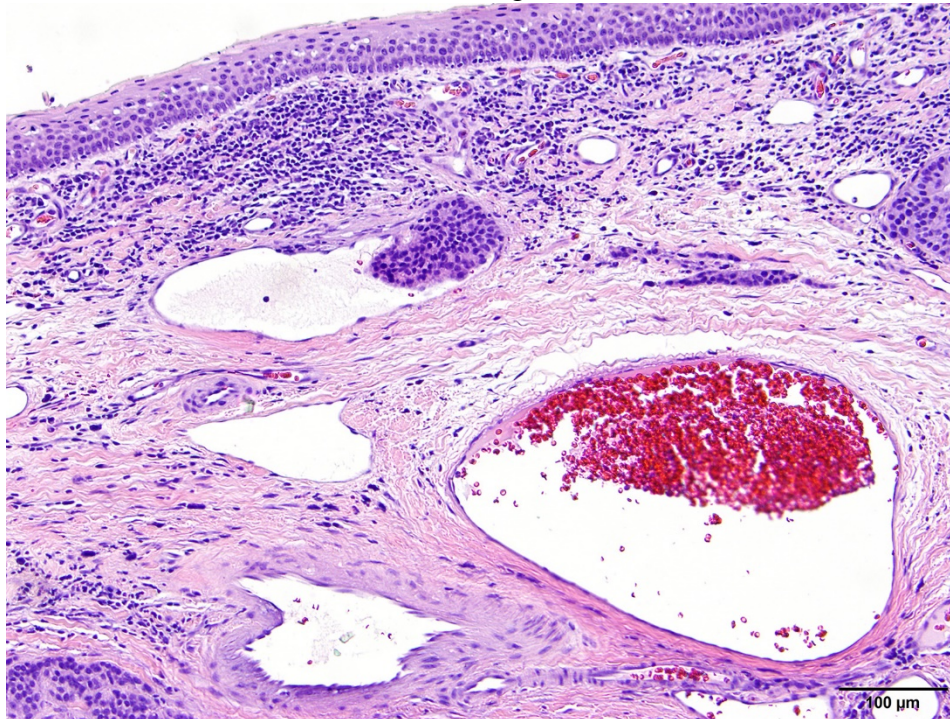
Contributing Institution:
University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences http://www.vet.utk.edu/departments/path/index.php

JPC Diagnosis: Mass from vaginal floor: Clitoral gland adenocarcinoma.

JPC Comment: The contributor has done an outstanding job summarizing the histologic and clinicopathologic features of this uncommon neoplasm of the vulva of the dog. A 2016 paper by Rout et al.[5] describes the salient features of this tumor on cytologic evaluation: loosely cohesive and acini of epithelial cells with numerous "naked" nuclei in the surrounding milieu, suggestive of neuroendocrine carcinoma. Cell clusters may include capillaries or pink amorphous material.[5] In addition, clitoral gland adenocarcinomas, like the closely related tumors of the apocrine glands of the anal sac often metastasize to regional nodes; close examination of regional nodes (including the inguinal as well as medial and internal iliac nodes) for gross evidence of metastasis is recommended.[8]

Neoplasms of the clitoral gland are well known in the rat and these glands, as well as the preputial glands of males, are usually sampled in carcinogenicity and xenobiotic studies in the mouse and rat.[6] These modified sebaceous glands are located adjacent to the vagina, and their ducts empty into the clitoral fossa. Their growth and activity are regulated by a number of hormones, including sex hormones, as well as ACTH, prolactin, and growth hormones. In general, a range of lesions is seen in this gland similar to those seen in the mammary gland. [6]

Vagina, dog. Neoplastic cells are occasionally present within submucosal lymphatics (HE, 246X) (Photo courtesy of: University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences http://www.vet.utk.edu/departments/path/index.php

In rodents, inflammatory changes of the clitoral gland may range from simple lymphocytic infiltrates to actual inflammation resulting from bacterial or fungal agents, or the presence of foreign bodies in adjacent tissue. Obstructed clitoral gland ducts may result in granulomatous inflammation. Hyperplasia, or less commonly atrophy, is seen in older animals. Clitoral gland neoplasms are often not large

enough to be observed grossly and necropsy, so this tissue (as well as preputial gland, mammary gland, and the rat Zymbal's gland) should be routinely harvested at necropsy.[6]

A range of neoplasms and subtypes have been observed in rodents. Adenomas of the clitoral glands, and are characterized as expansile growths with minimal atypia and few mitoses. [6] Clitoral gland carcinomas are generally larger with invasive growth, ulceration of the overlying skin, nuclear atypia and a high mitotic rate. Several subtypes (solid, cystic, papillary, papillary cystic, and mixed cell types) are described, but the current INHAND document does not recommend subtyping for regulatory activities. Squamous papillomas and malignant basal cell variants of clitoral gland adenocarcinomas are also described.[6]

In humans, the clitoris is covered by squamous mucosal epithelium without a glandular component.[2] The overwhelming majority of neoplasms arising in and around this organ in human are squamous cell carcinomas.[1] As the clitoris and surrounding tissues possess lymphatic drainage, malignancies from other areas of the body will rarely metastasize to this area.[1]

The moderator stated she chose this particular case as it not only is a new addition to the WSC database, but also has been recently published in the veterinary literature and would be very helpful for residents preparing for certifying examinations. She reviewed the various type of rosettes seen in various tumors including Homer-Wright (central region contains neuropil), Flexner-Wintersteiner (central region largely empty except cytoplasmic extensions from tumor cells), ependymal rosettes (central regions are empty), and pseudorosettes (surround a blood vessel). She also reviewed the various type of neoplasms whose cytologic appearance includes large numbers of "naked nuclei" to include a variety of neuroendocrine neoplasms, apocrine carcinomas of the anal sac, and clitoral adenocarcinoma.

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