Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #3 30 August 2023

CASE I:

Signalment:
14-year-old, American Paint gelding, horse (*Equus caballus*)

History:
The horse presented to its primary care veterinarian for an approximately 3 cm in diameter mass on the right facial crest that had been present for about two months. The mass began increasing in size two weeks prior to presentation. An in-house fine needle aspiration (FNA) cytology revealed 'macrophages and bacteria'. On ultrasonography the mass was noted to have a soft tissue echogenicity, lobulated appearance and several small fluid-filled pockets throughout.

Gross Pathology:
The mass was excised and submitted for histologic examination. The mass was transected prior to submission into two, approximately 5.0 x 2.0 cm, triangle-shaped sections of haired skin. On cut section there were multiple, variably sized and shaped foci that contained a yellow granular material.

Microscopic Description:
Haired skin, right facial crest: On histologic examination there is a poorly demarcated, nonencapsulated, non-compressive mass in the hypodermis. The mass extends to the lateral and deep surgical borders. The mass comprises islands and sheets of individualized round cells, supported by a dense collagenous stroma. The round cells have distinct cell borders and a moderate to high nuclear to cytoplasmic ratio. The cytoplasm is scant to moderate, grey blue and coarsely granular. The intracytoplasmic granules stained positively with toluidine blue stain (mast cell granules). The nucleus was centric, round to oval with a finely reticular chromatin pattern and a small to inconspicuous nucleolus. Mitotic figures are not present in the sections examined. Comprising approximately 30-40% of the area of the neoplasm are multiple, variably-sized (100-700µm), irregularly shaped, bright pink foci of necrosis with a central core

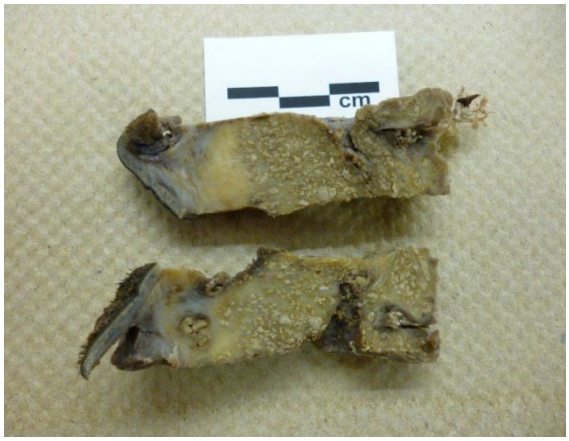

Figure 1-1. Haired skin, horse. Two sections of skin with a hypodermal mass with yellow foci scattered throughout were submitted to the contributor. (Photo courtesy of: Department of Veterinary Pathology and Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan)

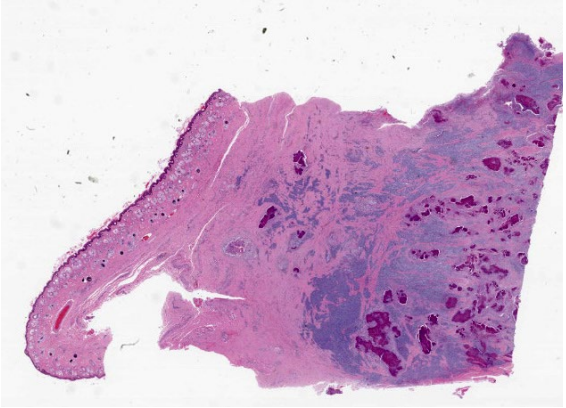

Figure 1-2. Haired skin, horse. A moderately cellular area with scattered foci of eosinophilic debris effaces the deep dermis and subcutis. (HE, 5X)

of disintegrating inflammatory cells (eosinophils) and karyorrhectic debris, a peripheralized layer of multinucleated giant cells, foamy macrophages and a few layers of fibroblasts. Neoplastic mast cells, admixed with moderate numbers of eosinophils and a few lymphocytes, surround these foci.

Contributor's Morphologic Diagnosis:
Cutaneous mast cell tumor.

Contributor's Comment:
Mast cell tumors are relatively uncommon in horses. They are usually benign tumors, with only a few reports where they show aggressive behaviour. Equine cutaneous MCTs are reported to commonly affect head, neck, trunk and limb.[1,2] They are rarely reported to affect other areas such as upper respiratory tract, oral cavity and eye.[2] There is a predilection in males, and no apparent breed predilection. The majority of these tumors are non-pruritic and non-painful.[3] They show slow, progressive growth or become static, even over a course of two years.[2] Rarely a lesion may show sudden rapid growth.[1] Equine mast cell tumors are also called mastocytosis or mastocytoma due to their clinical features and benign behaviour. There is some discussion in the literature regarding their representation as a true neoplastic process.[1,2,3]

Histologically, they manifest as well demarcated masses in the dermis or subcutis, comprised of sheets of well-differentiated mast cells admixed with a few eosinophils and prominent areas of necrosis, fibrosis and dystrophic mineralization and rare mitotic figures.[2,3] The mast cell granules are often numerous but difficult to visualize with routine hematoxylin and eosin staining.[2] Histochemical stains to highlight the mast cell granules were performed in this case (toluidine blue and Luna stains) and confirm the diagnosis. Histological grading schemes do not exist for equine cutaneous MCTs. Prognosis is usually good and complete surgical excision is curative. Recurrence after surgical excision is uncommon. Spontaneous regression has been reported in cases with incomplete surgical excision.

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JPC Diagnosis:
Haired skin: Mast cell tumor.

JPC Comment:
Equine mast cell tumors (MCTs) may present a diagnostic challenge when first encountered due to a few unique histologic features. As in other species, equine MCTs contain round cells with pale eosinophilic cytoplasm often separated by collagen fibers and accompanied by eosinophils. Unlike in other species, equine MCTs may contain large lakes of eo-

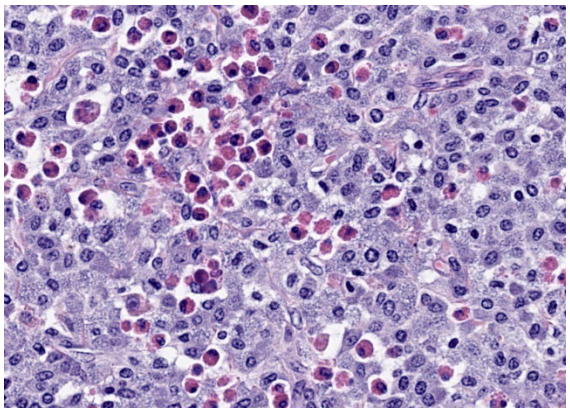

Figure 1-3. Haired skin, horse. The neoplasm is composed of sheets of moderately granulated mast cells with numerous scattered eosinophils. (HE, 571X)

sinophilic debris surrounded by fibrosis. Mast cells may be fewer than in MCTs from other species and cytoplasmic granules may be inapparent until revealed by toluidine blue or Giemsa staining. Tumors may also mineralize extensively with chronicity, leading to radiologically visible calcification.[2]

The development and proliferation of mast cells is regulated by stem cell factor (SCF), the ligand for the *c-kit* gene product KIT, a tyrosine kinase receptor also known as CD117.[4] In many canine MCTs, *c-kit* mutations cause activation of KIT in the absence of SCF, resulting in aberrant stimulation of pro-growth signaling pathways. Studies suggest that *c-kit* mutations may play a substantial role in the neoplastic transformation of mast cells in dogs and that differing KIT expression patterns can be used to assess grade, prognosis, and risk of recurrence.[4]

KIT expression, assessed with IHC staining using anti-KIT antibodies, is classified into three patterns, denoted by the numerals I-III. KIT staining pattern I consists of a membranous staining pattern, the expected localization for a transmembrane receptor tyrosine kinase; pattern II consists of focal to stippled cytoplasmic staining; and pattern III is characterized by diffuse cytoplasmic staining.[5] In well-differentiated canine MCTs, KIT expression is generally in a type I pattern, while high grade tumors have predominantly type II and III labelling; thus, translocation of KIT from the plasma membrane to the cytoplasm is associated with tumor recurrence and shorter overall survival times.[5]

In contrast to canine MCTs, in which grading and prognostic indices are well established, only two studies have assessed how morphologic features, proliferative activity, or KIT expression patterns can be used to predict biologic behavior in equine MCTs.[1,4] These two studies reached contradictory conclusions. The first found that KIT staining patterns and histologic features were not associated with poor clinical outcome or abnormal tumor behavior.[1] By contrast, the most recent study found that most equine MCTs were composed of mast cells with mild anisokaryosis, a low proliferative rate, and a dominance of KIT pattern I staining, representing well-differentiated MCTs and benign behavior.[4] Approximately one-third of cases in this study were comprised of mast cells exhibiting more infiltrative growth, moderate to marked anisokaryosis, a higher degree of proliferation, and KIT staining patterns II and III, representing poorly-differentiated MCTs associated with more potentially aggressive behavior.[4] These results indicate that it may be possible, as in canine MCTs, to make hay out of differential KIT staining to identify the small subset of potentially more aggressive equine MCTs. More research is needed to define the associations among biological behavior, morphology, proliferative indices, and KIT expression patterns in equine MCTs.[4]

This week's moderator, JPC's very own Dr. Bruce Williams, discussed this entity as one in which diagnosis is typically made on H&E evaluation alone. Toluidine blue and Giemsa stains can be used to visualize initially inapparent mast cell granules, and equine MCTs

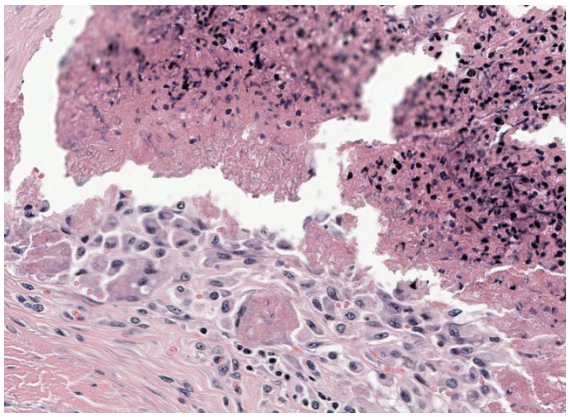

Figure 1-4. Haired skin, horse. Lakes of eosinophilic cellular debris are surrounded by epithelioid macrophages and rare multinucleated cells. (HE, 530X)

do show expected positive immunoreactivity to CD25, CD117, and tryptase, but the unique histologic appearance of equine MCTs usually obviates the need to pony up the time and money for histochemical and immunohistochemical stains.

The primary differential diagnosis for equine MCTs is equine collagenolytic granulomas, which contain, as the name implies, large numbers of eosinophils; however, collagenolytic granulomas often contain flame figures and do not contain the lakes of eosinophilic debris and sheets of mast cells characteristic of equine MCTs.

References:

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CASE II:

Signalment:
6-year-old, male neutered domestic short hair, cat (*Felis catus*)

History:
Presented to referring vets for acute onset swelling and lameness of right forelimb with subsequent development of generalised subcutaneous oedema on the right side of the body including the right hindlimb. The cat has outdoor access and no travel history (from the UK). The case was referred for medical investigation following further deterioration. Initial haematology and biochemistry revealed marked leukocytosis, neutrophila and basophilia, and a marked hypoalbuminaemia. Platelet count was normal but with a prolonged PT and aPTT and elevated D-dimers. Full body CT scan demonstrated marked multifocal subcutaneous and intramuscular oedema. Hepatic, splenic and peripheral lymph node cytology demonstrated neutrophilic inflammation and reactive lymphoid tissue. Culture of oedema fluid revealed a moderate growth of alpha-haemolytic colonies resistant to amoxicillin/clavulanate, cephalexin, penicillin G, TMPS and erythromycin, and sensitive to

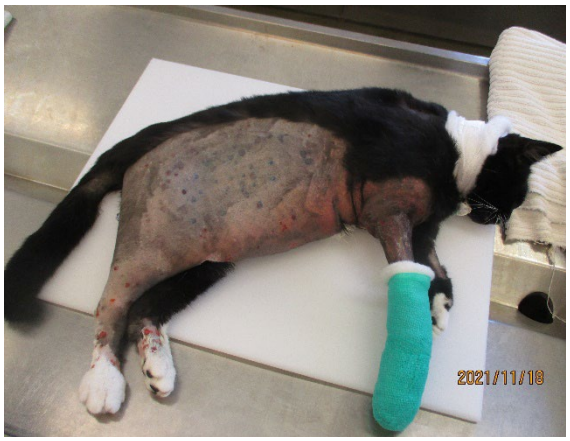

Figure 2-1. Presentation, cat. The cat has generalized subcutaneous edema of the right side of the body. (Photo courtesy of: Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge, Cambridge CB3 0ES, UK, https://www.vet.cam.ac.uk)

doxycycline, gentamicin and oxytetracycline. Further clinical destabilisation and an uncertain prognosis prompted elective euthanasia.

Gross Pathology:
Extensive subcutaneous oedema, multifocal small dermal papules, erosions and ulcers, areas of fascial haemorrhage overlying antebrachial muscles, thoracic effusion and right prescapular lymphadenomegaly.

Laboratory Results:
Feline poxvirus qPCR: detected, 19.08 Ct units. (the higher the Ct, the lower amount of DNA present in the sample; Ct values typically range from 15 to 40; 40 indicates much lower DNA levels than 15).

Microscopic Description:
Haired skin and subcutaneous adipose tissue: Affecting 100% of the tissue, the epidermis has lost normal structure. Multifocally there is extensive epidermal necrosis, characterised by keratinocytes with karyorrhectic and karyolytic nuclei, loss of cellular detail, and replacement by eosinophilic debris (necrotic debris and fibrin), nuclear debris and haemorrhage. In areas with viable keratinocytes, keratinocytes demonstrate hypereosinophilia and ballooning (ballooning degeneration), with cytoplasm often expanded by eosinophilic inclusion bodies (type A inclusion bodies). Keratinocytes are also disassociated, with keratinocyte necrosis and loss resulting in reticular degeneration. There are frequent viral syncytia, and these often have nuclei peripheralized by intracytoplasmic eosinophilic inclusion bodies. The epidermis and dermis are expanded by haemorrhage, fibrin and wispy eosinophilic material (oedema).

The same epidermal changes, including the necrosis, extend into hair follicles, affecting all levels of the follicular epithelium. At the lower levels of the hair follicles, where there are more viable cells, follicular epithelial cells demonstrate cytoplasmic ballooning that is often accompanied by eosinophilic intracytoplasmic inclusion bodies, and viral syncytial cells. Small sebaceous glands are

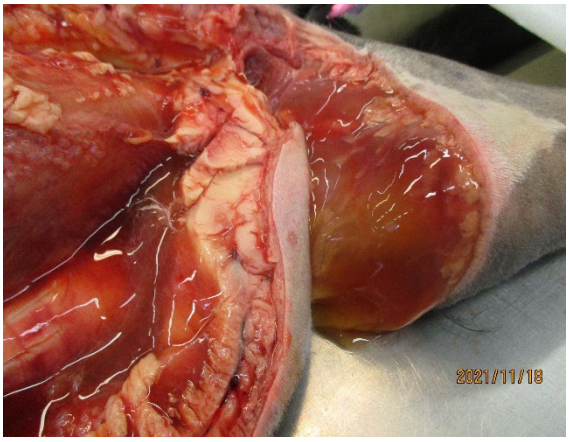

Figure 2-2. Right forelimb and neck, cat. Incision of the skin demonstrates the extent of the subcutaneous edema. (Photo courtesy of: Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge, Cambridge CB3 0ES, UK)

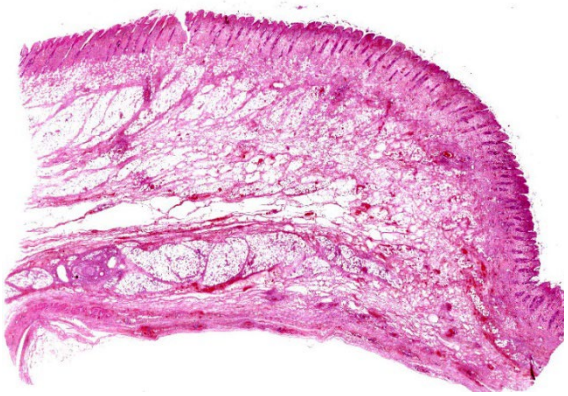

Figure 2-3. Haired skin, cat: One section of infarcted skin and subcutis are submitted for examination. There is diffuse severe edema visible at this low magnification. (HE, 6X)

almost completely effaced by necrosis of the sebocytes. Diffusely throughout the dermis and epidermis, there are low to moderate numbers of neutrophils and lymphocytes, with fewer eosinophils and macrophages, and scattered leukocytoclastic debris. Throughout the dermis, multifocally veins and to a lesser extent arteries have mural-associated neutrophils, and/or walls replaced by smudgy eosinophilic debris (fibrinoid necrosis), with intramural and perivascular leukocytoclastic debris. Perivascular spindle-cells and occasionally endothelial cells contain eosinophilic cytoplasmic inclusion bodies. Within the subcutis there are similar vascular changes to those described in the dermis, although frequently larger vessels are affected, resulting in marked expansion of the subcutaneous adipose tissue by haemorrhage, fibrin, and wispy eosinophilic material (oedema).

Contributor's Morphologic Diagnosis:
Haired skin and subcutis, ventrum: Dermatitis, folliculitis and cellulitis, necrotising, severe, diffuse, with leukocytoclastic vasculitis, keratinocyte intracytoplasmic eosinophilic inclusion bodies, keratinocyte viral syncytia, and epithelial ballooning degeneration.

Contributor's Comment:
This case demonstrates a systemic form of pox virus infection in cats, resulting in a clinical presentation of progressive marked cellulitis and necrotising dermatitis. This case lacked an observed primary cutaneous lesion or respiratory clinical changes, and prior to post-mortem examination, whilst a vasculitis was suspected, pox-viral related vasculitis was not a considered differential. On microscopic examination of the dermal and subcutaneous lesions, the frequent intracytoplasmic inclusion bodies were noted prompting qPCR for feline pox virus on stored frozen tissues. Feline poxvirus qPCR reveals moderately high levels of feline pox virus within the tissues, therefore pox viral infection is considered the cause of the necrotising process that has resulted in the clinical and histological features described in this case. Immunohistochemistry for pox virus demonstrated specific immunoreactivity within epithelial cells in affected areas. This cat had an extended post-mortem interval in cool storage, which is likely to have contributed to the poor preservation of some inflammatory cells.

Cow pox/cat pox virus is a large, enveloped, double-stranded DNA, cytocidal virus that is part of the Orthopoxviridae genus, within the Poxviridae family. The orthopox genus also includes ectromelia virus, monkeypox virus, smallpox virus (variola), vaccinia virus, and rabbitpox virus. Cowpox is endemic in Europe and Northern and Central Asia with a wide host range that includes people.[7,17,32] Cowpox infection in most species results in localised dermal lesions or systemic disease that may be fatal. Wild rodents, especially bank voles (*Clethrionmys*

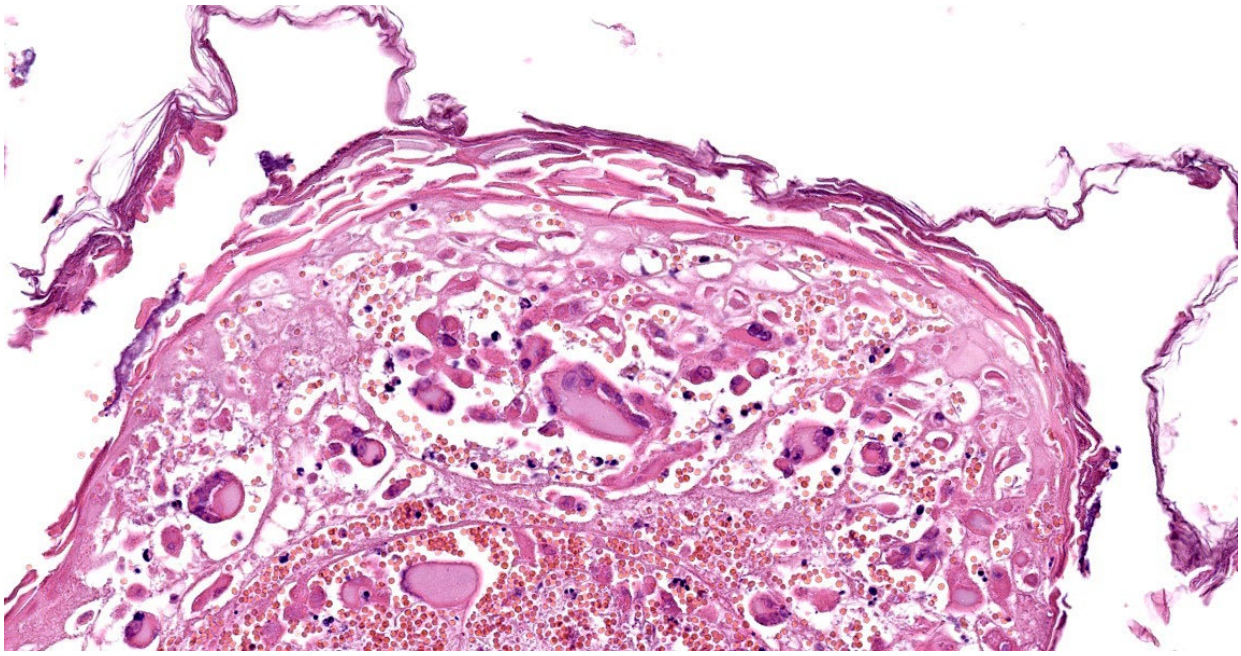

Figure 2-4. Haired skin, cat. There is diffuse necrosis of the epidermis. There are numerous viral syncytia in the epidermis. (HE, 302X)

glareolus), serve as the viral reservoir and infection of rodents, and subsequently secondary species such as humans and cats, varies seasonally with a peak in autumn and subsequently secondary transmission to cats.[1,4]

Cow pox is the most common poxviral infection of cats, with the common presentation involving the skin, often initially with a single primary lesion, which is assumed to be secondary to intradermal inoculation via a rodent bite.[26] Systemic infections can occur following the viraemic phase, and include pyrexia and lethargy, and occasionally pneumonia.[20,26] Feline cowpox infection is occasionally concurrent with other feline viruses, including viruses expected to cause immune dysfunction. At this stage the relationship between the clinical progression and severity of feline cowpox infection in cats co-infected with other viruses is not clear.[8] FIV has been suggested to exacerbate feline cowpox infection, with larger and more persistent primary lesions, however up to 30% of cats infected with cowpox are also seropositive to FIV with no change in disease course.[1] Feline herpesvirus and feline cowpox co-infection resulted in a necrotising pneumonia involving both viruses and a pox-viral dermatitis, however the cat successfully recovered with supportive treatment and antimicrobials.[14] A case of a cat with cowpox and feline parvovirus infection resulting in a dermatitis, panniculitis and enteritis was not associated with more severe poxvirus-associated disease.[24]

The FIV/FeLV status of the cat in this case was not defined. Special stains did not highlight bacteria within the subcutis, however a secondary bacterial infection, as indicated by the positive culture of the oedema fluid, is considered likely. Rare areas of ulcerated skin were associated with mats of superficial fungal hyphae, and deeper invasion was not observed. For both secondary infections, a

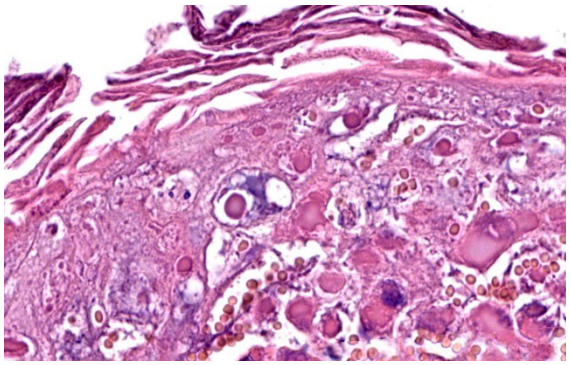

Figure 2-5. Haired skin, cat. Epithelial cells contain large eosinophilic viral inclusions. (HE, 887X)

systemic role for either the agents or elaborated toxins is considered a possible co-factor in the severe presentation in this case.

A systemic form of feline cowpox, primarily presenting with severe dermal oedema is unusual. This presentation has been previously reported in a 4 year old male neutered domestic short haired cat that exhibited known hunting behaviours, and ultimately resulted in pox-viral associated neurological lesions and euthanasia of the cat.[2] More localised dermal oedema was described in a series of five cats that presented with dermal plaques, oedema and hyperaemia affecting the hindlimbs,[16] and two cases of localised forelimb oedema in a four-cat feline cowpox case series from the UK.[9] Of the five-cat case series, all cats were geographically and temporarily grouped and in four of the cases, the cowpox viruses had an identical haemagglutinin gene sequence suggesting a common source of infection.[16]

*Pathogenesis***.** Epidermal lesions are the result of viral replication in keratinocytes resulting in cellular dysfunction and eventual lysis either due to host-cell related mechanisms or to the actions of immune cells such as NK cells.[3,30] Viral proteins involved in viral entry to cells also induce host cell fusion, resulting in syncytia formation.[28] Cowpox, similar to other pox viruses, contains an epidermal growth factor homologue that is able to influence cellular differentiation and proliferation of keratinocytes.[27] The virus can infect and damage endothelial cells and in some experimental models produces a consumptive coagulopathy, resulting in more widespread dermal or submucosal necrotising lesions as a consequence of vascular damage, thrombosis and tissue infarction.[5,15,23] Cowpox virus has a range of immunomodulatory proteins that include IFN-gamma receptor and TNF-alpha receptor homologues, IL-1beta binding proteins, and anti-inflammatory Serpins.[27]

*Typical and atypical clinical findings***.** Cats often present with a single primary skin lesion on the head, neck, forelimb or paws.[1] Secondary bacterial infection of the lesion may induce confounding clinical signs. A viremia with pyrexia, lethargy and inappetence may follow 1-3 weeks following the initial infection.[26] Widespread skin lesions may follow the viremia with papules and nodules that ulcerate and crust, but skin healing is ultimately expected.[1] The mucocutaneous junction and oral cavity may be affected, forming ulcerative foci.[9] Pneumonias in the absence of skin lesions has also been reported rarely with both cases resulting in a fatal pneumonia that was caused by a genetically similar but not identical viral strain.[11,25] Exotic felids, especially cheetahs, can be infected with cowpox virus.[18] The clinical signs range from dermal and oral lesions to a necrotising pneumonia, of which two out of nine cases (22%) of cheetah infections were fatal.[31]

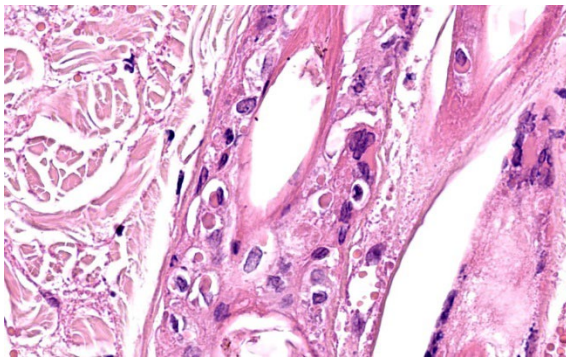

Figure 2-6. Haired skin, cat. Viral inclusions, syncytia, and epithelial changes extend into the follicles. (HE, 757X)

Typical gross findings. The typical macroscopic epidermal changes include erythematous macules developing into papules and then pustules that rupture and crust.[1] In this case, and in other reported atypical feline cowpox presentations, there was marked dermal and subcuticular oedema due to, in this case at least, widespread pox-viral associated vasculitis/vascular dysfunction. Marked subcutaneous oedema is a clinical feature more associated with Mousepox (ectromelia) virus infection.[22]

Typical microscopic findings. The typical microscopic findings in cases of feline cowpox are well defined. Epidermal lesions start with keratinocyte cytoplasmic swelling and vacuolation (intracellular oedema and ballooning degeneration), and progress through cell rupture to multiloculated vesicles (reticular degeneration) and epidermal necrosis.[1,12] Epidermal hyperplasia is often present and may be marked. Accompanying dermal changes include oedema, mononuclear cell infiltrate and a variable neutrophilic infiltrate. With time, the neutrophilic infiltrate extends into the epidermis creating pustules. Cox pox, like other pox viruses, creates intracytoplasmic inclusion bodies that vary in number and size. Large brightly eosinophilic inclusions are identified as A-type inclusions, and are predominantly composed of viral protein in which mature virions are embedded.[6] A-type inclusions are thought to protect infectious particles after release into the environment, and increase in size throughout the infection by coalescence of multiple smaller bodies.[13] B-Type pox inclusions are smaller, more basophilic, and are the sites of viral replication (viral factories).

Ultrastructure. On electron microscopy Poxviral particles are enveloped and are brick shaped. Single virions have multiple protruding surface spicules.[11]

Additional diagnostic tests. Confirmation of the diagnosis can be made using electron microscopy, immunofluorescence, virus isolation, and PCR on tissues, which also includes skin scabs and pulmonary or thoracic aspirates.[21,26]

Treatment. Treatment focuses on controlling/preventing secondary bacterial infections and immunomodulation with recombinant feline interferon omega.[2,20]

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JPC Diagnosis:
Haired skin and subcutis: Vasculitis, necrotizing, diffuse, severe, with cutaneous infarction, epithelial ballooning degeneration, numerous epithelial viral syncytia and intracytoplasmic inclusions.

JPC Comment: The contributor provides an excellent, thorough overview of cowpox infection in cats. While the cat is the most commonly recognized incidental host, cowpox

Genus	Representative Viruses
Orthopoxvirus	• Cowpox virus • Vaccinia virus (buffalopox virus, rabbitpox virus) • Horsepox virus • Camelpox virus • Ectromelia virus (mousepox virus) • Monkeypox virus
Parapoxvirus	• Orf virus (contagious pustular dermatitis virus, contagious ecthyma virus) • Pseudocowpox virus (milker's nodule virus) • Bovine papular stomatitis virus • Parapox virus of red deer
Avipoxvirus	• Fowlpox virus • Pigeonpox virus
Capripoxvirus	• Sheeppox virus • Goatpox virus • Lumpy skin disease virus
Leporipoxvirus	• Myxoma virus • Rabbit fibroma virus
Suipoxvirus	• Swinepox virus
Molluscipoxvirus	• Molluscum contagiosum virus
Yatapoxvirus	• Tanapox virus • Yaba monkey tumor virus

Table 2-1. Selected Poxviridae genera and representative members.

also infects dogs, rats, mice, humans, horses, and various exotic mammals.[10] Perhaps surprising given its name, cowpox infection is rare in cattle.

The *Poxviridae* is a large family of enveloped DNA viruses that are highly epitheliotropic and cause cutaneous and systemic disease.[19] Poxviruses encode their own replication machinery, including an RNA polymerase, which enables their characteristic cytoplasmic replication. Animal poxviruses exist within the subfamily *Chordopoxvirinae*, which is further subdivided into 22 different genera (see Table 2-1 for a sampling of genera and representative members).

The orthopoxviruses, including cowpox, are notable for their wide host spectrum and are among the most important and well-characterized poxviruses due to their impact on human and animal health. Their most notable and feared member, the *Variola* virus, is the causative agent of smallpox.[29]

Poxviruses damage cells in multiple ways. Degenerative changes are caused by direct cytotoxic damage due to poxviral replication and subsequent rupturing of the host cell membrane, leading to typical vesicular lesions.[19] As in this case, the dermis and submucosa may be subject to ischemia due to vascular damage caused by viral

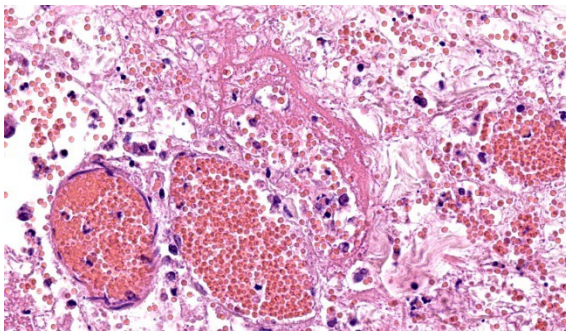

Figure 2-7. Haired skin, cat. Within the dermis and subcutis, widespread vasculitis gives rise to marked hemorrhage and edema within these areas. (HE, 360X)

replication within endothelial cells.[19] Poxviruses also induce proliferative lesions due, in some poxviruses, to a viral gene that encodes a mitogenic protein with significant similarity to epidermal growth factor.

Pox lesions tend to develop in a particular sequence, with erythematous macules becoming papular, and then vesicular.[19] The vesicles become "pocks," pustules with a depressed center and raised border which then rupture, forming crusts.[19] Histologically, poxvirus lesions are characterized by marked epithelial hyperplasia with serocellular crusts and intracytoplasmic inclusion bodies. Both the gross and histologic lesions are characteristic, facilitating easy diagnosis in most cases.

In conference, participants discussed the remarkable coagulative necrosis present in the epithelium and dermis in this case. The moderator emphasized that this indicator of infarction, when present, should precipitate a close evaluation of deep vessels. While we noted the contributor's description of cytoplasmic inclusion bodies within affected endothelium, no convincing endothelial cytoplasmic inclusions were found in the sections we received in this submission. The unrewarding hunt for endothelial inclusions did not dampen the participants' enthusiasm for the robust, multifocal vasculitis, believed by the moderator to be the main driver of the striking pathology in this case; thus, the JPC morphologic diagnosis leads with vasculitis to reflect the centrality of this histologic finding.

References:

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CASE III:

Signalment:
6-month-old, intact male Göttingen minipig (*Sus scrofa*)

History:
A 6-month-old, intact male, single-housed, 12 kg, Göttingen minipig presented with bilateral ear erythema at the apical margins 4 hours post repetitive auricular venipuncture. The pig had open cage bar communication with conspecifics. The pig had a jugular venous access port (VAP) and telemetry implanted 2 months prior to presentation. The pig was enrolled in a pharmacokinetics study 1 month prior and received 2 intramuscular doses of scopolamine. On exam, the pig was bright, alert, and responsive (BAR) with 10 needle punctures per ear and extensive bruising. At the 24 hour recheck, the pig was BAR but the erythema expanded the entire pinnae with ulceration, crusting, epidermal thickening, and apical margin necrosis. Aerobic culture revealed Staphylococcus aureus and povidone-iodine scrub with silver sulfadiazine therapy was initiated. Primary differentials included research/conspecific-induced trauma, bacterial, and/or viral systemic disease. At the 48 hour recheck, the pig presented with depression, inappetance, dyspnea, cold extremities, petechiation on snout, lips, rectum, and prepuce, and red-purple discoloration on all limbs distal to hock/elbow. Blood was taken for clinical pathology and was still watery 20 minutes later. The pig was euthanized due to poor study candidacy and disease severity.

Gross Pathology:
This 12 kg, male, Göttingen minipig is in lean body condition with adequate subcutaneous and visceral fat, 2.5/5 body condition score, and well-hydrated. There are multifocally extensive petechiae, purpura, and ecchymoses on the nose, ears, lips, lower limbs, ventrum, prepuce, anus, and tail. Bilaterally, the ear tips are dark & necrotic. The right pinna has a hole in the middle from an ear tag. There is a subcutaneous VAP port on the right lateral neck and a subcutaneous telemetry device on the right side of the ventral neck. Both have no significant gross lesions. The gums and conjunctiva are pale and there are multifocal petechiae on the palate. There is abundant hemorrhagic foam in the trachea. The lungs are noncollapsed and extensively dark with multifocal petechiae and purpura. There

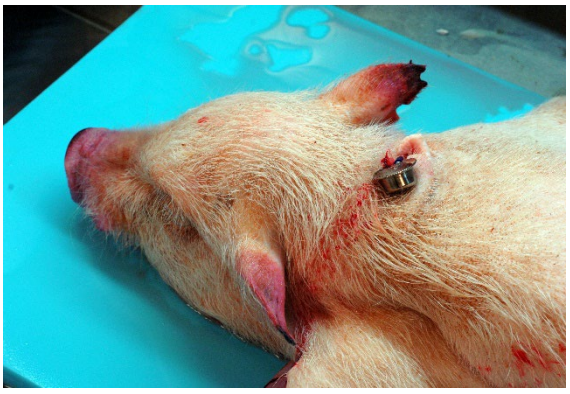

Figure 3-1. Presentation, Göttingen minipig. There are petechiae and ecchymoses on the face, nose, and skin of the head and neck. There is bilateral infarction of the ear tips. (Photo courtesy of: Comparative Pathology Department, Research Support Division, USAMRICD, Aberdeen Proving Ground, MD, https://usamricd.amedd.army.mil/)

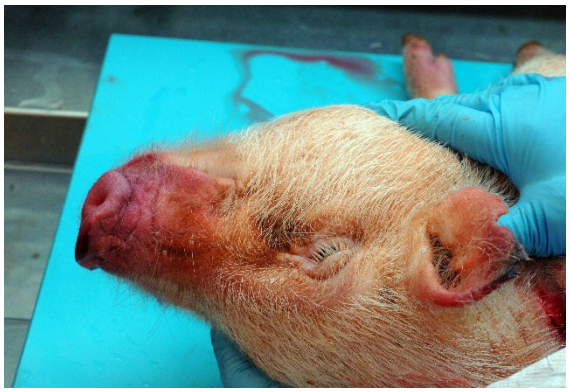

Figure 3-2. Ear pinna, Göttingen minipig. Higher magnification of the infarcted ear tip. (Photo courtesy of: Comparative Pathology Department, Research Support Division, USAMRICD, Aberdeen Proving Ground, MD)

are multifocal petechiae on the heart ventricles and there is a small amount of serosanguinous fluid in the pericardial sac. The liver, spleen, and kidneys are dark and enlarged (post-euthanasia congestion). The liver, kidneys, and intestinal serosa have multifocally extensive petechiae. The gallbladder contains a small amount of hemorrhagic bile. The stomach contains a moderate amount of normal ingesta and gas and there are multifocal petechiae and erosions/ulcers on the fundic mucosa. The small intestine contains a moderate amount of bile-stained digesta and gas. The cecum contains a moderate amount of dark hemorrhagic contents. The colon contains a small amount of normal feces. The urinary bladder is empty. No significant gross lesions were observed in any other organs.

Laboratory Results:
Clinical pathology revealed a regenerative anemia, low hemoglobin, marked thrombocytopenia, and elevated ALT, AST, GGT, and albumin. A differential count supported thrombocytopenia with schistocytosis, nucleated RBCs, acanthocytes, and target cells.

Microscopic Description:
Ear pinna: There is focally extensive full-thickness coagulative necrosis affecting the distal end. Multifocally there are vascular thrombi present and various arteriosclerotic changes to the arterioles and small muscular arteries (endothelial cell hypertrophy, myointimal proliferation, smooth muscle cell vacuolation, medial thickening, concentric laminar wall thickening ("onion-skinning"), luminal stenosis, and/or periarteritis). Inflammation is scattered and primarily composed of macrophages, lymphocytes, eosinophils, and neutrophils. There is also multifocal areas of periarticular hemorrhage and occasional hemosiderin-laden macrophages.

Changes in other organs (not submitted):
Heart: Multifocal severe myocardial fiber degeneration, necrosis, and loss with hemorrhage, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

Kidney, bilateral: Diffuse severe membranoproliferative glomerulonephritis with tubular degeneration, necrosis, and proteinosis, hemorrhage, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

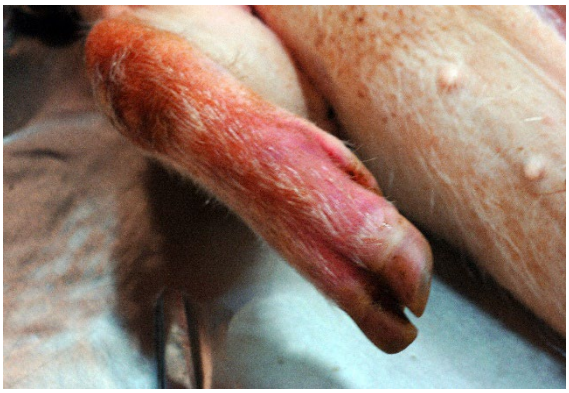

Figure 3-3. Hindlimb, Göttingen minipig. There are petechiae and ecchymoses on the skin of the hindlimbs. (Photo courtesy of: Comparative Pathology Department, Research Support Division, USAMRICD, Aberdeen Proving Ground, MD)

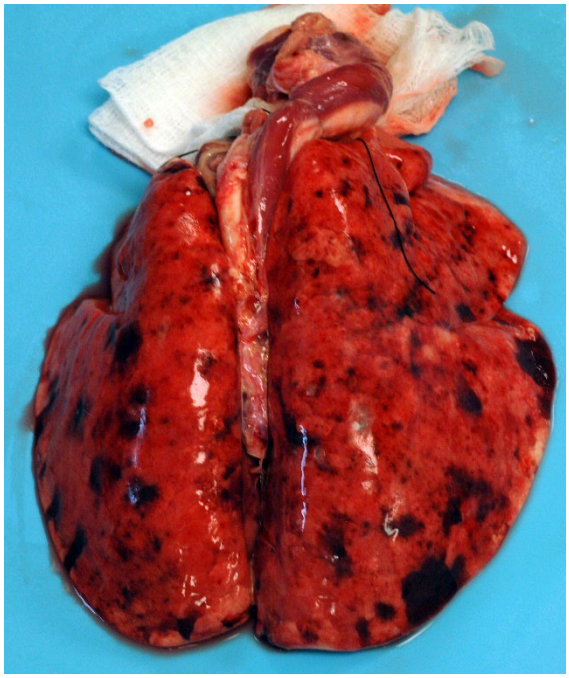

Figure 3-4. Lungs, Göttingen minipig. There are hemorrhages throughout the lungs. (Photo courtesy of: Comparative Pathology Department, Research Support Division, USAMRICD, Aberdeen Proving Ground, MD)

Lung: Severe multifocally extensive intraairway and interstitial hemorrhage, fibrin, and edema with vascular thrombi and various arteriosclerotic changes to the arterioles and small muscular arteries.

Liver: Moderate to severe multifocal centrilobular to midzonal hepatocellular degeneration and necrosis with hemorrhage and vascular thrombi.

Stomach, fundus: Severe focally extensive mucosal necrosis with submucosal inflammation, edema, hemorrhage, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

Pancreas: Multifocal moderate acinar degeneration and necrosis with hemorrhage, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

Diaphragm: Multifocal moderate myofiber degeneration and necrosis with hemorrhage, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

Spleen: Diffuse moderate extramedullary hematopoiesis (EMH) with vascular thrombi and various arteriosclerotic changes to the arterioles and small muscular arteries.

Bone marrow: Diffuse moderate megakaryocytic hyperplasia.

Adrenal gland, bilateral: Multifocal moderate hemorrhage.

Gallbladder: Multifocal moderate submucosal hemorrhage and edema.

Ileum: Diffuse moderate GALT lymphocytolysis with vascular thrombi and various arteriosclerotic changes to the arterioles and small muscular arteries.

Duodenum: Multifocal vascular thrombi and various arteriosclerotic changes to the arterioles and small muscular arteries.

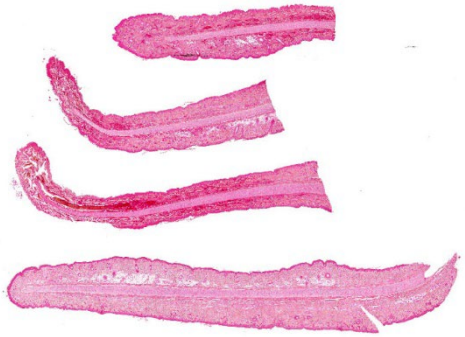

Figure 3-5. Ear pinnae, Göttingen minipig. Four sections of ear tip are submitted for examination. (HE 6X)

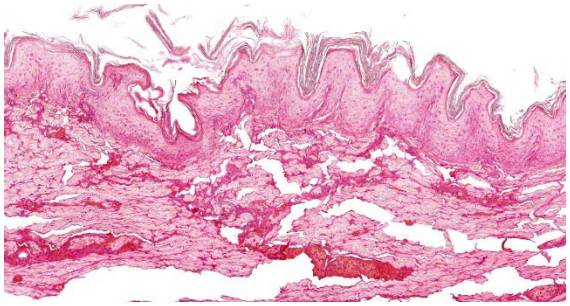

Figure 3-6. Ear pinna, Göttingen minipig. There is diffuse coagulative necrosis (infarction) with lack of stain affinitiy of the ear tip. (HE, 123X)

Lymph node, various: Multifocal moderate draining hemorrhage and lymphocytolysis with histiocytosis, erythrophagocytosis, hemosiderin-laden macrophages, vascular thrombi, and various arteriosclerotic changes to the arterioles and small muscular arteries.

Contributor's Morphologic Diagnosis:
Ear pinna: Necrosis, full-thickness, focally extensive, severe, with vascular thrombi and various arteriosclerotic changes to the arterioles and small muscular arteries.

Contributor's Comment:
Severe thrombocytopenia and anemia are consistent clinical findings with thrombocytopenia purpura syndrome of Göttingen minipigs, along with extensive hemorrhages in subcutaneous tissues and various other organs.[1,6] This syndrome is a spontaneous degenerative vasculopathy that rarely occurs in European and North American herds of Göttingen minipigs.[1,6] It primarily affects sexually mature males and females but has been seen in animals as young as 7 weeks of age.[6] Histologically, there are distinctive features of arteriosclerosis in various tissues, primarily in small- to medium-sized arteries and arterioles. These features include endothelial cell hypertrophy, myointimal proliferation, smooth muscle cell vacuolation, medial thickening typically by myxoid deposits, concentric laminar wall thickening ("onion-skinning"), luminal stenosis, and/or periarteritis.[6-8] Unlike spontaneous atherosclerosis of aged swine, lipid accumulation is not a feature.[6] Membranoproliferative glomerulonephritis and myocardial infarcts also feature prominently in this disease.[6,7] The pathogenesis is unknown at this time but it is proposed to be due to a type III hypersensitivity causing the widespread degenerative vasculopathy.[1,6,7]

Other rule-outs for cutaneous hemorrhages in pigs, although less likely in research bred animals, include: (1) neonatal thrombocytopenic purpura – hypersensitivity due to incompatible colostral antibodies; (2) vitamin K deficiency/anticoagulant rodenticide poisoning; and (3) viral infections, such as porcine circovirus, classical swine fever, and African swine fever.

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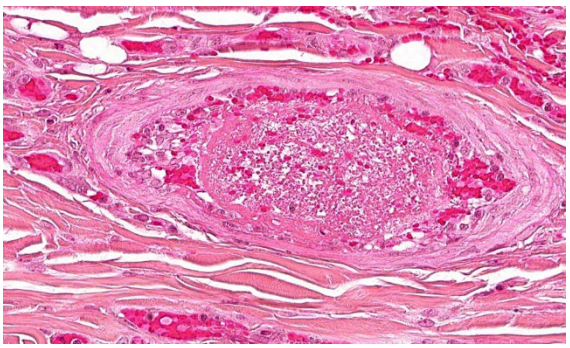

Figure 3-7. Ear pinna, Göttingen minipig. Dermal arterioles demonstrate mural thickening as a result of intimal hyperplasia and adventitial fibrosis and often contain non-occlusive fibrin thrombi. (HE, 387X)

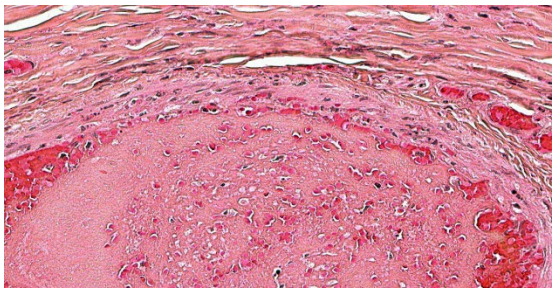

Figure 3-8. Ear pinna, Göttingen minipig. Occasionally, arteriolar walls contain exuded protein, necrotic smooth muscle, mural and adventitial necrotic neutrophils, and cellular debris. (HE, 508X)

JPC Diagnosis:
Haired skin, ear tip: Arteriolosclerosis, multifocal, moderate, with thrombosis, occasional arteritis and periarteritis, and focally extensive cutaneous infarction.

JPC Comment:
The syndrome nicely described by the contributor and illustrated by this case is a rare syndrome with an uncertain pathogenesis that is specific to Göttingen minipigs. As the contributor notes, one of the major differential diagnoses for this condition in swine is neonatal thrombocytopenia purpura, an immune-mediated condition in suckling pigs that has been described in veterinary literature for 50 years.[5] The case presentation is similar to the syndrome in Göttingen minipigs, but with a much younger age of onset and a different pathogenesis.

In neonatal thrombocytopenic purpura, suckling pigs typically become symptomatic around 3 days of age, recover, and then experience a re-emergence of symptoms around day 14 which quickly leads to death.[5] Piglets are pale and inactive and post-mortem exam reveals severe hemorrhage and edema in the lungs, heart, kidneys, subcutis, joints, gastrointestinal and respiratory tracts, and skeletal muscle. Histologic findings include hemorrhages in various tissues and depletion of megakaryocytes in the bone marrow and spleen.[5]

Thrombocytopenia develops due to isoimmune antibodies developed by the sow during gestation against boar thrombocyte antigens present in the piglets' blood. Piglets ingest these isoantibodies with colostrum which render existing platelets useless and have a cytotoxic effect on bone marrow megakaryocytes.[5] Matings between Landrace and Large White pigs increase the risk that the resulting piglets will develop neonatal thrombocytopenia purpura.

Due to the need for prior sensitization, neonatal thrombocytopenia purpura occurs only on subsequent matings of a sow to the same boar. Because of this, the disease fell out of fashion with the advent of modern artificial insemination and the concomitant reduction in multiple identical matings.[5] However, in a case of good intentions gone awry, the disease is being seen more frequently now that animal welfare preferences have catalyzed changes in livestock rearing practices. Of particular salience here is the use of natural mating as part of a larger push to allow animals to exhibit natural behaviors in all aspects of their lives.[2] A recent outbreak occurred on a Swiss organic farm that practiced natural mating of pigs using one of only three boars kept on the property, increasing the chances of repeat matings.[5] Thirteen piglets from two separate litters died of neonatal thrombocytopenia purpura before management practices, including exchanging boars periodically, documenting and managing matings, and switching all boars to the Duroc breed, were implemented.

The syndrome is similar to neonatal isoerythrolysis in foals in which anemia and subsequent organ failure result when foals inherit a blood type from the stallion against

which the mare has previously developed antibodies. Similar to the syndrome described above, foals ingest the isoantibodies with colostrum which end up in the bloodstream and attach to the foal's erythrocytes, causing their immune destruction.

Conference discussion centered on the pathogenesis of thrombocytopenia purpura of the Göttingen minipig. As the contributor notes, the current literature suggests that the degenerative vasculopathy characteristic of this disease is caused by a type III hypersensitivity reaction; however, causality has not been established and the moderator remains skeptical that the condition is solely immune-mediated. This case also provided a second opportunity to discuss the importance of a conscientious examination of vasculature, which here was multifocally thrombosed due presumably to intimal damage and the resulting exposure of collagen and tissue factor. The widespread thrombosis described by the contributor in various tissues likely resulted in a consumptive coagulopathy that explains the systemic, widespread hemorrhage seen in this animal. Due again to the centrality of the vasculature to the histologic presentation, the JPC morphologic diagnosis once again leads with the vascular changes.

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CASE IV:

Signalment:
11-year-old, spayed female bassett hound, dog (*canis familiaris*)

History:
The patient was observed over a period of several months to develop a fluctuant soft tissue swelling associated with the upper eyelid, which was indicated to be subconjunctival/ subcutaneous with extension posteriorly towards the orbit. Interpretation of a fine needle aspirate was consistent with a neoplasm of epithelial origin. The entire submitted tissue was expressible through a small conjunctival incision over the mass at surgery, and the submitting veterinarian described a tissue with the gross appearance of fat.

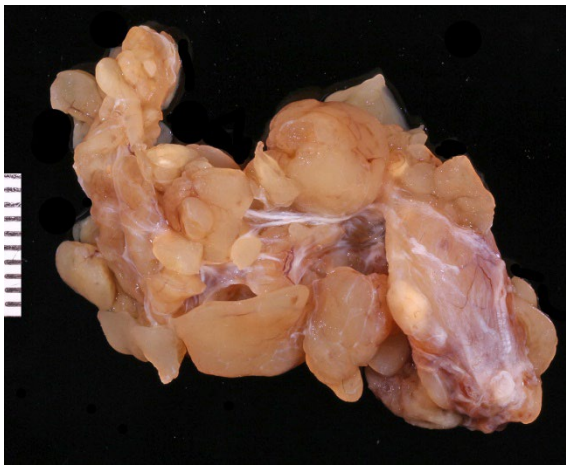

Figure 4-1. Periocular tissue, dog. A friable 3.5 x 1.8 x 1.0 cm mass of interconnected lobules of tan tissue was submitted to the contributor. (Photo courtesy of: University of Wisconsin, School of Veterinary Medicine, Madison WI. https://www.vetmed.wisc.-edu/departments/pathobiological-sciences/)

Gross Pathology:
The submitted specimen consisted of multiple well demarcated and interconnected lobules of tan tissue and measured 3.5 x 1.8 x 1.0 cm. The consistency of the tissue was friable, with lobules falling away from the main mass during manipulation.

Laboratory Results:
Fine needle aspirate suggested an epithelial neoplasm.

Microscopic Description:
The tissue is mainly composed of an unencapsulated, poorly-delineated, densely cellular and multilobular neoplastic mass infiltrating and expanding the adjacent connective tissue. The mass is composed of cuboidal epithelial neoplastic cells, arranged into acini, cords and trabeculae supported by a delicate fibrovascular stroma. The neoplastic cells present moderate amounts of granular, lightly basophilic cytoplasm with indistinct cell borders, and round to oval nuclei with homogenous to condensed chromatin and 1-3 variably distinct nucleoli. Anisocytosis and anisokaryosis are mild, and no mitotic figures are seen. A homogenous basophilic secretory material is distributed between the trabeculae of neoplastic cells and sometimes throughout the extracellular space. There is a focal accumulation of foamy macrophages expanding the connective tissue adjacent to the mass. The neoplastic cells extend beyond the surgical margins.

Contributor's Morphologic Diagnosis:
Orbital connective tissue: Canine lobular orbital adenoma.

Contributor's Comment:
The gross and morphologic features of this mass are consistent with the diagnosis of canine lobular orbital adenoma. The key features attributed to this neoplasm include a well-differentiated glandular morphology, distinctive multi-lobular growth pattern, and a lack of glandular ducts and ductular differentiation of the neoplastic cells.[2] These neoplasms are frequently described clinically and/or grossly as being remarkably friable, which is attributed to the scant amounts of connective tissue supporting the dense glandular lobular units. This neoplasm is associated with a high rate of local recurrence, which is attributed to its friable nature and tendency to disperse within the orbit, causing difficulties in achieving clean surgical

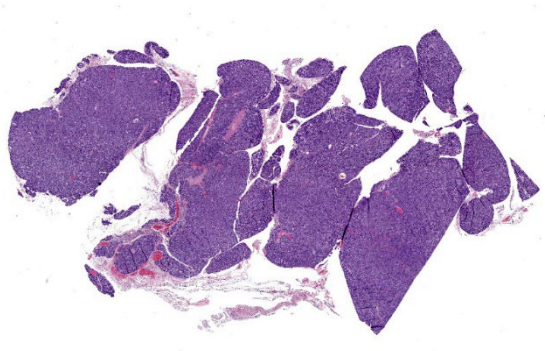

Figure 4-2. Periocular tissue, dog. A multilobular neoplasm is present. (HE 6X)

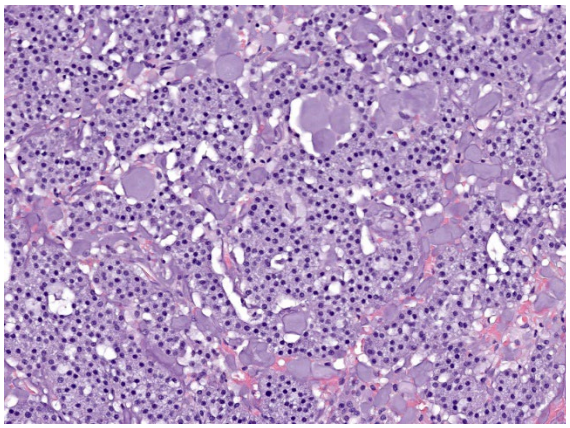

Figure 4-3. Periocular tissue, dog. Neoplastic epithelial cells are present in nests and packets and surround clear spaces that are often filled with a basophilic proteinaceous fluid. (HE 6X)

margins.[2] In the original case series describing this neoplasm, 10 out of 13 cases with available follow-up information experienced local recurrence (4 with multiple episodes of recurrence, with an average time after removal to recurrence of 395 days) with one case of recurrence associated with enucleation/exenteration, and the remaining 3 cases without recurrence being euthanized for reasons unrelated to this tumor.[2] The origin of this neoplasm is unknown. Empirical data from the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) suggests that accessory lacrimal glands observed throughout the subconjunctival and orbital connective tissue are the most likely tissue of origin; however, lacrimal, third eyelid, or zygomatic salivary glands are other possibilities.[2] Supporting evidence for a tissue of origin include location of the tumor within the orbit if it is relatively localized (e.g. supero-temporal suggesting lacrimal gland) and the morphologic and histochemical features of any adjacent non-neoplastic glandular tissue (e.g. primarily serous units suggesting lacrimal gland).[1,2,6] The dorsal localization of this case's neoplasm suggests it may be lacrimal in origin; however, this feature does not serve as definitive evidence.

The examined tissues in this case did not include any non-neoplastic glandular tissue to support a specific tissue of origin.

Orbital neoplasia has been cited as the most commonly described disease of the orbit in dogs, with many of these neoplasms representing primary disease.[2] However, metastatic tumors, neoplasia that extends into the orbit from nearby locations such as the oral and nasal cavities, and non-neoplastic orbital diseases such as orbital abscess also occur and must be differentiated.[3]

Contributing Institution:
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https://www.vetmed.wisc.edu/departments/pathobiological-sciences/

JPC Diagnosis:
Periocular tissue: Canine orbital lobular adenoma.

JPC Comment:
Canine lobular orbital adenoma (CLOA) is a rather descriptive name for an enigmatic tumor. First formally described in a University of Wisconsin case series in 2004, little progress has been made in positively identifying the tissue of origin and descriptions of the entity often focus on distinguishing it from

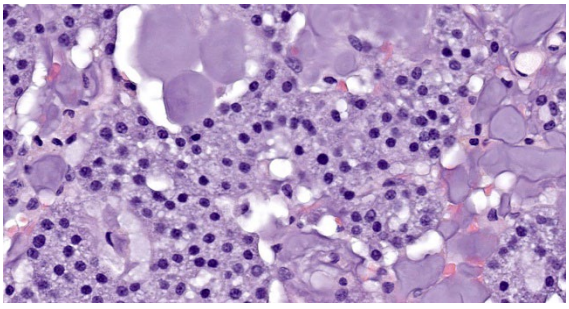

Figure 4-4. Periocular tissue, dog. High magnification of neoplastic cells with moderate amounts of granular cytoplasm. (HE, 520X)

more well-known entities such as lacrimal or salivary gland tumors.[2]

Pathogenesis likewise remains mysterious. One recent study investigating the pathogenesis of this entity found no association between canine papillomavirus and the development of canine lobular orbital adenoma.[4] Another recent study performed metagenomic analysis on 31 confirmed CLOAs looking for associations with an expanded group of DNA viruses in the neoplastic and normal conjunctival tissues and similarly found no associations.[5]

CLOAs occur in middle age to older dogs without breed predilection. The typical clinical presentation is a mass in the eyelid or subconjunctiva or, less commonly, in the retrobulbar tissues. Approximately 13% of cases are bilateral.[5] Grossly, the neoplastic tissue appears nodular, translucent, and friable.

The histologic appearance of the tumor in this case is characteristic of CLOAs; there are abundant acini composed of epithelial cells filled with PAS-positive granules without accompanying ductal differentiation. Tissue samples submitted for histopathologic evaluation typically consist, as in this case, of neoplastic tissue without any surrounding orbital structures or contextual tissues, perhaps due to the neoplasm's friable nature.[2] When surrounding tissues are present for evaluation, the lobular growth characteristic of this neoplasm extends into the connective tissues of the orbit.[2] The published immunohistochemical profile of CLOAs include positive immunoreactivity for CK 19 and AE1/AE3 and negative immunoreactivity for SMA, CK14, CALP, and p63, though this combination is not specific to this entity.[6]

Conference discussion focused mainly on the difficulty of tissue identification and histologic diagnosis. Conference participants offered a variety of differential diagnoses, including metastatic glandular tumors of the thyroid, lacrimal, sebaceous, and salivary glands, but even though diagnostically incorrect, all participants felt honored to be present for this novel tumor's Wednesday Slide Conference debut.

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