Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #1 — 14 August 2024

CASE I:

Signalment:
Aborted thoroughbred equine foetus (*Equus caballus*).

History:
A late gestation aborted thoroughbred foetus was submitted for postmortem examination. No placenta was submitted. According to the submitted clinical history, the mare had been vaccinated for equine herpesvirus and had no history of previous abortions.

Gross Pathology:
The equine foetus was presented dead, in a fair state of preservation. Fetal weight was 40 kg and body length 110 cm (estimated gestational age between 300 and 330 days). No placenta was available for examination. There were no gross lesions on external examination. Examination of the thoracic cavity revealed diffusely firm and rubbery lung lobes (non-aerated). No other lesions present.

Microscopic Description:
Liver: Within the hepatic parenchyma are multifocal, random areas of hepatocellular necrosis characterised by sinusoidal disruption, accumulation of karyorrhectic cellular debris, hepatocyte hypereosinophilia, cellular swelling, nuclear pyknosis and rare mixed inflammatory cells. Hepatocytes in adjacent areas frequently contain 2-4 um, pale eosinophilic intranuclear inclusions that displace the nuclear chromatin to the periphery.

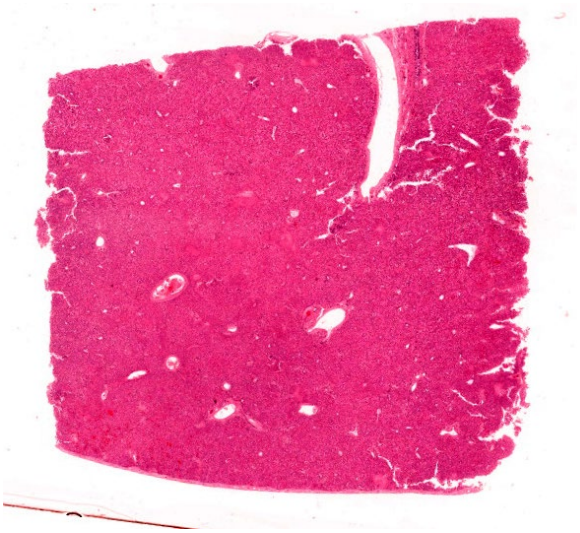

Figure 1-1. Liver, foal. One section of liver is submitted for examination. (HE, 5X)

Hepatic sinusoids are mildly congested. Periportal areas are moderately expanded by oedema and moderate numbers of lymphocytes, plasma cells and macrophages. Occasionally, small to medium calibre blood vessel walls are expanded by oedema, and necrosis of endothelial cells, characterised by pyknosis, cellular swelling, karyorrhexis and karyolysis.

Contributor's Morphologic Diagnosis:
Liver: Hepatocellular necrosis, acute, multifocal, random, with hepatocellular intranuclear inclusion bodies.

Contributor's Comment:
Equine herpesvirus type-1 (EHV-1) and equine herpesvirus type-4 (EHV-4) are important ubiquitous pathogens of all breeds of horses and other equids worldwide. In general,

EHV-1 strains are associated with respiratory disease, abortion, and paresis/paralysis, whereas EHV-4 strains are predominantly associated with respiratory disease.[2-4] In New Zealand, and similar to the rest of the world, most cases of equine herpesviral abortions are due to EHV-1. Equine herpesvirus type-1 (EHV-1) is a large, enveloped, DNA virus that is classified within the family *Alphaherpesviridae* in the order *Herpesvirales.* The virus is spread via multiple routes, including fomites, fetuses and placentas from EHV-1 induced abortions, and particularly by horse-horse contact and contamination. Horses shed EHV-1 in their respiratory secretions for up to three weeks post-infection.[3] Once infection is established, subclinical latency in the trigeminal ganglion occurs, and helps viral transmission between horses, which may or may not be associated with signs of illness.[3,5]

In clinically ill animals, following exposure, the virus replicates in a restricted plaque wise manner in the epithelial cells lining the upper respiratory tract,[5,6] including the pharynx, nasal turbinates, soft palate, and tracheal epithelium.[4] Entrance into epithelial cells is established either via direct fusion with the plasma membrane, or by endocytosis and fusion with an endosomal membrane.[3] After infecting the respiratory tract, the virus crosses the basement membrane via infected leukocytes ($CD172^+$ cells and T/B lymphocytes).[5] Once through the basement membrane, these infected leukocytes, penetrate connective tissues and enter the bloodstream and draining lymph nodes within 24-48 hours post infection.[4,6] Thus, viraemia via infected leukocytes disseminates the virus around the body, resulting in pyrexia and lymphadenomegaly.[5] Once in the circulation, EHV-1 can travel around the body and establish infection in target organs, including the pregnant uterus or the central nervous system.[3,5] This is established via strong adherence of EHV-1 infected leukocytes to endothelial cells, which subsequently results in an inflammatory environment that recruits additional monocytes to the endothelium.[5] The resulting inflammatory cascade may promote further endothelial cell infection, via increased expression of adhesion molecules (selectins secondary to release of IL-2 and TNF-alpha) on endothelial cells and stronger adherence of infected leukocytes.[7] After adherence, the virus replicates in the endothelial cells of the uterus, allantochorion, and umbilical vein.[8] The resulting vasculitis and thrombosis, damages the placenta and disrupts blood supply, leading to abortion[6]. This has been demonstrated in experimentally infected animals, where a lymphocytic vasculitis, focal thrombosis, and infarction of the microcotyledons results in abortion.[8] Abortion typically occurs, after 2-12 weeks in late gestation, and the incubation period varies widely from 9-120 days. [4,8] Interestingly, most cases result in no obvious uterine lesions, however in the presented case a severe metritis was diagnosed in the mare. However, it has been reported that there are no long term effects on reproductive performance in affected mares.[2]

As previously mentioned, viral latency can also develop, this occurs after replication in the respiratory epithelium. The virus enters nerve endings of the peripheral nervous system, including the trigeminal ganglia, and sympathetic and parasympathetic neurons that innervate the respiratory epithelium.[5] Here it travels via retrograde transport to the sensory and autonomic peripheral ganglia.[5] Along with this, it has been demonstrated to establish latency in the circulating T lymphocytes. Under stress, reactivation of EHV-1 replication occurs and particles spread from these infected T lymphocytes or trigeminal neurons through anterograde transport to the respiratory epithelium.[5]

Gross foetal lesions described in the literature include icterus, splenomegaly, perirenal oedema, pulmonary oedema or haemorrhage,

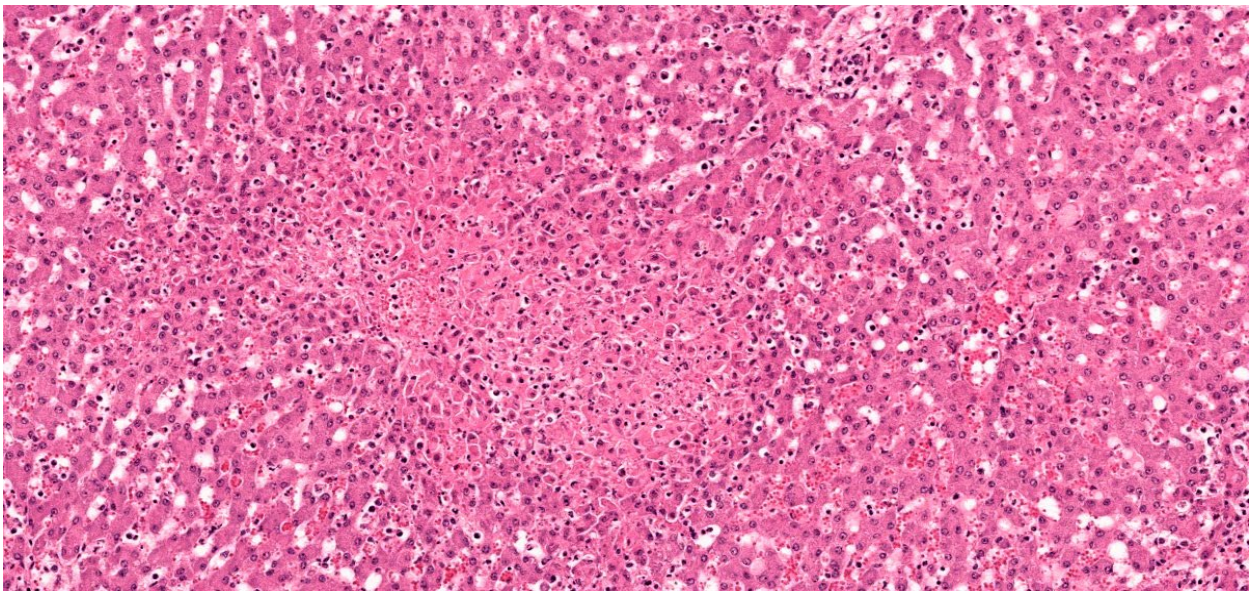

Figure 1-2. Liver, foal Areas of coagulative necrosis are scattered throughout the section. (HE, 144X)

and pale miliary foci on the surface of the liver.[8,9] In the present case, no gross lesions were present in the foetus, and a definitive cause for the abortion was not established during gross postmortem examination.

Confirmation of EHV-1 infection can be done via a number of ways, including immunohistochemistry of frozen or paraffin embedded tissues, and PCR for direct and rapid detection in frozen or paraffin embedded tissues.[8] Additionally, histological lesions, if present, can be used to support a diagnosis of EHV-1. Respiratory lesions include uniform pulmonary interlobular septal oedema and infiltration with mononuclear inflammatory cells, with fibrinous alveolar exudation and necrosis of bronchial and alveolar epithelial cells.[7] Hepatic lesions are not as common, but include focal necrosis, oedema, and leukocytes in the necrotic foci and portal triads. The acidophilic inclusion bodies in the nuclei of hepatic parenchymal cells surrounding these necrotic foci are specific for EHV.[6,7]

Preventative measures include management strategies and vaccination of breeding mares. Separating animals into groups or maintaining a closed herd can be an effective strategy. Quarantine of any new arrivals onto the farm for a minimum of 30 days is also highly recommended[4] as is investigation into the horses vaccination status, health certificate and negative tests results. All equipment used for new arrivals should be separate or disinfected and cleaned thoroughly if shared around the property.[4] Any personal involved in handling said animals, should also clean their bodies, boots and clothing thoroughly before handling other animals. Vaccines currently used include, inert or live vaccines. Current guidelines are to vaccinate foals over 3-5 months of age, with a booster within 4-6 weeks, along with vaccination every 3-6 months to enhance immunity.[4] Pregnant mares should also be vaccinated at the fifth, seventh, and ninth months of pregnancy.[4]

Contributing Institution:

Massey University
School of Veterinary Science
Private Bag 11 222
Palmerston North 4442
New Zealand

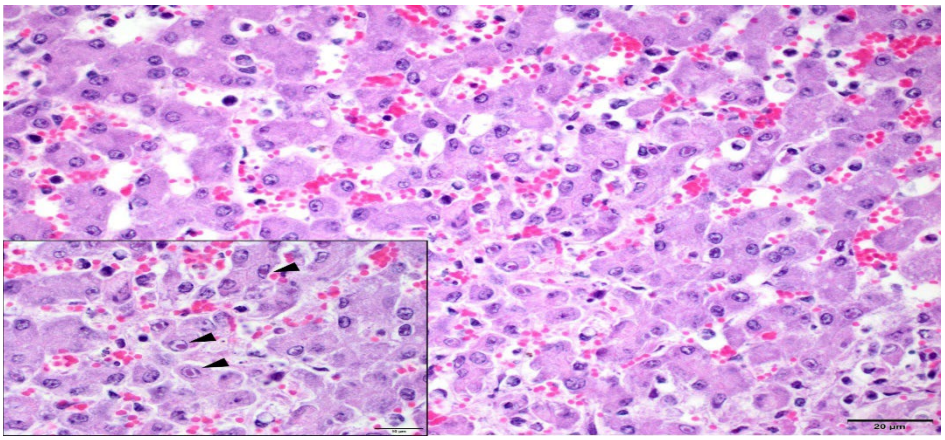

Figure 1-3. Liver, foal. At the edges of areas of necrosis, hepatocellular nuclei contain prominent viral inclusions. (HE, 400X) (*Photo courtesy of:* Massey University, School of Veterinary Science, Palmerston North 4442, New Zealand)

JPC Diagnosis:
Liver: Hepatitis, necrotizing, subacute, multifocal, random, moderate, with hetpacellular intranuclear viral inclusions.

JPC Comment:
This week's session was led by Colonel Sherri Daye, Director of JPC Veterinary Pathology (and all-around equine enthusiast). The cases for the first conference of WSC 2024-2025 were entirely an 'equine affaire' with 4 classic entities that have been seen in the conference before. This first particular case is diagnostically rewarding with multifocal, random areas of necrosis within the liver that provide a solid hint at the underlying pathogenesis. Likewise, intranuclear viral inclusions within hepatocytes are characteristic (figure 1-3) and help refine differentials for this case. Conference participants remarked at the distribution of necrosis in this case – there was a brief discussion of whether coagulative necrosis secondary to viral ischemic effects was a significant feature of this case. Ultimately, the group felt that endothelial cells lacked obvious viral inclusions and that the lesions were not predominately vasocentric and lacked significant thrombi. Additionally, the group slightly differed from the contributor in chronicity, noting that most cases of abortion take an extended period of time to develop given the cell-to-cell spread of alphaherpesviruses.[1] Moreover, a similar case of EHV in a neonatal horse was reviewed in a previous WSC (Conference 7, Case 3, 2015-2016) with extensive regions of hemorrhage and lytic necrosis within the lung, liver, and adrenal gland that reflects the expected distribution of lesions in animals that may be infected late in term and/or survive to parturition with EHV. Absent confirmation of the diagnosis via PCR and/or IHC of tissue in this case, we are left to favor EHV-1 over EHV-4.

The case contributor provides a nice overview of equine herpesviruses with emphasis on their role in equine abortion. Prevention of EHV-associated disease remains paramount; current consensus recommendations were recently updated by the ACVIM[6] and include vaccination in conjunction with a robust biosecurity program. To date, vaccination alone is not sufficient to prevent spread of EHV nor does it ameliorate clinical signs in sick horses. That stated, the ability of vaccines to prevent the development of viremia, at least in some animals, can serve to avoid devastating sequalae such as abortion or myeloencephalopathy.[6]

There are a number of other potential causes for abortion and stillbirth in foals. Bacterial causes include beta-hemolytic streptococci, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococ-*

cus aureus, *Klebsiella pneumoniae*, and *Actinobacillus equuli* among others.[1, 7] Infection may occur as a consequence of mare bacteremia or, more commonly, via ascending infection through the cervix. Fungal causes are typically attributed to *Aspergillus* spp., though other organisms may occasionally be isolated.[1,7] Rarely, equine viral arteritis has been isolated in abortion cases.[1,7] Non-infectious causes such as umbilical cord torsion and congenital malformation also remain common. Definitive cause of abortion and stillbirths remains elusive in many cases with only 29.2% of all submissions to a California diagnostic lab having a certain etiology identified.[1]

Finally, EHV-1 and EHV-4 should be distinguished from EHV-2 and EHV-5 which are both gamma herpesviruses that do not cause abortion, but are isolated both from healthy horses and those with equine multinodular pulmonary fibrosis (EMPF; EHV-5) and respiratory illness (EHV-2). There are multiple examples of EHV-5 in previous WSC proceedings. EHV-3 is associated with equine coital exanthema, and rarely, subclinical respiratory infections in yearling horses.[7]

References:

1. Cantón GJ, Navarro MA, Asin J, et al. Equine abortion and stillbirth in California: a review of 1,774 cases received at a diagnostic laboratory, 1990-2022. *J Vet Diagn Invest.* 2023 Mar;35(2):153-162.
2. Dunowska M. A review of equid herpesvirus 1 for the veterinary practitioner. Part A: clinical presentation, diagnosis, and treatment. *New Zeal Vet J.* 2014;62: 171-178.
3. Dunowska M. A review of equid herpesvirus 1 for the veterinary practitioner. Part B: pathogenesis and epidemiology. *New Zeal Vet J*. 2014;62: 179-188.
4. Khusro A, Aarti C, Rivas-Caceres RR, Barbabosa-Pliego A. Equine herpesvirus-1 infection in horses: Recent updates on its pathogenicity, vaccination, and preventive management strategies. *J Equine Vet Sci.* 2020;87: 102923.
5. Laval K, Poelaert KCK, Van Cleemput J, et al. The pathogenesis and immune evasive mechanisms of equine herpesvirus type 1. *Front Microbiol*. 2021;12: 662686.
6. Lunn DP, Burgess BA, Dorman DC, et al. Updated ACVIM consensus statement on equine herpesvirus-1. *J Vet Intern Med*. 2024 May-Jun;38(3):1290-1299.
7. Schlafer DH, Foster RA. Chapter 4: Female genital system. ln: Maxie MG ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 3. 6th ed. Missouri, USA: Elsevier; 2016:358-464.
8. Smith KC. Herpesviral abortion in domestic animals. *Vet J*. 1997;153: 253-268.
9. Smith KC, Blunden AS, Whitewell KE, Dunn KA, Wales AD. A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. *Equine Vet. J.* 2003;35: 496-501.
10. Van Maanen C. Equine herpes virus 1 and 4 infections: an update. *Vet Q*. 2002;24: 57-78.

CASE II:

Signalment:
8-year-old, male castrated, Arabian horse (*Equus ferus caballus*).

History:
This is a mass surgically removed from the left thorax.

Gross Pathology:
N/A

Microscopic Description:
Subcutis (mass from the left thorax): Expanding the deep dermis and extending to the subcutis are numerous multifocal to coalescing pyogranulomas. The pyogranulomas are com

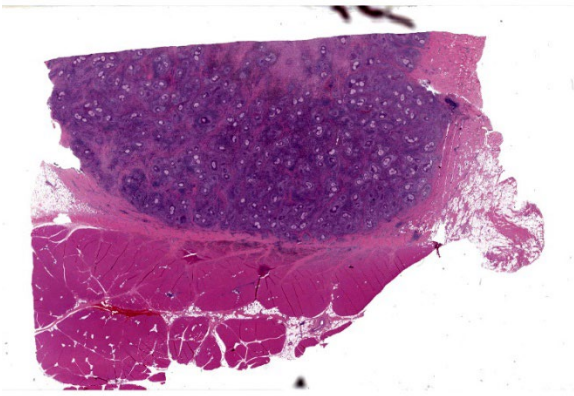

Figure 2-1. Panniculus and subcutis, horse. The subcutis is effaced by an inflammatory nodule which extends into the underlying skeletal muscle. (HE, 5X)

posed of intact and necrotic neutrophils surrounded by epithelioid macrophages, occasional multinucleated giant cells with up to 20 nuclei, fewer lymphocytes and plasma cells. In the center of the pyogranulomas are irregular pools of lightly eosinophilic amorphous material forming grain or granular structures measuring up to 500 um that contain countless tight clusters of polymorphic, refractile, fungal hyphae with frequent bulbous dilations. Surrounding the pyogranulomas are variably thick bands composed of fibroblasts and collagen (fibrosis). Multifocally, the surrounding dermis and subcutis are infiltrated by moderate numbers of lymphocytes and plasma cells, and rare macrophages and neutrophils.

PAS and GMS stains highlight the approximate 2-5 um, polymorphic, fungal hyphae with frequent and bulbous dilatations up to 30 um, consistent with dermatophytic pseudomycetoma.

Contributor's Morphologic Diagnosis:
Subcutis (mass from the left thorax): Multifocal to coalescing, marked, chronic, pyogranulomas with numerous intralesional fungal hyphae (pseudomycetoma).

Contributor's Comment:
A pseudomycetoma represents a subcutaneous infection with either bacteria or dermatophytes present in the tissue as granules or grains.[7] Dermatophytic pseudomycetoma in horses is a rare form of dermatophytosis with colonization beyond the superficial layers of the skin to the live tissues of the deep dermis and subcutis. The lesions are thought to form following the rupture of an infected hair follicle that results in a granulomatous or pyogranulomatous reaction around the dermatophyte hyphae.[8]

The most common cause of dermatophytosis in horses is *Trichophyton equinum*, with less frequent infection by *T. mentagrophytes, T. verrucosum, Microsporum equinum,* and *M. gypseum*.[10] Dermatophytosis is a common and contagious fungal infection of equine skin affecting horses of all ages, and young animals in crowded environments, and those in hot and humid climates may be over represented.[9,10] Infection rarely invades beyond the superficial layers of the skin in healthy animals.[9] Although the health status of this horse is unknown, it can be speculated that an immunocompromised state may have predisposed it to the deeper infection and subsequent psudeomycetoma formation. Pseudomycetomas, while rare, are documented in other domestic

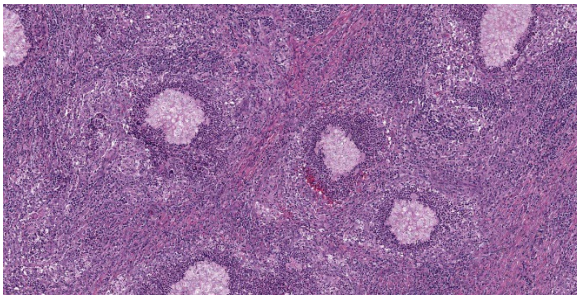

Figure 2-2. Panniculus and subcutis, horse: The nodule is composed of coalescing pyogranulomas centered on aggregates of poorly staining fungal hyphae. (HE, 86X)

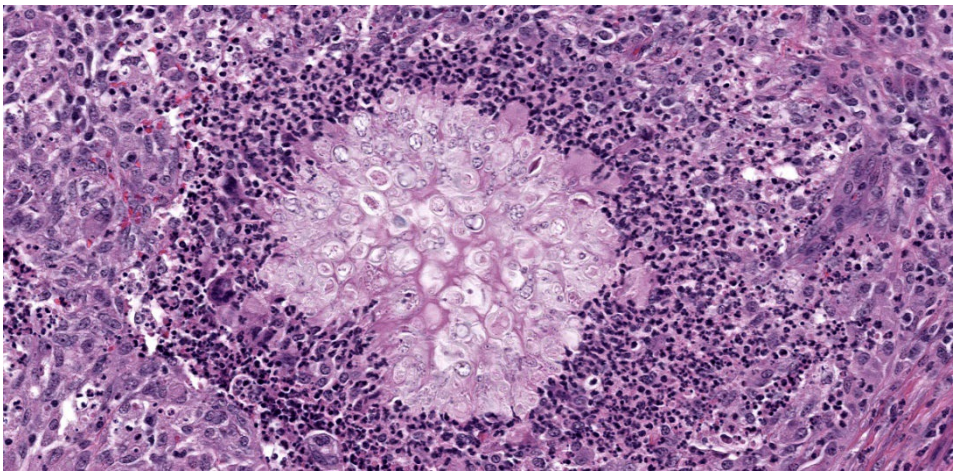

Figure 2-3. Panniculus and subcutis, horse. High magnification of an aggregate of fungal hyphae. Bulbous dilations are numerous within this aggregate. Fungal hyphae are surrounded by numerous neutrophils which are in turn surrounded by a layer of robust epithelioid macrophages and rare multinucleated foreign body-type macrophages. (HE 380X)

pecies, with a predisposition for Persian cats and Yorkshire terriers with *Microsporum canis* as the most common etiologic agent.[8] Given the predominance in Persian cats over other domestic feline species, a genetic deficit in immunity has been a proposed cause.[6]

With standard hematoxylin and eosin staining, grain or granule-like structures composed of fungal hyphae that are embedded in an amorphous eosinophilic matrix presumed to be antigen-antibody complexes[5] are seen to be surrounded by granulomatous or pyogranulomatous inflammation with multinucleate giant cells, and variable lymphoplasmacytic inflammation that are separated by varying degrees of fibrosis. Differential diagnoses include causes of true mycetomas including actinomycetes (actinomycetoma) or fungi (eumyoctic mycetoma).[2]

Contributing Institution:
University of Illinois at Urbana-Champaign, Veterinary Diagnostic Laboratory

http://vetmed.illinois.edu/vet-resources/veterinary-diagnostic-laboratory/

JPC Diagnosis:
Panniculus and underlying skeletal muscle: Pyogranulomas, multiple with numerous fungal hyphae, Splendore-Hoeppli material, and marked panniculular fibrosis.

JPC Comment:
This second case is striking from subgross, with identification of the lesion distribution hardly a diagnostic quandary (Figure 2-1). Although special stains were not necessary to arrive at the diagnosis for this case, we found that the methenamine silver (GMS) and periodic acid-Schiff (PAS) stains highlighted fungal elements nicely (Figure 2-4, Figure 2-5). Significant features include the presence of septate hyphae, chain-like pseudohyphae, and large (12um) pseudogranules.[10] Conference participants remarked that the H&E features were convincing for a fungal etiology, but exact speciation was not possible without additional ancillary diagnostics such as PCR or fungal culture. We differed from the contributor therefore in this case by omitting the mycetoma/pseudomycetoma distinction from the morphologic diagnosis. As a final treat for conference participants, Dr. Bruce Williams reminded the group that PAS is pronounced 'per-eye-OD-ik acid – Schiff' as the name of the reagent that we use 'periodically' for cases has decidedly different pronunciation that is easy to confuse.

As pointed out by the contributor, the location of these fungal pyogranulomas in the deep dermis with extension into the pectoral muscle is unusual for this entity however. Mycetoma

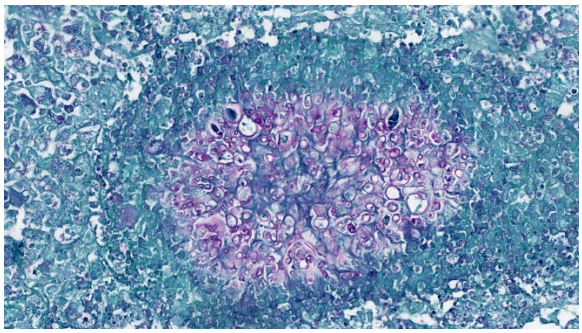

Figure 2-4. Panniculus and subcutis, horse. Fungal hyphae stain positively with periodic-acid Schiff stain. (PAS, 400X)

should be distinguished from pseudomycetoma by several key features. Mycetomas are fibrotic inflammatory nodules with draining fistulous tracts, and tissue grains of fungal aggregates with fewer hyphal elements surrounded by minimal amounts of Splendore-Hoeppli material and more cement material.[5] In contrast, pseudomycetomas are typically multifocal, may lack overt skin changes, are associated with dermatophytes, and typically have more abundant fungal hyphae and Splendore-Hoeppli material present histologically than true mycetomas do.[5] The abundant fungal hyphae, multifocal distribution of lesions, and abundant amorphous eosinophilic material (Splendore-Hoeppli) in this case is most consistent with dermatophytic pseudomycetoma, absent other confirmatory diagnostics. Wider distribution of lesions, to include regional draining lymph nodes, is an occasional finding.[1,5]

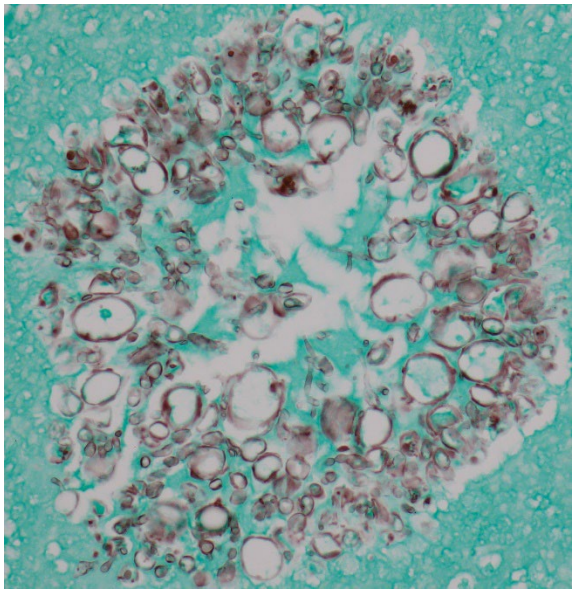

Figure 2-5. Panniculus and subcutis, horse. The cell wall of the fungal hyphae stain positively with a silver stain. (GMS, 400X)

Dermatophytic pseudomycetoma has been rarely reported in the cat[1,3,5] and has been previously covered in WSC (see Conference 11, Case 2, 2019-2020 and Conference 11, Case 3, 2008-2009). Persian, and to a lesser extent, Himalayan cats appear to have a predilection that may be explained by a longer hair coat and/or increased susceptibility to dermatophytes secondary to variation in the calprotectin gene which encodes an antimicrobial peptide.[1] Dermatophytic pseudomycetoma in shorthair cats has also been described, to include a recent case report from Korea.[3] Pseudomycetomas have also been described in the ferret[4], though case reports in the horse are sparse in the literature.

References:

1. Barrs VR, Bęczkowski PM, Talbot JJ, et al. Invasive fungal infections and oomycoses in cats: 1. Diagnostic approach. *Journal of Feline Medicine and Surgery*. 2024;26(1).
2. Cafarchia C, Figueredo L, Otranto D. Fungal diseases of horses. *Veterinary Microbiology*. 2013;167: 215-234.
3. Cho J, Park C, Park J, Yoon JS. Case report: Dermatophytic pseudomycetoma in a domestic Korean short hair cat treated with intralesional injection of amphotericin B and oral terbinafine administration. *Front Vet Sci*. 2024 Jun 13;11:1402691.
4. Giner J, Bailey J, Juan-Salles C, Joiner K, Martinez-Romero EG, Oster S. Dermatophytic pseudomycetomas in two ferrets. *Vet Derm* 2018: 29:452-e154.
5. Gross TL, Ihreke PJ, Walder EJ, Affolter VK. *Skin Diseases of the Dog and Cat*

Clinical and Histopathologic Diagnosis. 2nd ed. Ames, IA: Blackwell Publishing; 2005: 288-291.
6. Hargis AM, Ginn P, The integument. Ln: McGavin MD, Zachary JF eds. *Pathologic Basis of Veterinary Disease.* 6th ed. St. Louis, MO:Mosby Elsevier; 2017: 1079-1144.
7. Maudlin EA, Peter-Kenney J. Integumentary. ln: Maxie MG ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol. 1. 6th ed. Philadelphia, PA: Elsevier Saunders. 2016: 649-653.
8. Nardoni S Franceschi A & Mancianti F. Identification of *Microsporum canis* from dermatophytic pseudomycetoma in paraffin-embedded veterinary specimens using a common PCR protocol. *Mycoses.* 2007;50(3): 215-217.
9. Pilsworth R, Knottenbelt D. Common Equine Skin Diseases. Equine Veterinary Journal Ltd, Newmarket, Suffolk, UK; 2006: 52-53.
10. Scott, DW, Miller WH. *Equine Dermatology.* 2nd ed. St. Louis, MO: WB Saunders Co; 2011: 161-163, 171-201.

CASE III:

Signalment:
7 years of age, female, Selle Français, *Equus caballus*, Horse.

History:
The mare was initially presented to the referral clinic due to symptoms of chronic, recurrent diarrhea and intermittent exercise intolerance. General clinical examination revealed no abnormalities, with the exception of an enlarged, non-painful, retropharyngeal lymph node on the left side. Hematology, basic blood chemistry, and parasitological examination were normal. The horse was sent home after oral treatment with prednisolone (Equisolon®).

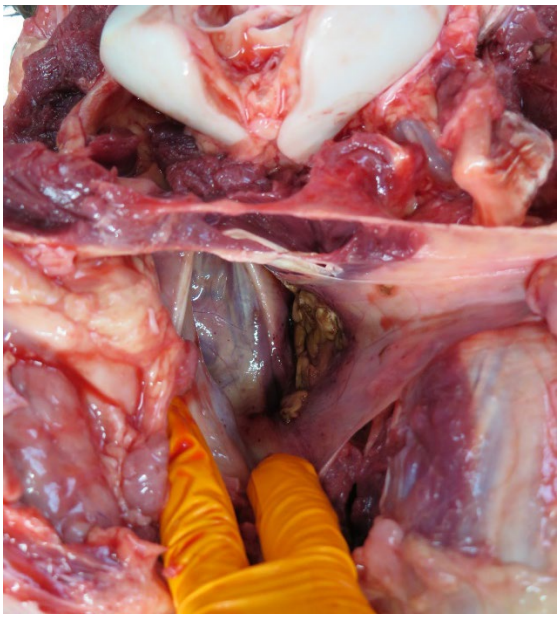

Figure 3-1. Guttural pouch, horse. The right guttural pouch was filled with clotted blood and contained a sharply delineated slightly raised irregular white plaque ca. 6 cm in diameter (suggestive of mycosis) at the level of the medial wall. (*Photo courtesy of:* Department of Pathology, Microbiology and Avian Diseases, Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, 9820 Merelbeke, BELGIUM, veterinaire.pathologie@ugent.be, + 32 9 264 77 41, https://www.ugent.be/di/di05/nl).

1 month later the horse suffered from severe, acute, right-sided epistaxis and was re-admitted to the clinic and hospitalized. Endoscopy of the upper airways revealed the presence of large amounts of blood in pharynx and trachea, as well as a large blood clot filling the lumen of the right guttural pouch. The left guttural pouch was evaluated as normal.

Surgery under general anesthesia was performed with fluoroscopy-guided placement of 7 intra-arterial coils in carotid and maxillary arteries. During recovery excitation and epistaxis developed. An emergency ligation of the right-sided a. carotis communis was attempted but proved ineffective. The horse was subsequently euthanized.

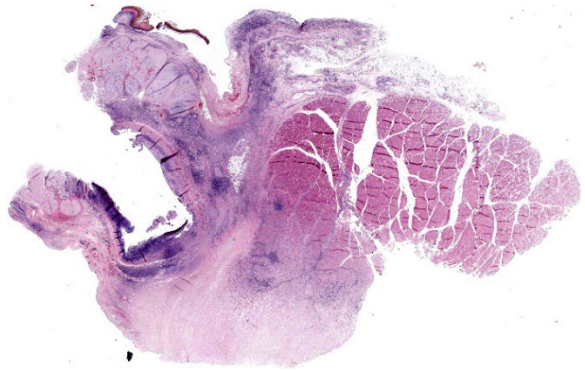

Figure 3-2. Guttural pouch, horse. An excellent section from the guttural pouch is submitted for examination. The internal carotid artery is at left, and the guttural pouch proper is at top center. (HE, 4X)

Gross Pathology:
The right guttural pouch was filled with clotted blood and contained a sharply delineated slightly raised irregular white plaque ca. 6 cm in diameter (suggestive of mycosis) at the level of the medial wall, with several smaller blood clots adherent to this area. Which, when removed, revealed a focal depression in the wall and a frayed transmural defect of a large thick-walled blood vessel (a. carotis interna).
The left side guttural pouch did not contain any blood but a smaller, ca. 4 cm diameter, pale plaque was found adherent to the wall.
Other significant gross findings included pale oral mucosae, blood exuding from both nostrils, and hemorrhagic contents of stomach and duodenum.

Microscopic Description:
Guttural pouch, part of large vessel wall, associated nerves and muscle.
The endothelium of the large artery is multifocally eroded and replaced by a layer of fibrin, admixed with erythrocytes (hemorrhage), eosinophilic necrotic cell and nuclear debris, and few partially degenerate neutrophils (arteritis).

At the level of the eroded surface and penetrating deeper into the tunica media and connective tissues a moderate amount of ca. 5-8µm long deeply basophilic structures with parallel walls can be found, sometimes seen to be dichotomously branching, and often endospore-forming (fungal hyphae). The severity of the arteritis and presence of hyphae is dependent on the level of tissue section.

Smaller vessels show hyalinization of the wall and contain mixed nuclear dust and a dense infiltration of neutrophils (thrombosis and vasculitis).

Multifocal areas of coagulative necrosis surround and infiltrate muscle fibers and nerve fibers deep in the tissue, along with the presence of occasional fungal hyphae.

Contributor's Morphologic Diagnosis:
Mycotic air sacculitis, chronic, severe, erosive, necrotizing, with fungal hyphae, obliterating necrosuppurative arteritis, multifocal vascular thromboses, and local necrotizing fungal myositis and neuritis.

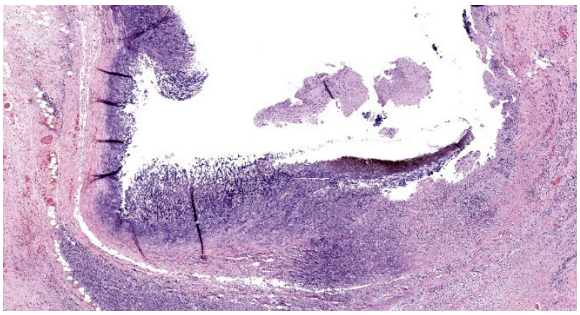

Figure 3-3. Internal carotid artery, horse. There is segmental loss of the endothelium and necrosis of the underlying arterial wall with growth of fungal hyphae into the lumen. (HE, 39X)

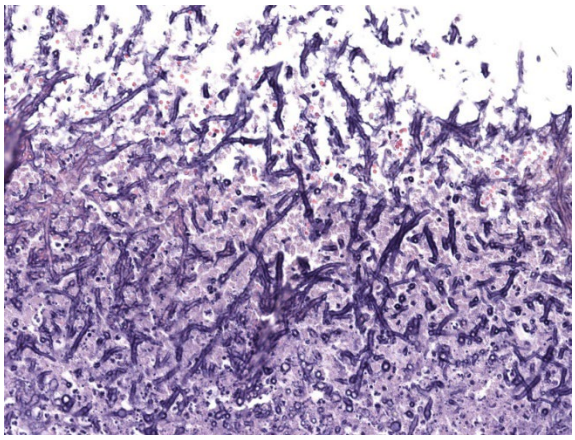

Figure 3-4. Internal carotid artery, horse. Higher magnification of septate, dichotomously branching, 4-6um fungal hyphae within the internal carotid artery. (HE, 240X)

Contributor's Comment:
The equine guttural pouches are a somewhat unique bilaterally symmetrical anatomical structure which can be interpreted as a local diverticulum of the inner auditory tube or Eustachian tube in which several important structures can be found.[10] It is longitudinally divided into two communicating compartments, lateral and medial, by the stylohyoid bone. Their functionality has been a topic of discussion for many decades or even centuries, but Ninomiya & Kuwano confirmed them to play a role as an effective countercurrent heat exchanger cooling arterial blood flowing towards the brain.[10]

Equine guttural pouch mycosis is a rare but potentially highly morbid and possibly fatal disease, most often caused by *Aspergillus* spp. in temperate climates, which are fairly ubiquitous opportunistic pathogens present in upper airways of healthy equids as well as in their immediate surroundings.[3,4,5,6,8]

There appears to be no predilection in regard to breed, sex, age, or left versus right side air sacs.[3] The fungal hyphae, especially of *A. fumigatus*, are typically angiotropic/angioinvasive and therefore often form infiltrative mycotic plaques associated with the large blood vessels lying closely beneath the guttural pouch surfaces.[1,8,5]

It can be considered challenging to diagnose, in part due to the wide spectrum of possible clinical signs, but also due to the high risks associated with confirmation of the pathogen in situ.[7,11] In this particular case, based on the history, gross lesions, and microscopic findings, infection with *Aspergillus spp*. was found to be most likely, though the presence of other non-pigmented hyphae producing fungi cannot be excluded.[4]

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JPC Diagnosis:
Guttural pouch: Eustachitis and arteritis, necrotizing, chronic, diffuse, severe, with numerous fungal hyphae, fibrin thrombi, and infarction of skeletal muscle and nerves.

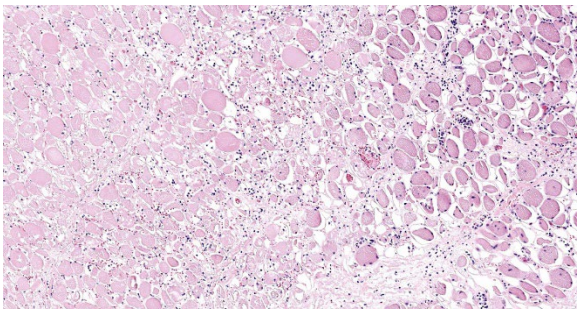

Figure 3-5. Guttural pouch horse. Infarcted skeletal muscle (at left) adjacent to the inflamed internal carotid artery. Viable muscle and inflammatory cells are present at right.) (HE, 75X)

JPC Comment:
The exact tissue identification for Case 3 is subtle, though the presence of a large blood

vessel next to nerves, deeper muscle and a thin layer of squamous epithelium is suggestive of the equine guttural pouch (Figure 1-1). That stated, the magnificent fungal arteritis that the contributor has shared with us quickly pares down the differential list. Like the previous case, fungal elements are also a major diagnostic feature though the distribution and tissue response is quite different from Case 2. GMS and PAS stains were less remarkable for this case, though this may simply reflect luck and plane of section for the slides we selected. Conference participants felt that the features on H&E were highly suggestive of *Aspergillus*, though they did remark on the pigmentation present along the guttural pouch mucosa and whether this might be related to another fungal species instead (Figure 3-7).

As the contributor notes, guttural pouch mycosis is often caused by *Aspergillus* species and the large fungal mats along the surface of and expanding into large blood vessels is a helpful diagnostic feature.[2] While hemorrhage and epistaxis are common clinical manifestations of guttural pouch mycosis, they are not the only significant ones to consider. Marked guttural pouch inflammation can extend into adjacent structures to include the vagosympathetic trunk, bones , middle ear, brain, or

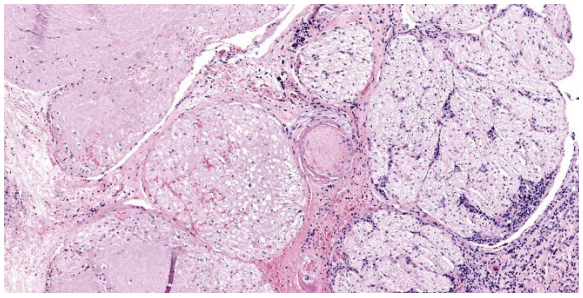

Figure 3-6. Guttural pouch horse. There is infarction (left) of bundles of nerves, with multifocal inflammation and hemorrhage. A thrombosed arteriole is present in the center of this field. (HE, 175X)

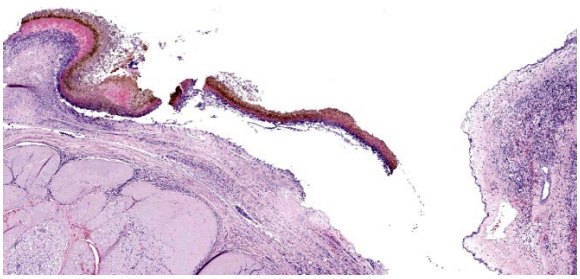

Figure 3-7. Guttural pouch, horse. A large mat of fungal hyphae extends along the ulcerated surface of the guttural pouch as well. (HE, 39X)

joints.[2] Dysphagia, Horner's syndrome, and laryngeal hemiparesis have all been reported secondary to guttural pouch mycosis.[2] Although the animal in the present case did not have reported neurologic deficits at the time of euthanasia, there is marked necrotizing arteritis that spills over to adjacent skeletal muscle (myositis) and nerves (neuritis) quite spectacularly. For a similar case from the WSC archives, see Conference 21, Case 2, 2012-2013.

Other diseases of the guttural pouch to consider include guttural pouch tympany, guttural pouch empyema, temporohyoid osteoarthropathy, and neoplasia.[2,11] Tympany is uncommon and is seen in young horses secondary to congenital conformational abnormalities or inflammation that leads to retention of air and expansion of the guttural pouch. Empyema is seen secondary to respiratory infection, most commonly *Streptococcus equi*, and purulent nasal discharge (not hemorrhage) is the typical finding. Neoplasia of the guttural pouch is rare, but squamous cell carcinoma has been reported.[2] Temporohyoid osteoarthropathy reflects remodeling of either/both the stylohyoid bone and petrous temporal bone; this may occur secondary to otitis media, respiratory infection, or degenerative conditions.[11] Diagnostic imaging, to include, radiography, endoscopy, and computed tomography are helpful at distinguishing these diseases amid a complex anatomical landscape. [11]

References:

1. Brandão ISL, Oliveira-Moraes H, Souza Motta CM, Oliveira NTD, Magalhães O. Elastin increases biofilm and extracellular matrix production of *Aspergillus fumigatus*. *Brazilian Journal of Microbiology*. 2018; 49: 675–682.
2. Caswell JL, Williams KJ. Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. St. Louis, MO: Elsevier; 2016:480-481.
3. Dobesova O, Schwarz B, Velde K, Jahn P, Zert Z, Bezdekova B. Guttural pouch mycosis in horses: a retrospective study of 28 cases. *Veterinary Record*. 2012; 171: 561-561.
4. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clinical Microbiology Reviews*. 2011; 24: 247-280.
5. Kipar A, Frese K. Hypoglossal neuritis with associated lingual hemiplegia secondary to guttural pouch mycosis. *Veterinary Pathology*. 1993; 30: 574-576.
6. Lepage OM, Perron MF, Cadoré JL. The mystery of fungal infection in the guttural pouches. *The Veterinary Journal*. 2004; 168: 60-64.
7. Lepage OM. Challenges associated with the diagnosis and management of guttural pouch epistaxis in equids. *Equine Veterinary Education*. 2016; 28: 372-378.
8. Ludwig A, Gatineau S, Reynaud MC, Cadoré JL, Bourdoiseau G. Fungal isolation and identification in 21 cases of guttural pouch mycosis in horses (1998–2002). *The Veterinary Journal*. 2005; 169: 457-461.
9. Nadkarni T, Goel A. Aspergilloma of the brain: an overview. *Journal of Postgraduate Medicine*. 2005; 51: 37-41.
10. Ninomiya H, Kuwano A. Microvasculature of the Guttural Pouch Mucosa and Relationship With the Internal Carotid Artery in Horses. *Journal of Equine Veterinary Science*. 2019; 74: 84-89.
11. Pollock PJ. Diagnosis and management of guttural pouch mycosis. *Equine Veterinary Education*. 2007; 19: 522-527.
12. Thomas-Cancian A, Ségard-Weisse E, Drumond B, Cadoré J-L. Diagnostic Imaging of Diseases Affecting the Guttural Pouch. *Veterinary Sciences*. 2023; 10(8):525.

CASE IV:

Signalment:
9-year-old, gelding, warmblood, horse, Equus caballus.

History:
This horse presented with a 1-year history of a bleeding rostral nasal mass.

Gross Pathology:
N/A

Microscopic Description:
Slide A: Examined are three sections of the nasal vestibule lined by a multifocally eroded stratified squamous epithelium that has numerous individualized neutrophils percolating throughout (neutrophilic exocytosis). The ves

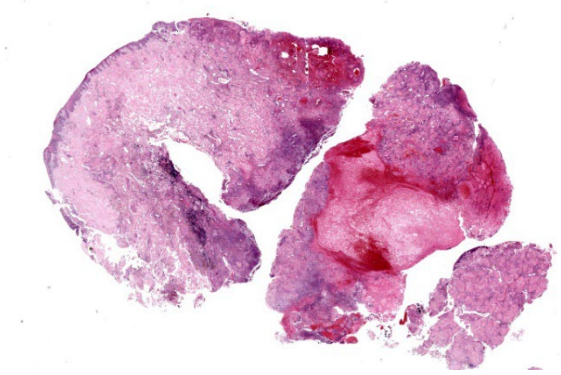

Figure 4-1. Nasal mucosa, horse. Multiple hemorrhagic and ulcerated fragments of nasal mucosa and underlying lamina propria are submitted. (HE, 5X).

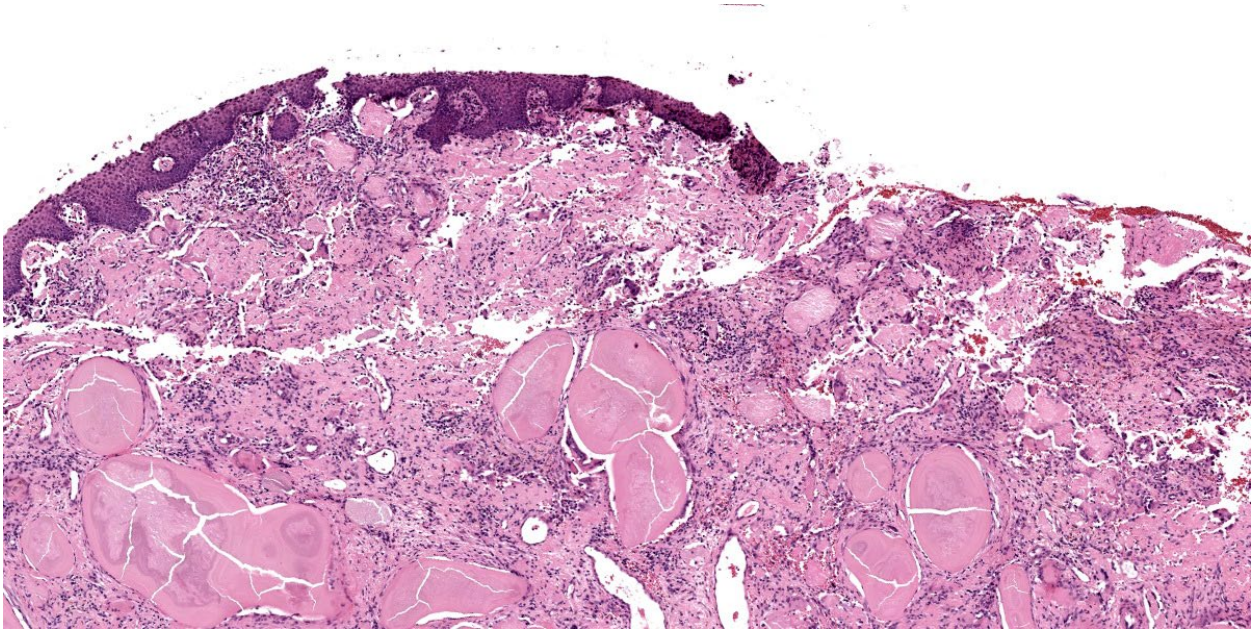

Figure 4-2. Nasal mucosa, horse. There is segmental ulceration of the nasal mucosa. Within the underlying lamina propria, there are aggregates of amyloid. (HE, 60X).

tibular lamina propria is markedly expanded by numerous, large, multinodular deposits of pale eosinophilic, amorphous, smudgy, extracellular material (amyloid). Associated with this amyloid deposition are thick anastomosing bands of fibrous connective tissue that are punctuated by plump reactive fibroblasts and branching small-caliber vessels lined by plump endothelium (granulation tissue). Numerous neutrophils, lymphocytes, plasma cells, macrophages, and multinucleated giant cells percolate throughout this granulation tissue and are interspersed between amyloid deposits. The vestibular lamina propria is additionally expanded by large pools of brightly eosinophilic amorphous to fibrillar material (necrosis) admixed with numerous extravasated red blood cells (hemorrhage), degenerate leukocytes, and hemosiderin and hematoidin-laden macrophages.

Congo red: The pale eosinophilic, amorphous extracellular material stains orange-red (congophilic) and exhibits apple-green birefringence under polarized light.

Contributor's Morphologic Diagnosis:
Nasal vestibule: Severe, chronic, locally extensive amyloidosis with neutrophilic and histiocytic nasal vestibulitis, granulation tissue, epithelial erosion, multifocal necrosis, and chronic-active hemorrhage.

Contributor's Comment:
Equine nasal amyloidosis is an uncommon manifestation of local amyloidosis. Its most common presentation is a single or multiple, often ulcerated, mass-like lesions in the rostral nasal cavity or nasal vestibule; however, amyloidosis can occur in any portion of the nasal cavity and can present as diffuse swelling and not a discrete mass.[1,5-8,12] Concurrent cutaneous, conjunctival, and/or corneal amyloidosis has also been documented.[5,12] Clinical signs often include epistaxis and larger masses may lead to respiratory difficulty and exercise intolerance.[1,5-8] Grossly, the masses caused by amyloidosis can be soft to firm, are often ulcerated, and can have a smooth to waxy appearance.[5-8] Histologic findings are similar amongst all described cases with the presence

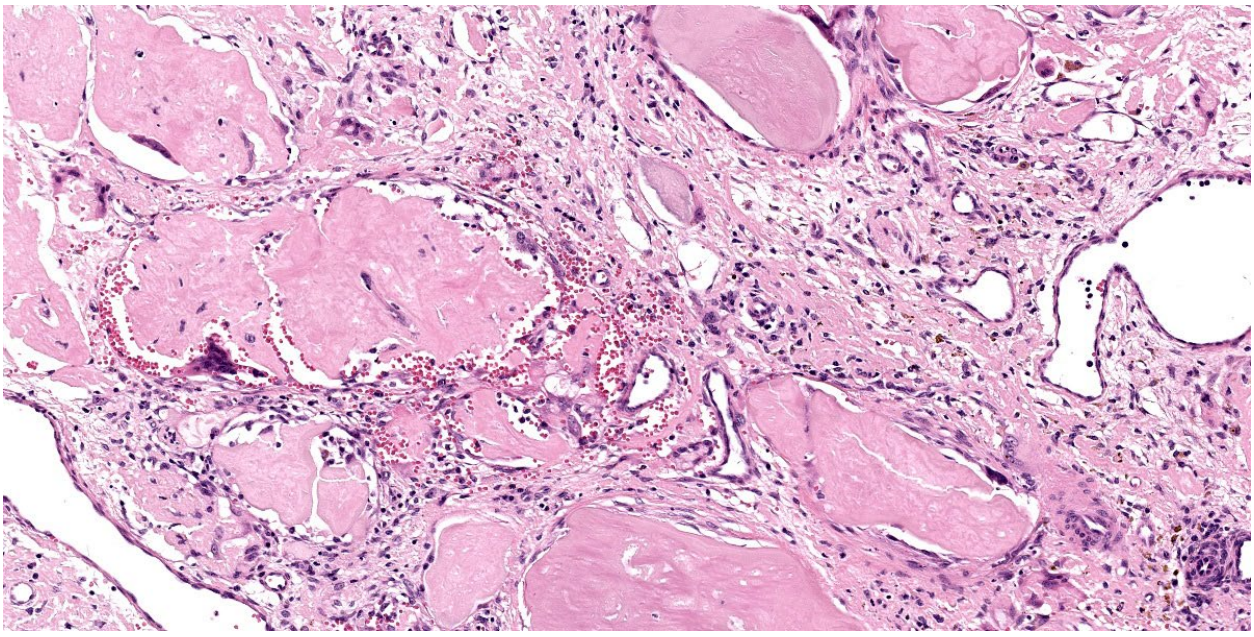

Figure 4-3. Nasal mucosa, horse. High magnification of aggregates of amyloid, which is surrounded by macrophages and foreign body macrophages. (HE, 157X)

of eosinophilic, amorphous material, often associated with granulation tissue, hemorrhage, lymphoplasmacytic histiocytic inflammation, multinucleated giant cells, and occasionally mineralization. The histopathologic findings in this case were similar to those in previous reports.

Amyloid is an extracellular, hyalinized, proteinaceous material composed of polypeptides arranged in beta-pleated sheets forming fibrillar proteins.[4] Due to the conformation of amyloid, it is resistant to degradation leading to accumulation within tissues. Thirty different types of types of amyloid fibril proteins have been discovered, but only nine have been studied in domestic animals and only two, amyloid light chain (AL) and amyloid A (AA), have been described in horses.[1,2,9] Equine nasal amyloidosis is caused by accumulation of the amyloid light chain (AL) type.[1]

Diagnosis of amyloid is commonly achieved through histochemical staining of with Congo Red, which results in orange to red coloration that exhibits apple-green birefringence when observed under polarizing light.[4] Additionally, amyloid light chain (AL) and amyloid A (AA) can be differentiated using a pre-treatment with potassium permanganate. Amyloid A (AA), when pre-treated with potassium permanganate will lose its affinity for Congo red and subsequently lacks the typical birefringent properties.[10]

Differentials on gross examination include fungal granuloma, ethmoid hematoma, habronemiasis, sarcoids/soft tissue sarcomas, and maxillary dental tumors that extend into the nasal cavity; however, all of these differentials can be differentiated histologically by the absence of amyloid.

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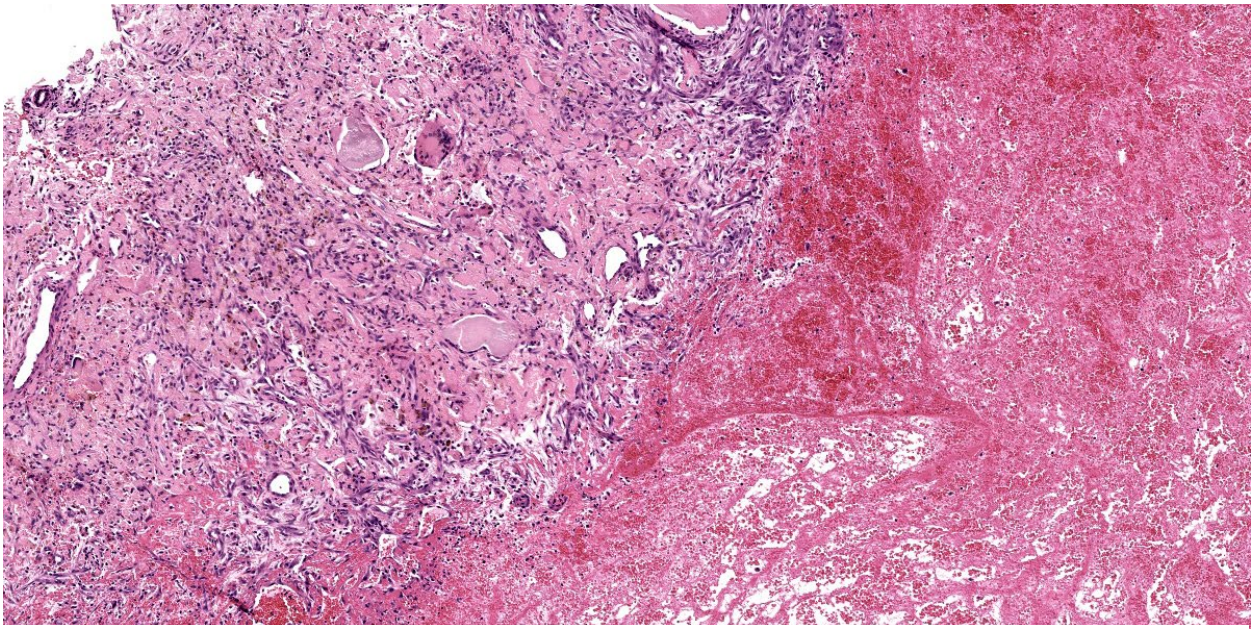

Figure 4-4. Nasal mucosa, horse. In some fragments, amyloid is enmeshed in hemorrhagic granulation tissue. (HE, 78X)

JPC Diagnosis:
Nasal muscosa: Amyloidosis, multifocal, severe, with fibrin thrombi, chronic hemmorrhage, and ulceration.

JPC Comment:
Conference 1 concludes with a relatively straightforward case. The thick squamous epithelial layer that lacks underlying hair follicles helps to place the tissue as the nostril (specifically within the nasal vestibule). The contributor provides a nice description of the features of this case and reviews the salient points of equine amyloidosis as well. The diagnosis of amyloidosis was readily confirmed as the eosinophilic extracellular material was strongly congophilic and sharply birefringent under polarized light. There was discussion among conference participants about the degree of hemorrhage and fibrin present in this case. The major takeaway was that these changes were the direct result of amyloid being deposited within the walls of blood vessels.

This case is an example of immunoglobulin-derived (AL) amyloidosis which is rare in animals though relatively common in humans.[11] Other reported causes of primary amyloidosis include immune dyscrasias such as extramedullary plasmacytomas and multiple myeloma where overproduction of immunoglobulin light chains (or fragments) by plasma cells coalesce to form insoluble fibrils.[1,11] One case of systemic AL amyloidosis secondary to multiple myeloma in a horse has been described to date.[3] In contrast, secondary (reactive; AA) amyloidosis is more common in veterinary species and familial forms of AA amyloidosis have also been described. Reactive amyloidosis occurs secondary to chronic inflammatory stimulus and production of serum amyloid A protein, or less commonly, due to nonimmunocyte dyscrasia or idiopathic causes.[11] Renal and/or hepatic involvement with deposition of extracellular amyloid A protein disrupts normal function and eventually may cause organ failure; others tissues may similarly be affected.[11] This is seen in both domestic animals as well as captive wildlife species such as flamingos and cheetahs among many

others. Familial forms of AA amyloidosis occur in Shar Pei dogs and Abyssinian cats, though other breeds have also been identified.[11]

Amyloidosis has been a frequent feature in slide conferences over the years, though horses are not as well represented as the dog in this regard. Ocular amyloid secondary to recurrent uveitis ('moon blindness') was covered in Conference 7, Case 3, 2018-2019 and represents AA amyloid. Cutaneous amyloidosis in the horse represents a localized AL amyloidosis that is distinct from the nasal form.

References:

1. Caswell JL, Williams KJ. Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. St. Louis, MO: Elsevier; 2016:483.
2. Johnson KH, Westermark P, Sletten K, O'brien TD. Amyloid proteins and amyloidosis in domestic animals. Amyloid. 1996 Jan 1; 3(4):270-89.
3. Kim DY, Taylor HW, Eades SC, Cho DY. Systemic AL amyloidosis associated with multiple myeloma in a horse. *Vet Pathol*. 2005 Jan;42(1):81-4.
4. Kumar V, Abbas AK, Aster JC. Diseases of the Immune System. Kumar V, Abbas AK, Aster JC, eds. In: *Robbins and Cotran Pathologic Basis of Disease.* 10th ed. Philadelphia, PA: Elsevier Saunders; 2021:259-264.
5. Mould JR, Munroe GA, Eckersall PD, Conner JG, McNeil PE. Conjunctival and nasal amyloidosis in a horse. *Equine Veterinary Journal*. 1990 Sep;22(S10):8-11.
6. Nappert G, Vrins A, Doré M, Morin M, Beauregard M. Nasal amyloidosis in two quarter horses. *The Canadian Veterinary Journal*. 1988 Oct;29(10):834.
7. Portela R, Dantas A, Melo D, Marinho J, Neto P, Riet-Correa F. Nasal Amyloidosis in a Horse. *Brazilian Journal of Veterinary Pathology*. 2012; 5: 86-88.
8. Shaw DP, Gunson DE, Evans LH. Nasal amyloidosis in four horses. *Veterinary Pathology*. 1987 Mar;24(2):183-5.
9. Sipe JD, Benson MD, Buxbaum JN, et al. Amyloid fibril protein nomenclature: 2012 recommendations from the Nomenclature Committee of the International Society of Amyloidosis. *Amyloid*. 2012;19(4):167-70.
10. Van Rijswijk MH, van Heusden CW. The potassium permanganate method. A reliable method for differentiating amyloid AA from other forms of amyloid in routine laboratory practice. *Am J Pathol*. 1979;97(1):43-58.
11. Woldemeskel M. A concise review of amyloidosis in animals. *Vet Med Int.* 2012;2012:427296.
12. Østevik L, Gunnes G, de Souza GA, Wien TN, Sørby R. Nasal and ocular amyloidosis in a 15-year-old horse. Acta Vet Scand. 2014 Aug 27;56(1): 50.