Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #14 — 3 January 2024

CASE I:

Signalment:
Adult female Norwegian Fjord horse (*Equus caballus*)

History:
This horse was born near Hanover, Germany. It was kept solely in northern Germany and has never been abroad. It was presented to the local veterinarian due to lameness. During the clinical examination, the veterinarian detected multiple cutaneous nodules. Two representative samples (elbow and periorbital skin) were surgically excised, fixed in 10% formalin, and submitted for histological examination.

Gross Pathology:
In both samples, the haired skin showed focal alopecia, focal ulceration, and poorly demarcated lumps with a size of up to 1.2 cm in diameter.

Laboratory Results:
Genome fragments of *Leishmania* sp. were detected in the cytoplasm of multinucleated giant cells and macrophages using *in situ* hybridization.

Microscopic Description:
Haired skin: The superficial and deep dermis shows a multifocal to coalescing periadnexal and a multinodular to coalescing severe inflammatory cell infiltrate consisting of myriad

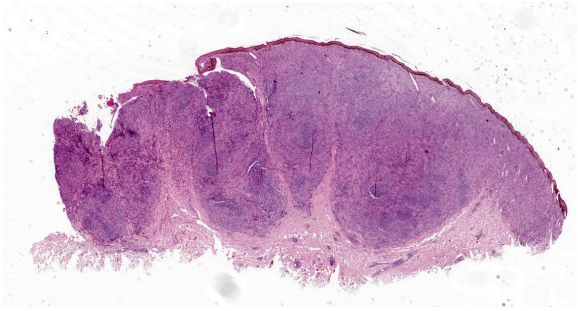

Figure 1-1. Haired skin, horse. A submitted section of skin contains coalescing inflammatory nodules within the dermis. (HE, 5X)

macrophages admixed with low to moderate numbers of plasma cells and lymphocytes, as well as single eosinophilic granulocytes. Randomly distributed throughout the whole lesion, there are multinucleated giant cells with a size of up to 50 µm in diameter, containing up to 10 predominantly peripherally located nuclei (Langhans type). Occasionally in the extracellular space, but predominantly and frequently within the cytoplasm of macrophages, there are multiple protozoal structures (amastigotes) with a diameter of 2 to 3 µm often surrounded by a clear halo suggestive of a clear parasitophorous vacuole. The round organisms are characterized by a clear cytoplasm and a single eccentric nucleus of approximately 1-2 µm diameter. Furthermore, in the superficial dermis, fibroblastic connective tissue is moderately hyperplastic. In addition, low numbers of macrophages with brown-black intracytoplasmic pigment are present in

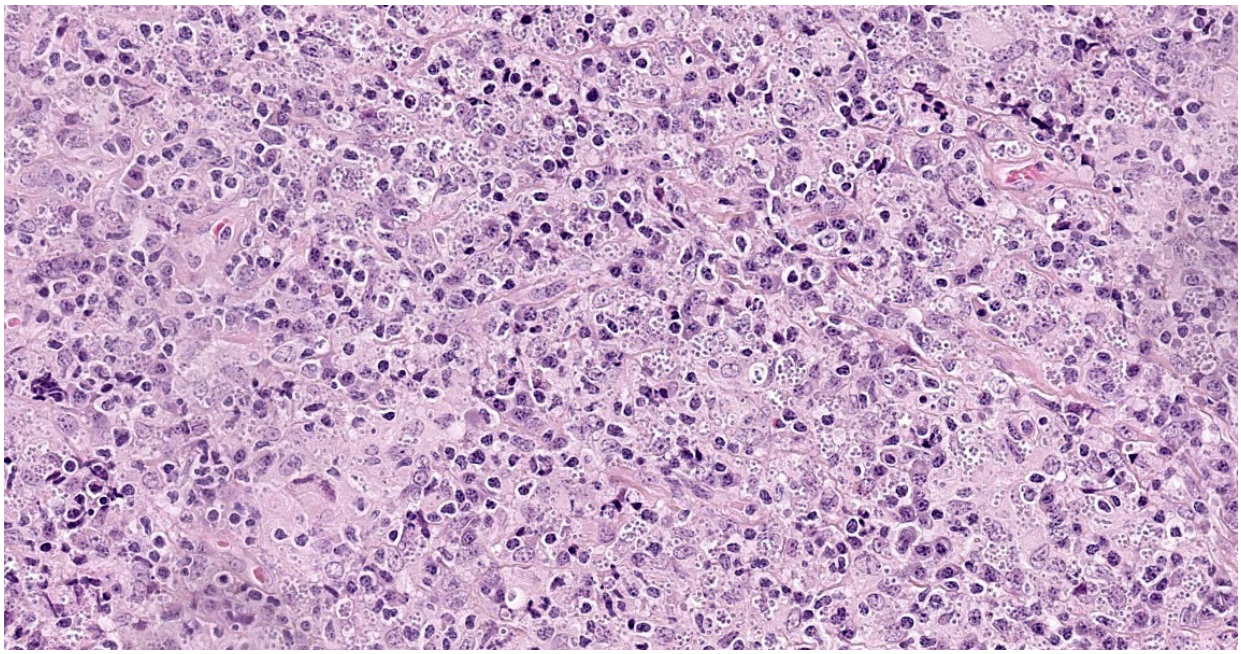

Figure 1-2. Haired skin, horse. Inflammatory nodules are composed primarily of histiocytes with large numbers of lymphocytes scattered throughout, often in aggregates. Macrophages contain multiple round protozoal amastigotes within their cytoplasm. (HE, 381X)

the subepidermal tissue (pigmentary incontinence). Occasionally, hair shafts and sebaceous glands are missing.

Contributor's Morphologic Diagnosis:
Haired skin: Dermatitis, chronic, severe, focally extensive, periadnexal to diffuse, granulomatous, with multinucleated giant cells (Langhans type) and intrahistiocytic protozoal amastigotes, equine.

Contributor's Comment:
Leishmaniasis is a globally occurring infectious disease in humans and animals caused by obligate intracellular protozoan parasites of the genus *Leishmania*. The disease is endemic in Southern Europe, North Africa, South and Central America, and Asia.[13] In Europe, it occurs mainly in the Mediterranean region. Due to increased travel activities with companion animals, the number of animals infected with *Leishmania* sp. in Northern Europe has increased. Additionally, since the 1980s, the sandflies that transmit leishmaniasis have increasingly appeared in Northern Europe, especially in Germany, possibly due to climate change.[1,8] This has initiated the discussion about the occurrence of autochthonous cases of leishmaniasis in Europe north of the Alps.[7]

Leishmania sp. require both arthropods and vertebrates for their development. Sandflies such as *Phlebotomus* sp. and *Lutzomyia* sp. play an important role as vectors of *Leishmania* sp. in Europe and South and Central America.[1] The amastigote form of *Leishmania* sp. is found in the cells of the mononuclear phagocyte system of vertebrates, predominantly in liver, spleen, bone marrow, and lymph nodes. Occasionally, the organisms are detected in other leukocytes, endothelial cells, fibroblasts, and neoplastic cells.[2,10] After ingestion of macrophages containing *Leishmania* amastigotes by a female arthropod during a blood

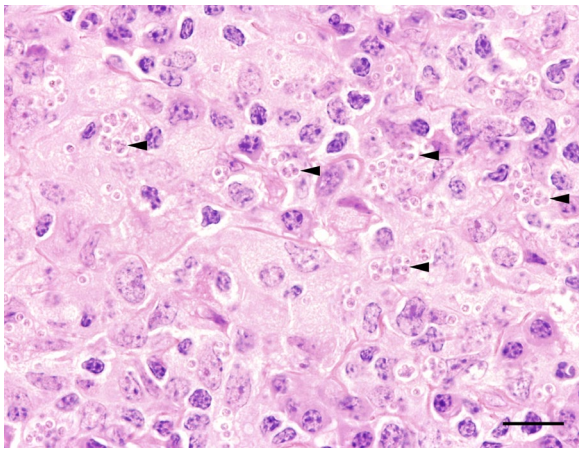

Figure 1-3. Haired skin, horse. Amastigotes are characterized by a clear cytoplasm and a single eccentric nucleus of approximately 1-2 µm diameter. (*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany. http://www.tiho-hannover.de/ kliniken-institute/institute/institut-fuer-pathologie)(HE, 600X)

meal, the amastigotes are transformed into promastigotes in the intestine and subsequently enter the biting mouthparts of the arthropod.[13,18] Transmission of the pathogen through either mechanical vectors such as bloodsucking insects and ticks or a direct and vertical transmission has also been reported.[6,11,18]

There are more than 50 species of *Leishmania* based on a complex classification scheme. The species of *Leishmania* vary by region, and different species are thought to cause different disease manifestations.[1] Currently,three different *Leishmania* sp. have been confirmed to cause cutaneous leishmaniasis in horses in Europe: *Leishmania braziliensis, Leishmania siamenis,* and *Leishmania infantum*.[14]

In animals and humans, leishmaniasis can cause three different disease patterns: cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis.[13] Clinical signs of equine cutaneous leishmaniasis are usually less severe than in other host species and the disease in equids is typically not life-threatening. The most common lesions are single or multiple papules or nodules characterized by hyperkeratosis with prominent scaling, alopecia, depigmentation, and ulceration in the areas where sand flies commonly feed, including periorbital skin, muzzle, neck, pinnae, scrotum, and legs.[13,14]

Histologically, a nodular to diffuse lymphohistiocytic dermatitis is a consistent feature of cutaneous leishmaniasis.[14] The inflammation mainlyvoccurs in one of the following three patterns: granulomatous perifolliculitis, superficial and deep perivascular dermatitis, or interstitial dermatitis.[13] However, the burden of the protozoa may vary. This reflects the dynamics of the interaction between the host and the pathogen. Moreover, the incubation time of cutaneous leishmaniasis varies from several months up to seven years.[13] Spontaneous regression and recurrence of cutaneous leishmaniasis have been reported in cats and horses.[15]

Not all infected animals become ill. In infected animals, leishmania-specific and nonspecific polyclonal B-cell activation may occur, which can lead to deposition of immune complexes and formation of autoantibodies against erythrocytes, platelets, and nuclear antigens. In diseased animals, a Th2-immune response predominates. In contrast, a type IV hypersensitivity reaction and a Th1-immune response are found in asymptomatic carriers or in animals after successful chemotherapy.[13]

Due to the variety of possible lesions and the various infectious and noninfectious

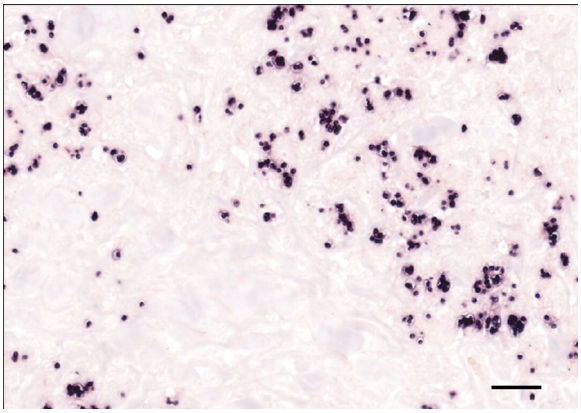

Figure 1-4. Haired skin, horse. In-situ hybridization against *Leishmania* sp. (*Photo courtesy of:* Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany.) (anti-*Leishmania* ISH.)

differential diagnoses, the diagnosis of leishmaniasis requires direct cytologic or histological identification of amastigotes in the cytoplasm of macrophages, best visible by Giemsa staining.[13] Furthermore, pathogen detection is possible by immunohistological, culture, and molecular (PCR and *in situ*-hybridization) detection methods.[3,12] A positive serological test indicates that an infection has occurred, but does not imply that the parasite is still present in the infected host.[3]

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JPC Diagnosis:
Haired skin: Dermatitis, granulomatous, multifocal to coalescing, severe, with numerous intrahistiocytic amastigotes.

JPC Comment:
The contributor provides an excellent overview of leishmaniasis, a zoonotic disease most consistently found in humans and dogs and which has not historically been considered endemic in the United States. In recent decades, cutaneous and visceral leishmaniasis has been reported among certain canine breeds in the United States and Canada, and cases of cutaneous leishmaniasis have been reported in U.S. horses without a history of travel to endemic areas.[16,17]

The first documented report of a domestically acquired case of equine leishmaniasis occured in a Morgan horse mare in Florida in 2011.[4,16] As noted by the contributor, the canonical mode of transmission of *Leishmania* sp. is via the bite of sandflies from the genera *Phlebotomus* and *Lutzomyia*, and Florida is host to two possible sand fly vectors, *Lutzomyia shannoni* and *Lutzomyia vexator*.[17] *Lutzomyia shannoni* is known to feed on both humans and other mammals, and it has been experimentally shown capable of infection with *Leishmania infantum* when fed on infected dogs; however, it is not known if the infective amastigote forms develop within *Lutzomyia shannoni*.[17] The search for a competent sandfly vector may be superfluous, however, as recent research has demonstrated that the species of *Leishmania* isolated from infected Florida horses, *Leishmania martiniquensis*, is likely transmitted by *Culicoides* spp. biting midges.[5,9,17] Due to the presence of competent vectors in the United States and a small but growing number of confirmed cases of domestically-acquired equine leishmaniasis, the American Association of Equine Practitioners recently released guidelines on the diagnosis and treatment of the disease.[4]

Equine cutaneous leishmaniasis commonly presents as solitary or multiple ulcerated nodules on the head, pinnae, scrotum, legs, and neck.[17] To date, equine leishmaniasis has been confined to the skin and the more serious and

potentially fatal visceral form of the disease seen in dogs and humans has not been reported in horses; most clinical equine cases spontaneously resolve in 3-6 months and the prognosis is therefore generally good.[4,17]

This week's conference was moderated by Joint Pathology Center Director and equestrian enthusiast LTC(P) Sherri Daye. LTC(P) Daye led conference participants in a discussion of the differences among molecular diagnostic techniques such as *in situ* hybridization and immunohistochemistry before moving to differential diagnoses. Participants were evenly split between *Histoplasma capsulatum* and *Leishmania* spp. as the likely etiologic agent of this lesion, and significant conference discussion focused on how to differentiate between the two organisms when presented with only an H&E section. Participants noted that leishmaniasis typically presents with abundant plasmacytic inflammation, which is absent in this case. In addition, the clear capsule-like rings surrounding the organisms are unusual in leishmaniasis and further contributed to the diagnostic confusion.

Though cutaneous leishmaniasis has not historically been top of mind in the United States and Northern Europe, the incidence of this disease is likely to increase as competent vectors, driven by migratory movements of people and animals and climate change, move into newly favorable geographic niches.

References:

1. Akhoundi M, Kuhls K, Cannet A, et al. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLOS Negl Trop Dis*. 2016;10**:**e0004349.
2. Albanese F, Poli A, Millanta F, et al. Primary cutaneous extragenital canine transmissible venereal tumour with *Leishmania*-laden neoplastic cells: a further suggestion of histiocytic origin? *Vet Dermatol.* 2002;13**:**243–246.
3. Albuquerque A, Campino L, Cardoso L, et al. Evaluation of four molecular methods to detect *Leishmania* infection in dogs. *Parasit Vectors*. 2017;10**:**57.
4. American Association of Equine Practitioners. *AAEP Infectious Disease Guidelines:Equine Cutaneous Leishmaniasis*. 2022. Available at https://aaep.org/document/equine-cutaneous-leishmaniasis.
5. Becvar T, Vojtkova B, Siriyasatien, et. al. Experimental transmission of *Leishmania* (*Mundia*) parasites by biting midges (Diptera: Ceratopogonidae). *PLos Pathog*. 2021;17(6):e1009654.
6. Bogdan C, Schönian G, Bañuls AL, et al. Visceral leishmaniasis in a German child who had never entered a known endemic area: case report and review of the literature. *Clin Infect Dis*. 2001;32**:**302-306.
7. Dujardin JC, Campino L, Canavate C, et al. Spread of vector-borne diseases and neglect of leishmaniasis, Europe. *Emerg Infect Dis*. 2008;14**:**1013–1018.
8. Harms G, Schönian G, Feldmeier H. Leishmaniasis in Germany. *Emerg Infect Dis*. 2003;9:872-875.
9. Kaewmee S, Mano C, Phanitchakun T, et al. Natural infection with *Leishmania (Mundia) martiniquensis* supports *Culicoides peregrinus* (Diptera: Ceratopogonidae) as a potential vector of leishmaniasis and characterization of a *Crithidia* sp. isolated from the midges. *Front Microbiol*. 2023;14:1-17.
10. Kegler K, Habierski, A, Hahn, H, et al. Vaginal canine transmissible venereal tumour associated with intra-tumoural *Leishmania* spp. amastigotes in an asymptomatic female dog. *J Comp Pathol.* 2013;149:156-161.

11. Koehler K, Stechele M, Hetzel U, et al. Cutaneous leishmaniosis in a horse in southern Germany caused by *Leishmania infantum*. *Vet Parasitol*. 2002;109:9-17.
12. Maia C, Campino L. Methods for diagnosis of canine leishmaniasis and immune response to infection. *Vet Parasitol*. 2008;158:274–287.
13. Mauldin EA, Peters-Kennedy J. Integumentary System. In: Maxie G, ed. *Jubb, Kennedy and Palmer`s Pathology of Domestic Animals*. 6th ed. Vol 1. Elsevier; 2016:240.
14. Mhadhbi M, Sassi A. Infection of the equine population by Leishmania parasites. *Equine Vet J*. 2020;52:28-33.
15. Müller N, Welle M, Lobsiger L, et al. Occurrence of *Leishmania* sp. in cutaneous lesions of horses in Central Europe. *Vet Parasitol*. 2009;166:346-351.
16. Petersen CA, Barr SC. Canine leishmaniasis in North America: emerging or newly recognized? *Vet Clin North Am Small Anim Pract*. 2009;39(6):1065-1074.
17. Reuss SM. Review of equine cutaneous leishmaniasis: not just a foreign animal disease. *AAEP Proceedings*. 2013;59:256-260.
18. Serafim TD, Coutinho-Abreu IV, Dey R, et al. Leishmaniasis: the act of transmission. *Trends Parasitol*. 2021;37: 976-987.

CASE II:

Signalment:
10-year-old, intact female Tennessee Walking Horse (*Equus caballus)*

History:
The patient had a history of a mass in the nose and troubled breathing. A mass was confirmed on radiographs and via endoscopic examination.

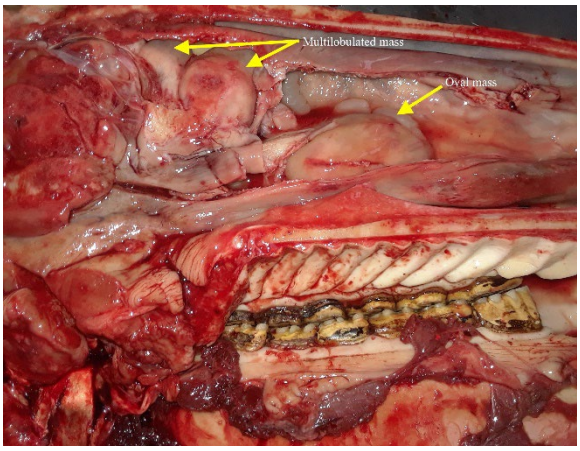

Figure 2-1. Nasal turbinates, horse. There are multiple firm to hard, cream-colored masses. in the ventral nasal concha there was an oval 7cm x 4 cm x 2 cm mass with a linear cobblestone appearance in the rostral aspect of the mass (*Photo courtesy of:* Tuskegee University, College of Veterinary Medicine, Department of Pathobiology, https://www.tuskegee.edu/programs-courses/colleges-schools/cvm)

Gross Pathology:
The carcass was in adequate body condition (5.5/9). A mild creamy yellow viscous mucus-like nasal discharge was present. Saggital sectioning of the head revealed multiple firm to hard cream-colored masses. In the ventral nasal concha there was an oval 7 cm x 4 cm x 2 cm mass with a linear cobblestone appearance in the rostral aspect of the mass. Invading the dorsal concha was another irregular multilobulated mass, measuring 5.5 cm x 4 cm x 5 cm, which contained several tan to gray soft, rounded cyst-like fluid-filled structures. The left ethmoid turbinates contained a similar 5 cm x 5 cm mass.

On the surface of the right caudal lung lobe at the caudal border, there was a 6.5 cm x 7 cm, slightly elevated, cream-colored, gelatinous, irregular, focally extensive area with short thick projections. The surface of these projections had multifocal to coalescing pinpoint to

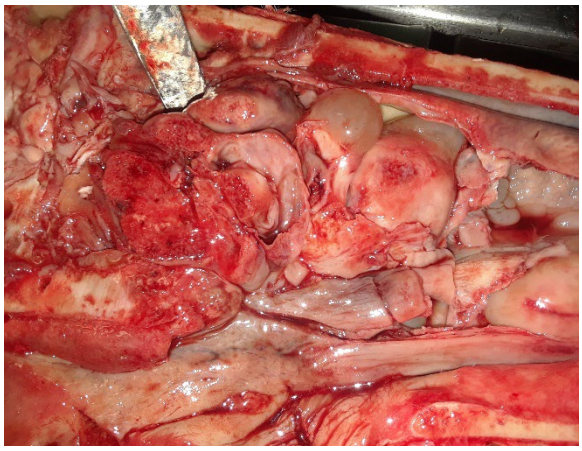

Figure 2-2. Nasal turbinates, horse. The sample is from an oval 7 cm x 4 cm x 2 cm mass with a linear cobblestone appearance. (*Photo courtesy of:* Tuskegee University, College of Veterinary Medicine, Department of Pathobiology)

2 cm red foci, and cut section of this area revealed two round, semi-firm, opaque yellow nodules that extended 1 cm into the lung parenchyma. Twelve centimeters cranially to the aforementioned lesion there was another similar (cream colored, gelatinous with pinpoint red discolorations) area measuring 1 cm x 0.5 cm.

Microscopic Description:
Nasal turbinates: Infiltrating and effacing >90% of the nasal turbinates are multifocal to coalescing nodular foci of inflammation centered on myriad dematiaceous mycotic agents.The inflammation consists of numerous macrophages with Langhans and foreign body type macrophages, numerous plasma cells, fewer lymphocytes, and random aggregates of neutrophils, which are surrounding numerous round to oval, 30-60 µm spherules with a 2-3 µm clear to brown stained capsule. These structures are frequently filled with several 3-6 µm clear to lightly basophilic rounded structures that are clustered together. The mycotic agents are frequently within multinucleated giant cells. Rarely, the organisms form 5-6 µm septate hyphae which were observed with Periodic Acid-Schiff (PAS) stain.

Contributor's Morphologic Diagnosis:
Nasal cavity: Severe multifocal granulomatous rhinitis with intralesional dematiaceous fungi (Phaeohyphomycosis).

Contributor's Comment:
On macroscopic examination it was thought that the lesion in the nose was a neoplastic process; however, histologic examination revealed the presence of dematiaceous (pigmented) mycotic agents associated with a granulomatous inflammatory process.

Dematiaceous fungi are naturally pigmented molds whose hyphae and conidia contain melanin.[2] They are ubiquitous and can be found in soil, plants, and organic debris.[2]

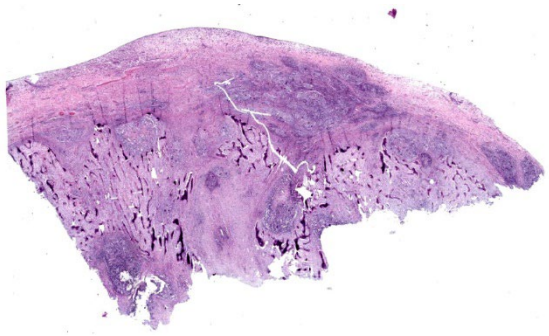

Figure 2-3. Nasal turbinate, horse. There is loss of normal turbinate architecture. The submucosa is expanded and the lytic turbinate bone is largely effaced by large areas of inflammation. (HE, 9X)

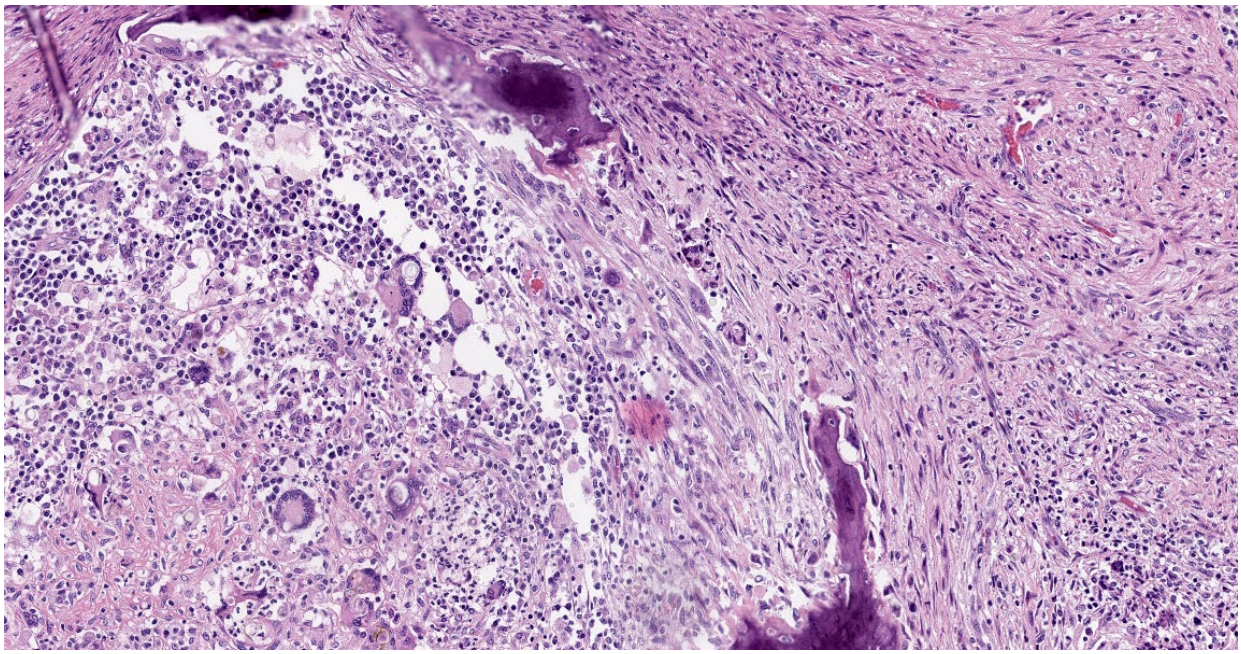

Figure 2-4. Nasal turbinate, horse. There is marked granulomatous inflammation with multinucleated foreign body macrophages against a background of fibrosis and lytic turbinate bone. (HE, 190X)

Melanin is what gives the dematiaceous fungi their pigmentation and it is believed to contribute to the organism's ability to elude the host immune response.[5,6] Melanin contributes to the evasion of the immune response by blocking the effects of hydrolytic enzymes on the cell wall and scavenging free radicals released by phagocytic cells during the oxidative burst.[6] There are several clinical presentations of dematiaceous molds, including eumycetoma, chromoblastomycosis,and phaeohyphomycosis. Our case is an example of phaeohyphomycosis.

Phaeohyphomycosis is an infection caused by dematiaceous (pigmented) fungi which typically affects immunocompromised individuals, but can also affect those that are immunocompetent.[2,5] Clinically, infections range from superficial colonization to systemic abscess formation and dissemination.[6] The fungi can be unicellular as well as yeast-like, but the key feature is the melanin pigment which distinguishes them from other fungi that may have similar morphology.[6] There are many causes of phaeohyphomycosis; the ones most commonly reported in equines are *Alternaria* sp., *Dreschslera* sp., and *Curvularia* sp.[1]

The presence of pigmentation on histopathology warrants a diagnosis of phaeohyphomycosis. In cases with dematiaceous fungi that are poorly pigmented, Fontana-Masson can be used to accentuate the melanin.[5] As with other mycotic agents, Periodic Acid-Schiff (PAS) and Grocott-Gomori methenamine silver (GMS) are common stains used to visualize the agents. Culture as well as molecular analysis can also be used to further narrow the identification down to the species level.[6]

Contributing Institution:
Tuskegee University
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https://www.tuskegee.edu/programs-courses/colleges-schools/cvm

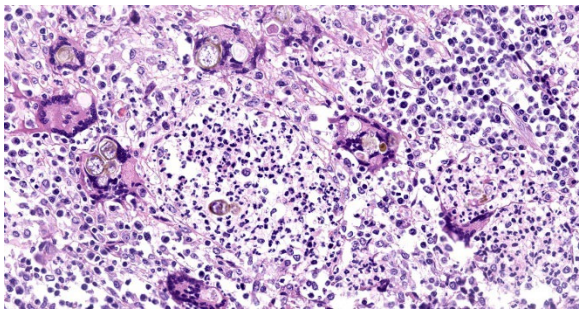

Figure 2-5. Nasal turbinate, horse. Pigmented hyphae and large round "yeast-like cells" are phagocytized by macrophages and multinucleated foreign body macrophages. (HE, 381X)

JPC Diagnosis:
Nasal mucosa: Rhinitis, granulomatous, multifocal to coalescing, severe, with turbinate bone lysis and numerous pigmented fungal yeast-like cells and hyphae.

JPC Comment:
This case provides both an excellent example of dematiaceous fungi and a reason to revisit the various classifications of cutaneous fungal infections, particularly in light of an excellent, recently published review article on the subject.[3]

In general, cutaneous infection with opportunistic fungi presents as nodules or masses in the dermis and/or subcutis which may become ulcerated and develop draining tracts.[3] Histologically, fungal lesions are typically characterized by pyogranulomatous or granulomatous inflammation; some fungal infections may have a significant eosinophilic component, depending on species.[3] Fungi generally grow as hyphae in tissue and can be demonstrated by PAS and GMS stains to highlight histomorphologic features useful in narrowing differential diagnoses.[3]

Infection with non-dermatophytic, non-dimorphic, nonpigmented saprophytic fungi that grow as hyphae in tissue is referred to broadly as hyalohyphomycosis. Note that *Aspergillus* sp., fungi of the order *Mucorales*, and *Conidiobolus* spp and *Basidiobolus* spp, the agents of entomophthoromycosis, while all nonpigmented hyphae-forming fungi, are typically categorized separately from general hyalohyphomycosis.[3]

Phaeohyphomycosis refers to infection with *pigmented* saprophytic fungi that grow as hyphae and yeast-like cells in tissue. The term chromoblastomycosis is used for infection with a subset of pigmented fungi that predominately form large, round, thick-walled cells with prominent septations, called sclerotic bodies or Medlar bodies.[3] While small numbers of hyphae may be observed in chromoblastomycosis, sclerotic bodies are the predominant fungal form in the lesion.[3] Note that, as in this case, phaeohymphocosis may contain yeast-like cells; these may be differentiated from the sclerotic bodies of chrombolastomycosis by their thinner walls, septations in a single plane, and typical arrangement in chains.[3]

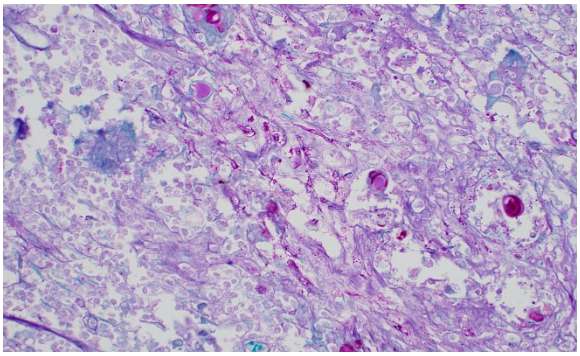

Figure 2-6. Nasal turbinate, horse. A PAS stain demonstrates the fungal hyphae and yeast-like cells within macrophages and multinucleated foreign body macrophages. (PAS, 400X)

Agent Category	Description	Representative Species
White-grain eumycotic mycetoma	• Septate, nonpigmented hyphae • Infrequent dichotomous branching • Frequent terminal cystic dilations	• *Pseudallescheria boydii* • *Pencillium dupontii*
Black-grain eumycotic mycetoma	• Septate, pigmented hyphae • Infrequent dichotomous branching • Frequent terminal cystic dilations	• *Curvularia* spp. • *Cladophialophora bantiana*
Hyalohyphomycosis	• Septate hyphae with parallel walls and occasional acute-angle branching	• *Fusarium* spp. • *Scedosporium* spp.
Phaeohyphomycosis	• Pigmented hyphae, +/- branching • Yeast-like cells • Fontana-Masson may highlight melanin	• *Alternaria* spp. • *Curvularia* spp. • *Bipolaris* spp.
Chromoblastomycosis	• Pigmented sclerotic bodies with transverse and longitudinal septation	• *Cladophialophora bantiana* • *Curvularia* spp.
Entomophthoromycosis	• Sparsely septate hyphae • Nonparallel walls, rare irregular branching, terminal bulbous dilations • Eosinophilic sleeve around hyphae	• *Conidiobolus* spp. • *Basidiobolus* spp.

Table 2-1. Selected organisms causing dermal to subcutaneous fungal infections in animals. Adapted from Hoffman, et al. *Vet Pathol*. 2023;60(6):814.[3]

It is unknown what factors cause a particular fungus to cause phaeohyphomycosis versus chromoblastomycosis, though most cases of chromoblastomycosis in veterinary medicine have been described in amphibians and horses.[3]

Separate from the categorization of pigmented vs. non-pigmented fungi is the clinical presentation referred to as mycetoma. A mycetoma is a dermal and/or subcutaneous nodule with three typical features: swelling, an exudate containing *grossly apparent grains* or *granules* that represent large aggregates of the infectious agent, and a draining tract.[3] Mycetomas are divided into those caused by bacteria (termed actinomycotic mycetomas) or fungi (eumycotic mycetomas). Eumycotic mycetomas may be further subdivided into black-grain eumycotic mycetomas and white-grain eumycotic mycetoma, depending on whether they are caused by pigmented or non-pigmented fungi, respectively.[3] Confusingly, eumycotic mycetoma is a morphologic classification that is not based on the inciting organism, so the same fungal species that may cause hyalohyphomycosis or phaeohyphomycosis may alternatively cause eumycotic mycetomas if tissue grains are present.[3] Common representative species of each of the categories discussed above are presented in Table 2-1.

Participants discussed the yeast-like cells present throughout the section and considered a diagnosis of chromoblastomycosis given the prominence of the yeast-like cells on H&E section; however, PAS staining revealed numerous hyphae and participants noted that the yeast-like cells are frequently arranged in chains, making phaeohyphomycosis the appropriate diagnosis. Participants also discussed the arrangement and character of the bone present in section, particularly the arrangement of the bone fragments perpendicular to the nasal mucosa. Participants felt that the bony fragments represent periosteal new

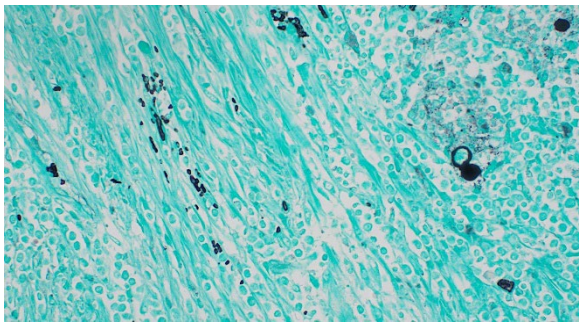

Figure 2-7. Nasal turbinate, horse. A silver stain demonstrates the fungal hyphae and yeast-like cells within macrophages and multinucleated foreign body macrophages. (Grocott's methenamine silver, 400X)

bone growth rather than lysed turbinates, which be oriented parallel to the mucosal surface. Participants felt that the turbinates have been destroyed completely by this inflammatory process, leaving behind only remodeling woven bone. The morphologic diagnosis retains the emphasis on bony lysis, though, in the examined section, we are left with only its aftermath to appreciate.

References:

1. Cafarchia C, Figueredo LA, Otranto D. Fungal diseases of horses.*Veterinary Microbiology*. 2013:215-234.
2. Guarner J, Brandt ME Brandt. Histopathologic diagnosis of fungal infections in the 21st century. *Clinical Microbiology Reviews*. 2011:247-280.
3. Hoffman AR, Ramos MG, Walker RT, Stranahan LW. Hyphae, pseudohyphae, yeasts, spherules, spores, and more: A review on the morphology and pathology of fungal and oomycete infections in the skin of domestic animals. *Vet Pathol*. 2023;60(6):812-828.
4. Mauldin EA, Peters-Kennedy J. Integumentary System. In: Maxie G, ed. *Jubb, Kennedy and Palmer`s Pathology of Domestic Animals*. 6th ed. Vol 1. Elsevier; 2016.
5. Pumlee Q, Meason-Smith C, Dieterly A, et al. Chaetomiaceae fungi, novel pathogens of equine neurotropic phaeohyphomycosis. *Vet Pathol*. 2017;54(5):813-819.
6. Seyedmousavi S, Guillot J, de Hoog GS. Phaeohyphomycoses, emerging opportunistic diseases in animals. *Clin Microbiol Rev*. 2013;26(1):19-35.

CASE III:

Signalment:
19-year-old, Polo mare (*Equus caballus*)

History:
This 19-year-old Polo mare presented with a recent history of coughing and exercise intolerance.

Gross Pathology:
On endoscopy, several irregular, broad, pedunculated, polypoid lesions were detected at the laryngeal aditus and aryepiglottic folds. The masses were mottled cream to red-brown and slightly granular.

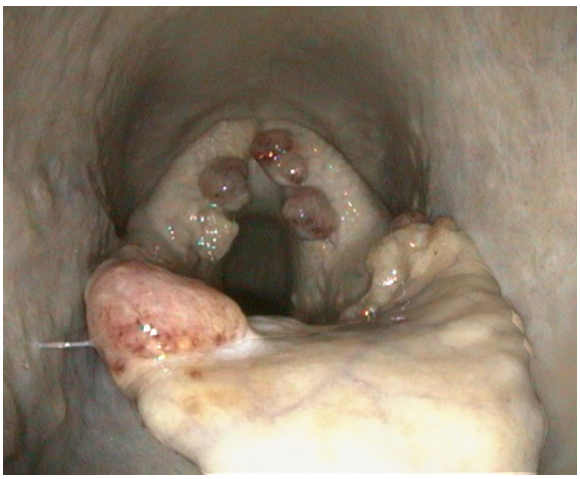

Figure 3-1. Larynx, horse. Endoscopy of the polypoid masses at the laryngeal aditus and aryepiglottic folds. (*Photo courtesy of*: Henry Tremaine, B&W Equine Vets, https://www.bwequinevets.co.uk/183/henry-tremaine-equine-vet-gloucester/)

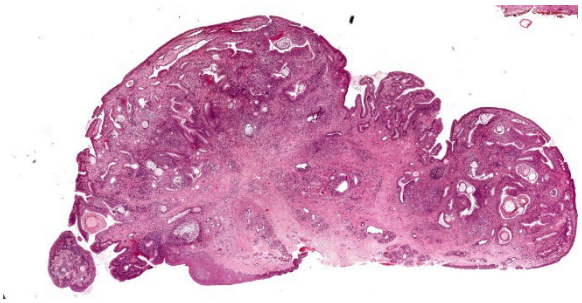

Figure 3-2. Larynx, horse. The laryngeal mucosa is markedly hyperplastic and thrown into numerous and deep folds. (HE, 12X)

Microscopic Description:

At low magnification, raised polypoid proliferations of loose fibrovascular tissue covered by an intact, mildly hyperplastic, non-keratinizing stratified squamous epithelium expand the mucosa and submucosa. Numerous spherical structures of varying size are randomly distributed throughout the lamina propria and submucosa, and are occasionally present within the laryngeal epithelium. These spherical structures represent sporangia in different stages of development. Sporangia are also present within submucosal glands that are dilated and are sometimes surrounded by deposits of amorphous eosinophilic material.

Small juvenile, immature sporangia (trophocytes) measure 10-100 µm, have a thick unilamellar, eosinophilic wall, and a single central round nucleus surrounded by granular amphophilic cytoplasm. Intermediate sporangia are characterized by the loss of the central single nucleus, a size of up to 300 µm, and the presence of numerous punctate eosinophilic granules. With progressing maturation, nuclei become more discrete 1-4 µm ovoid structures (prophase nuclei) and intermediate sporangia exhibit a mixture of punctate granules and small eosinophilic ovoid structures.[4]

Mature sporangia are spherical and 100-400 µm in diameter with a bilamellar, outer eosinophilic and inner amphophilic wall. The mature endospores are 10 µm, eosinophilic to basophilic, ovoid to round structures with a cell wall and single nucleus. Often a centripetal or centrifugal zone of small round eosinophilic structures (germinative zone) and mature endospores is present within the mature sporangia. Multifocally, the mature sporangia are ruptured or release endospores through their operculum (pore). Rupture of sporangia is accompanied by infiltration of large numbers of neutrophils and fewer macrophages.

Moderate numbers of lymphocytes and plasma cells and fewer neutrophils are multifocally present within the stroma of the lamina propria and the submucosa. Multifocally, small numbers of neutrophils migrate into the overlying mucosal epithelium (exocytosis).

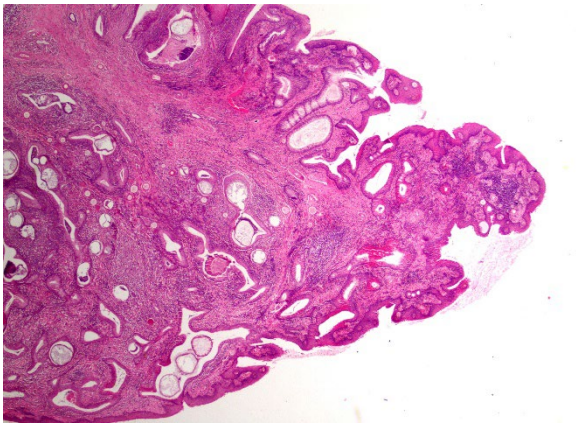

Figure 3-3. Larynx, horse. The polypoid proliferation of loose connective tissue of the laryngeal mucosa on subgross magnification. Multiple sporangia at different stages of development are present within the submucosa. The mass is covered by a hyperplastic epithelium (HE, 20X)(*Photo courtesy of:* Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge, Cambridge CB3 0ES, UK. https://www.vet. cam.ac.uk)

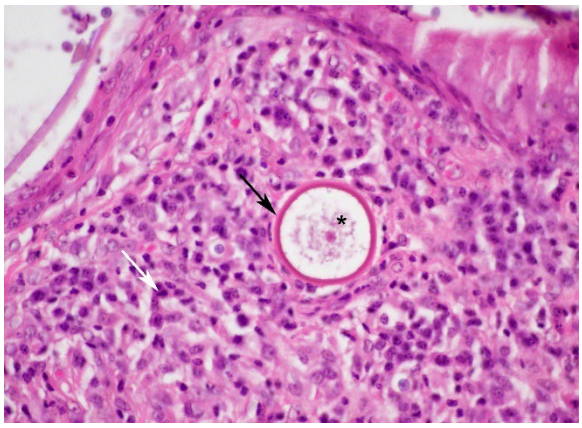

Figure 3-4. Larynx, horse. Juvenile sporangia (trophocytes) measuring ~50 µm, with a thick unilamellar, eosinophilic wall (black arrow), a single central round nucleus surrounded by amphophilic granular material (cytoplasm, asterisk). Lymphocytes and plasma cells infiltrate the connective tissue (white arrow) (HE, 400X)(*Photo courtesy of:* Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge. Cambridge CB3 0ES, UK)

A thin layer of coagulative necrosis (cautery artefact) is present around the margins and is often associated with mild multifocal oedema and haemorrhage. In some of the sections examined, sporangia are detected very close to the areas of cautery.

Contributor's Morphologic Diagnosis:
Laryngitis, proliferative, lymphocytic, plasmacytic, neutrophilic, and histiocytic, multifocal to coalescing, moderate, chronic, with intralesional juvenile and mature sporangia consistent with *Rhinosporidium seeberi*.

Contributor's Comment:
Rhinosporidium seeberi is a eukaryotic, hydrophilic organism from the Mesomycetozoa clade (DRIP clade) that also includes several fish pathogens such as *Dermocystidium* spp.[11,18] Its exact phylogenetic classification is unclear, with some discussion as to whether it should be grouped differently. The organism is generally found in stagnant waters and is the causative agent of rhinosporidiosis in humans.[11,17] In addition, rhinosporidiosis has been described in multiple other species, including cats, horses, cattle, buffaloes, dogs, mules, swans, and wild fowls.[3,14,17] Rhinosporidiosis is endemic in India, Sri-Lanka, in parts of the Americas, and Africa.[12,14,17] In horses, in addition to endemic areas, cases have also been recorded in countries traditionally free of rhinosporidiosis. Those cases have mostly been restricted to imported animals, such as four polo ponies that were imported from Argentina to the United Kingdom.[4,12,17] However, the potential for the disease to inhabit traditionally non-endemic areas was highlighted by a recent report from Europe. In that case, a Belgian warmblood horse, born in Belgium, that had never left the country, was diagnosed with rhinosporidiosis.[15] The source of infection remained unclear; however, as this organism is not contagious, the case suggests that the organism was present in the environment in Belgium.[16]

Due to difficulties with *in vitro* culturing of this organism, the life cycle of *Rhinosporidium seeberi* is still not fully understood.[18] It is believed that the infective stage is the mature endospore which enters the host through damaged epithelium of mucous membranes, mostly of the nose.[10,18] In the host tissue the endospore develops into a juvenile sporangium (trophocyte) with a single central nucleus. Subsequent reproduction takes place in intermediate sporangia via sole synchronized nuclear division. Cytokinesis occurs only once shortly before the formation of the mature sporangium. [9,13] Furthermore, it is suggested that mature sporangia are capable of additional "de

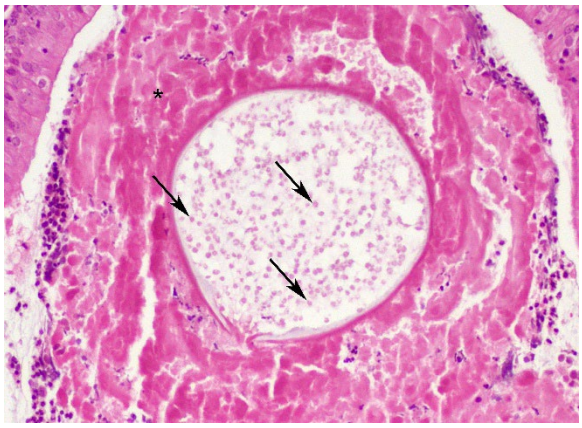

Figure 3-5. Larynx, horse. Intermediate sporangia in the lumen of a submucosal gland surrounded by eosinophilic material. At this stage of development sporangia measure up to 300 µm and contain numerous punctate eosinophilic granules or discrete 1-4 µm ovoid structures (prophase nuclei, black arrows). (HE, 200X)(*Photo courtesy of:* Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge. Cambridge CB3 0ES, UK)

novo" production of mature, cell-walled endospores.[9]

Based on the finding that only a few humans out of the many bathing in contaminated water become infected with *Rhinosporidium seeberi*, an individual host susceptibility has been suggested. The susceptibility is assumed to correlated to the presence of certain blood groups, but this relation has not be confirmed.[2]

Rhinosporidiosis most often localises in mucous membranes of the head region, with the nostrils and conjunctivae most commonly affected.[1,10] In humans, infection of the throat, eye, ear, oropharynx, larynx, trachea, bronchi, and genitourinary tract are also described.[7,16] Infected tissues develop single to multiple, pink, multilobular, broad-based, granulomatous, pedunculated, non-infiltrative masses. The affected mucosa is often red with stippled, pinpoint white granules (mature sporangia) that give the mass a mulberry-like or strawberry-like appearance.[5] The masses are slow growing and generally painless; however, irritation due to the presence of the masses can lead to sneezing, discharge, and when obstructing airways, may result in respiratory distress.[5,8]

In this horse, multiple polypoid masses were detected around the laryngeal aditus and at the aryepiglottic folds, resulting in coughing and exercise intolerance. The localisation in the laryngeal mucosa is rather unusual in horses. To our knowledge, two cases of equine laryngeal rhinosporidiosis have been described so far. In both of these cases, the masses were centred around the larynx, were surgical excised, and recurred.[4,15] So far, half a year after surgical excision, no recurrence was observed in this horse.

Rhinosporidiosis remains an uncommon infection that can be diagnostically challenging. The diagnosis of equine rhinosporidiosis is based on clinical history, characteristic macroscopic and histopathologic appearance, and potentially, confirmatory polymerase chain reaction.[4,12,15] Treatment of choice is surgical excision with broad, clear margins and electrocauterization or cryotherapy of adjacent tissues[4,5,15] In humans, additional administration of dapsone is used to prevent maturation.[10] Recurrence was reported to occur in 11% of human cases and is due to spreading of mature endospores.[10,12] In addition, due to a long incubation period reported to be at least 10 months in one pony, clinically inapparent animals may be transported from endemic areas and might shed the organism.[12] Given the long incubation period, increased international movement of animals, and climate change, rhinosporidiosis has the potential to be an

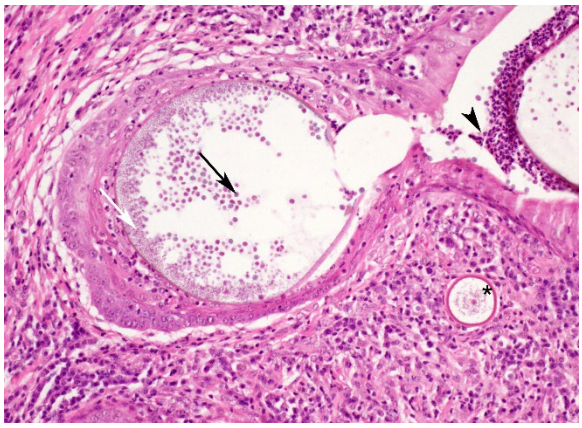

Figure 3-6. Larynx, horse. Mature sporangia have a bilamellar wall, are 100-400 µm in diameter. A zone of small round eosinophilic structures (germinative zone, white arrow) and mature endospores (black arrow) is present within the mature sporangia. The mature sporangia release endospores through their pore. The rupture of sporangia causes infiltration of large numbers of neutrophils (arrowhead). A juvenile sporangium is present in the connective tissue. (asterisk, HE, 200X)

emerging disease and to become endemic in areas that have traditionally been free of rhinosporidiosis.

Contributing Institution:
Department of Veterinary Medicine
The Queen's Veterinary School Hospital
University of Cambridge.
Cambridge CB3 0ES, UK.
https://www.vet.cam.ac.uk

JPC Diagnosis:
Larynx: Laryngitis, proliferative, chronic-active, diffuse, moderate, with numerous juvenile and mature sporangia and endospores.

JPC Comment:
The contributor provides an excellent, thorough summary of *Rhinosporidium seeberi* infection, a classic disease with a classic, virtually unmistakable histological appearance. As the contributor notes, the laryngeal location is uncommon; the more typical presentation of rhinosporidiosis is a soft, pink unilateral fleshy polyp, usually in the nose of a dog.[6]

While the gross appearance of *R. seeberi* may be confused for a variety of inflammatory or neoplastic conditions, particularly when found in an unusual location, once under the microscope, *R. seeberi* sporangia are not histologic wallflowers, but are large, striking, and distinctive. The juvenile sporangia are 15-75µm in diameter, have a unilamellar, PAS-positive wall, and a single nucleus.[6] The real stars, however, are the mature sporangia, which can grow to an astonishing 100-400 µm, have a PAS-positive bilamellar wall, and contain myriad 5-10 µm endospores. The presence of these eye-catching sporangia in the histologic section of a nasal polyp is essentially pathognomonic for rhinosporidiosis.

R. seeberi belongs to a class of organisms aptly termed endosporulators. Other endosporulators of veterinary importance include *Coccidioides immitis*, *Coccidioides posadasii, Prototheca* sp., *Chlorella* sp., and *Batrachochytrium dendrobatidis*. Of these, only *Coccidioides* is of a size and morphology that could be confused with *R. seeberi*. Immature *Coccidioides* spherules, the analogue to the *R. seeberi* sporangia, are approximately 10-20 µm in diameter and grow into mature spherules of up to 200 µm in diameter filled with numerous 2-5µm endospores.[6]

Participants struggled with tissue identification with this slide, with most defaulting to nasal mucosa based on the typical location. Conference discussion focused largely on the mor-

phologic diagnosis and how to capture the significant, mixed inflammatory component of the lesion. Some participant felt strongly that the morphologic diagnosis should emphasize the extensive lymphohistiocytic and plasmacytic character of the lesion, while others argued that the significant neutrophilic infiltrate should also be included. In the end, participants decided that "chronic-active" implied the existnence of both a neutrophilic (active) component and a lymphohistiocytic component with marked hyperplasia of the mucosa (a chronic change) and preferred the pared down diagnosis given above.

References:

1. Allison RW, Ramachandran A. What is your diagnosis? Ulcerative nasal lesion in a Quarter Horse. *Vet Clin Path*. 2015; 44: 455-456.
2. Arseculeratne SN. Recent advances in rhinosporidiosis and *Rhinosporidium seeberi*. *Indian J Med Microbiol*. 2002;20: 119-131.
3. Berrocal A, López A. Nasal rhinosporidiosis in a mule. *Can Vet J*. 2007;48: 305-306.
4. Burgess HJ, Lockerbie BP, Czerwinski S, Scott M. Equine laryngeal rhinosporidiosis in western Canada. *J Vet Diagn Invest*. 2012;24(4):77-780.
5. Caniatti M, Roccabianca P, Scanziani E, et al. Nasal rhinosporidiosis in dogs: four cases from Europe and a review of the literature. *Vet Rec*. 1998;142:334-338.
6. Caswell JL, Williams K. Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. 2016; Elsevier:579-584.
7. Costa EF, Pinto LM, Campos MAG, Gomes TM, et al. Partial regression of large anterior scleral staphyloma secondary to rhinosporidiosis after corneoscleral graft - a case report. *BMC Ophthalmology*. 2018;18:61.
8. Das S, Kashyap B, Barua M, et al. Nasal rhinosporidiosis in humans: new interpretations and a review of the literature of this enigmatic disease. *Medical Mycol*. 2011;49:311-315.
9. Delfino D, Mendoza L, Vilela R. *Rhinosporidium seeberi* nuclear cycle activities using confocal microscopy. *J Parasitol.* 2016;102(1):60-68.
10. Harissi-Dagher M, Robillard N, Corriveau C, Mabon M, et al. Histopathologically confirmed ocular rhinosporidiosis in two Canadians. *Can J Ophthalmol*. 2006;41: 226-229.
11. Herr RA, Ajello L, Taylor JW, Arseculeratne SN, Mendoza L. Phylogenetic analysis of *Rhinosporidium seeberi*'s 18S small-subunit ribosomal DNA groups this pathogen among mem-bers of the Protoctistan Mesomycetozoa Clade. *J Clin Microbiol*. 1999;37(9):2750-2754.
12. Leeming G, Smith KC, Bestbier ME, Barrelet A, Kipar A. Equine rhinosporidiosis in United Kingdom. *Emerg Infect Dis*. 2007;13(9):1377-1379.
13. Mendoza L, Vilela R. Presumptive synchronized nuclear divisions without cytokinesis in the *Rhinosporidium seeberi* parasitic life cycle. *Microbiology*. 2013; 159:1545-1551.
14. Moses JS, Balachandran C. Rhinosporidiosis in bovines of Kanyakumari district, Tamil Nadu, India. *Mycopathologia*. 1987;100:23-26.
15. Nollet H, Vercauteren G, Martens A, et al. Laryngeal rhinosporidiosis in a Belgian warmblood horse. *Zoonoses Public Health*. 2008;55:274-278.
16. Putthia H, Manjunatha BS, Astekar M, Taufiq S. Palatal rhinosporidiosis: an

unusual case report and review of the literature. *J Korean Assoc Oral Maxillofac Surg*. 2018;44:293-297.
17. Sudasinghe T, Rajapakse RPVJ, Perera NAND, et al. The regional sero-epidemiology of rhinosporidiosis in Sri Lankan humans and animals. *Acta Tropica*. 2011;120:72-81.
18. Vilela R, Mendoza L. The taxonomy and phylogenetics of the human and animal pathogen *Rhinosporidium seeberi*: A critical review. *Rev Iberoam Micol*. 2012;29(4):185-199.

CASE IV:

Signalment:
3-year-old, Thoroughbred filly (*Equus caballus*)

History:
Found dead in stall.

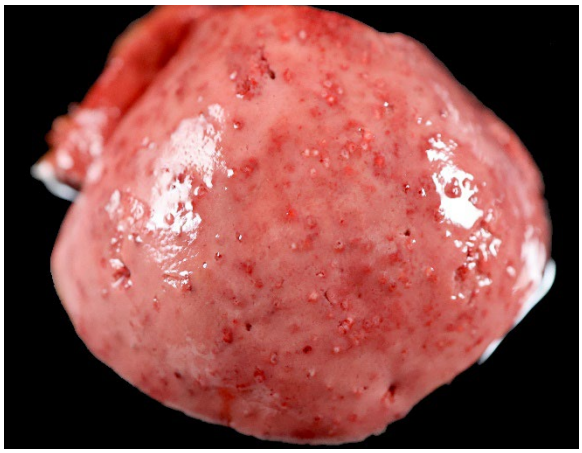

Figure 4-1. Kidney, horse. The kidney has raised white foci of inflammation on the capsular surface surrounded by red halos. *(Photo courtesy of* California Animal Health and Food Safety Laboratory San Bernardino Branch, https://cahfs.vetmed.ucdavis.edu/)

Figure 4-2. Kidney, horse. On cut section, the cortex has focal randomly scattered areas of suppurative inflammation surrounded by hemorrhage. *(Photo courtesy of* California Animal Health and Food Safety Laboratory San Bernardino Branch)

Gross Pathology:
Both kidneys have numerous pinpoint raised, tan foci with a red halo scattered throughout the renal cortices.

Laboratory Results:
Aerobic culture, kidney: *Actinobacillus* sp., mixed flora (*Actinobacillus* sp. were also isolated from the liver and lung).

DNA sequence analysis: DNA sequencing of a ~600 bp region of the 16S rRNA gene identified the isolate as most closely related to *Actinobacillus equuli* ssp. *equuli*.

Microscopic Description:
Within the renal cortical interstitium, there are randomly scattered, unencapsulated aggregates of neutrophils (measuring up to 3 mm in diameter), often with a central region of necrosis or hemorrhage, that efface the tubular and glomerular architecture. In some areas, these aggregates of neutrophils appear to be centered on glomeruli or blood vessels. There are aggregates of intra- and extravascular coccobacilli associated with these neutrophilic infiltrates. Glomeruli have similar bacteria and fibrin in the capillaries. There is also mild tubular degeneration. Coccobacilli are gram-negative.

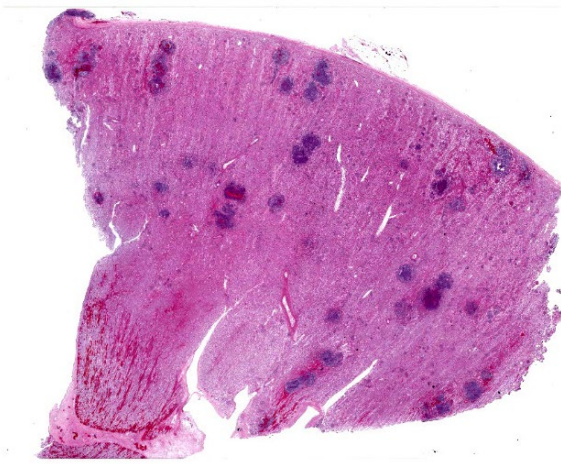

Figure 4-3. Kidney, horse. There are randomly scattered foci of inflammation within the cortex and medulla. (HE, 6X)

Contributor's Morphologic Diagnosis:
Kidney: Nephritis, embolic-suppurative, multifocal, with intra- and extravascular Gram-negative coccobacilli.

Contributor's Comment:
Actinobacillus spp. (Family: *Pasteurellaceae*) are pleomorphic, non-motile, non-spore forming, gram-negative, rod-shaped bacteria characterized by their ability to grow on MacConkey agar, produce β-galactosidase, and ferment carbohydrates without the production of gas.[8] There are a number of *Actinobacillus* species of significance within veterinary medicine, including *A. lignieresii* (causative agent of wooden tongue in cattle), *A. pleuropneumoniae* (causative agent of porcine contagious pleuropneumonia in pigs), *A. suis* (cause of septicemia in pigs), *A. capsulatus* (cause of joint disease in rabbits), and *A. seminis* (cause of epididymitis in rams).[8] As with many pathogenic bacteria within the family *Pasteurellaceae, Actinobacillus* often produce repeats-in-toxin (RTX) toxins, which are typically hemolytic and cytotoxic.[3]

In horses, *Actinobacillus* are common commensals and can be isolated from the gastrointestinal, respiratory, and urogenital tracts of healthy animals.[4] *Actinobacillus* spp. isolated from horses include: *A. equuli* ssp. *equuli*, *A. equuli* ssp. *haemolyticus*, *A. arthritidis, A. lignieresii, A. suis*, and *A. pleuropneumoniae.* Of these, *A. equuli* ssp. *equuli* and *A. equuli* ssp. *haemolyticus* are isolated most frequently from horses. These subspecies are differentiated based on the presence or absence of an RTX toxin, *A. equuli* toxin (or Aqx), encoded on the *aqx gene*.[3,8]

Actinobacillus equuli ssp. *equuli* is typically carried in the oral cavity and alimentary tract of juvenile and adult horses and is primarily associated with septicemia in neonatal foals (often referred to as "sleepy foal disease").[4,8] There are also reports of *A. equuli*-associated peritonitis and septicemia in adult horses, with gross and microscopic lesions similar to those described in foals.[4-7,9] Predisposing factors for "sleepy foal disease" include failure of passive transfer and concurrent bacterial/viral infections.[4]

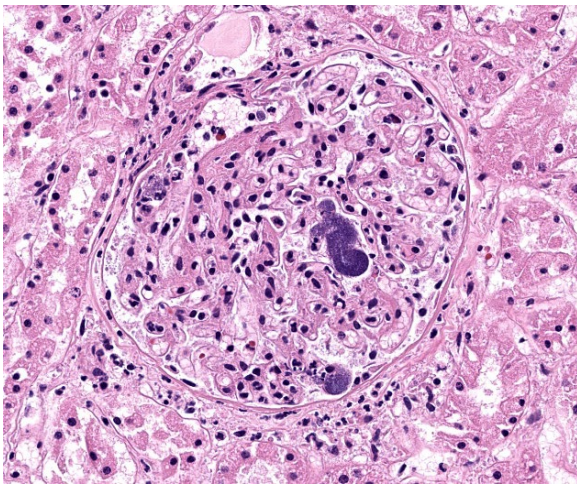

Figure 4-4. Kidney, horse. Glomerular capillaries contain colonies of coccobacilli. (HE, 317X)

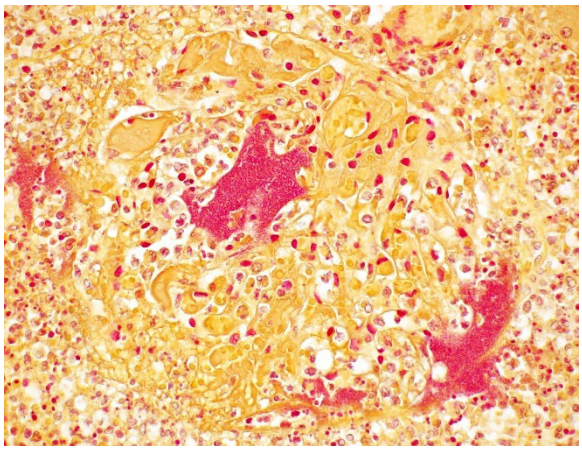

Figure 4-5. Kidney, horse. Coccobacilli within glomerular capillaries are gram-negative. (Gram, 400X)

Similarly, actinobacillosis in adult horses can be associated with stress (such as shipping) and concurrent bacterial/viral infections; there is, however, evidence that *A. equuli* can act as a primary pathogen in horses in the absence of stress/concurrent disease.[4]

Typical light microscopic findings of *A. equuli* infection include: suppurative embolic nephritis, suppurative embolic pneumonia, multifocal suppurative hepatitis, lymphoid necrosis, suppurative oomphalitis, and suppurative meningoencephalitis. *A. equuli* is zoonotic with sporadic reports of disease in people.[1]

Contributing Institution:
California Animal Health and Food Safety Laboratory (San Bernardino Branch)
https://cahfs.vetmed.ucdavis.edu/

JPC Diagnoses:
Kidney: Nephritis, suppurative, embolic, with numerous large colonies of bacilli.

JPC Comment:
This case is an excellent example of suppurative embolic nephritis, in which septic emboli lodge in glomerular and peritubular capillaries and produce variably sized abscesses, primarily in the renal cortex.[2] In horses, *Actinbacillus equuli* is the most common cause of embolic suppurative nephritis, generally seen in neonatal foals after acquiring the infection in uetero, during parturition, or shortly after birth via the umbilicus.[2]

As the contributor notes, there are two subspecies of *A. equuli* that differ based on the presence of absence of an RTX toxin. *A. equuli* subspecies *haemolyticus* produces Aqx, an RTX toxin similar to the leukotoxins and hemolysins produced by related *Pasteurellaceae* such as *Mannheimia haemolytica, A. pleuropneumonia, A. suis*, and hemolytic *E. coli*.[4] The Aqx toxin is highly cytolytic to equine erythrocytes and lymphocytes, and has less virulent lytic activity against the same cells in pigs.

The vast majority of *A. equuli* septicemia cases are cause by *A. equuli* subspecies *equuli*, which does not produce the Aqx toxin, indicating that other virulence factors likely play the key roles in neonatal septicemic actinobacillosis.[4]

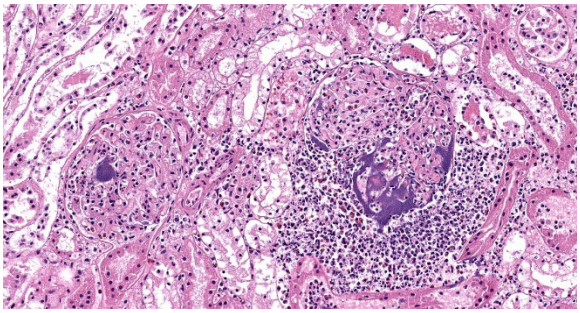

Figure 4-6. Kidney, horse. Glomerular capillaries contain colonies of coccobacilli. (HE, 317X)

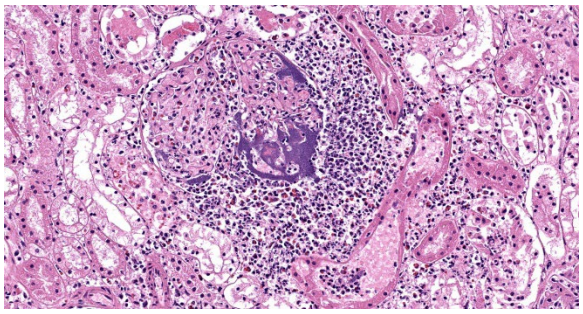

Figure 4-7. Kidney, horse. Rupture of Bowman's capsule allows extension of suppurative inflammation into the surrounding interstitium. (HE, 244X)

Like any self-respecting gram-negative bacterium, *A. equuli* subspecies *equuli* is armed with endotoxin which, upon the destruction of the bacterium, damages endothelium and causes the vasculitis and bacterial emboli that are characteristic of septicemic actinobacillosis.[4] As illustrated in this case, a particular quirk of equine actinbacillosis is the presence of large bacterial colonies present in affected vessels, unlike other gram-negative bacteria which do not form such obvious intravascular aggregates.[4] This has led to speculation that *A. equuli* subspecies *equuli* may express an adhesion for endothelium. Unfortunately, other than the well-characterized Aqx toxin present in *A. equuli* subspecies *haemolyticus*, little is definitively known about the virulence factors that enable the spectacular lesions of equine actinobacillosis.[4]

Conference discussion focused initially on the age of the animal since, as noted above, *A. equuli* embolic nephritis is typically a disease of neonatal foals. The moderator noted reports of this presentation in non-stressed, non-immunocompromised adult animals and reminded residents to think broadly about differential diagnoses, even when the signalment isn't a perfect fit. The moderator led conference participants in a discussion other famous *Actinobacillus* species of veterinary importance, including the species noted above by the contributor. Finally, participants discussed the presence of bacterial colonies within glomeruli and whether this would constitute glomerulonephritis. Participants felt that, since the pathogeneses behind the established categories of glomerulonephritis (membranoproliferative, membranous, etc.) did not pertain to a suppurative embolic nephritis, the term glomerulonephritis was inappropriate. Glomerulitis would be a more appropriate term; however, since suppurative inflammation was present within the glomeruli, tubules, and interstitium, the preferred morphologic diagnosis is the all-inclusive term "nephritis."

References:

1. Ashhurst-Smith C, Norton R, Thoreau W, Peel MM. *Actinobacillus equuli* septicemia: an unusual zoonotic infection. *J Clin Microbiol*. 1998;36(9):2789-90.
2. Cianciolo RE, Mohr FC. Urinary System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. Elsevier;206:433.
3. Frey J, Kuhnert P. RTX toxins in *Pasteurellaceae*. *Int J Med Microbiol*. 2002;292(3-4):149-58.
4. Layman QD, Rezabek GB, Ramachandran A, Love BC, Confer AW. A retrospective study of equine actinobacillosis cases: 1999-2011. *J Vet Diagn Invest*. 2014; 26(3):365-75.
5. Matthews S, Dart AJ, Dowling BA, Hodgson JL, Hodgson DR. Peritonitis associated with *Actinobacillus equuli* in horses: 51 cases. *Aust Vet J*. 2001;79(8):536-9.
6. Means K, Townsend K, Johnson P. Multicavitary septic effusions associated with actinobacillosis in an adult Tennessee Walking Horse with weight loss. *Equine Vet Educ*. 2022;34(1):e60-6.

7. Patterson-Kane JC, Donahue JM, Harrison LR. Septicemia and peritonitis due to *Actinobacillus equuli* infection in an adult horse. *Vet Pathol*. 2001;38(2):230-2.
8. Rycroft AN, Garside H. *Actinobacillus* species and their role in animal disease. *Vet J*. 2000;159(1):18-36.
9. Uchida-Fujii E, Niwa H, Kinoshita Y, Nukada T. *Actinobacillus* species isolated from Japanese Thoroughbred racehorses in the last two decades. *J Vet Med Sci*. 2019;81(9):1234-7.