Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 14

13 January, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: 41375 (4117060-00)

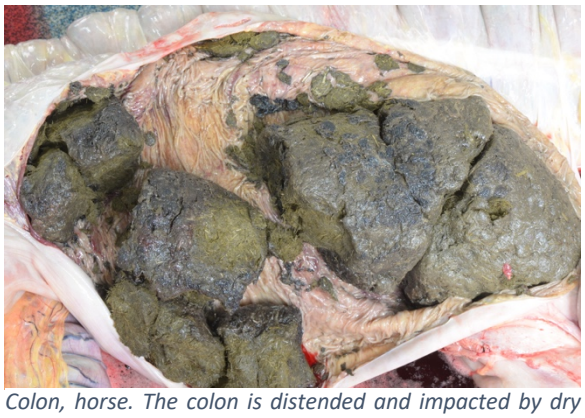
Signalment:
7-year-old male (gelded) cob, equine (*Equus caballus*)

History:
The gelding presented with a 48-hour history of colic. Signs of abdominal pain (colic) and mild ptosis were evident on clinical examination. Nasogastric reflux was present on intubation. Rectal examination and abdominal ultrasound revealed distended and non-motile small intestinal loops, and non-compressible impaction of the colon. Medical management was initiated however, due to unrelenting abdominal pain, and the clinical suspicion of grass sickness, euthanasia was carried out.

Gross Pathology:
The horse was in good body condition. Gross findings were limited to the gastrointestinal tract. All 4 divisions of the ascending colon contain firmly compacted and dry content (impaction) that has a variably present black coating (most prominent in the more distal regions, especially the right dorsal colon). The small intestine was dilated by gas and a moderate amount of liquid.

Laboratory results:
Not supplied.

Microscopic description:
Coeliac/ cranial mesenteric ganglion. Multifocally, high numbers of neurons (estimated 70-80%) have degenerative changes characterized by central to complete cytoplasmic clearing of Nissl substance (chromatolysis), with an eccentrically positioned nucleus. Some of these neurons contain a central cytoplasmic eosinophilic body. Many neurons with chromatolytic changes have eccentric nuclei with condensed chromatin and shrunken nuclei (apoptosis). Axonal spheroids are seen



Colon, horse. The colon is distended and impacted by dry fecal material. (Photo courtesy of: Division of Pathology, Public Health and Disease Investigation, Veterinary Diagnostic Services, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow (Garscube Campus), 464 Bearsden Road, Glasgow G61 1QH, Scotland, https://www.gla.ac.uk/schools/vet/cad/)

Colon, horse. The impacted fecal material is covered by a black crust. (Photo courtesy of: Division of Pathology, Public Health and Disease Investigation, Veterinary Diagnostic Services, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow (Garscube Campus), 464 Bearsden Road, Glasgow G61 1QH, Scotland, https://www.gla.ac.uk/schools/vet/cad/)

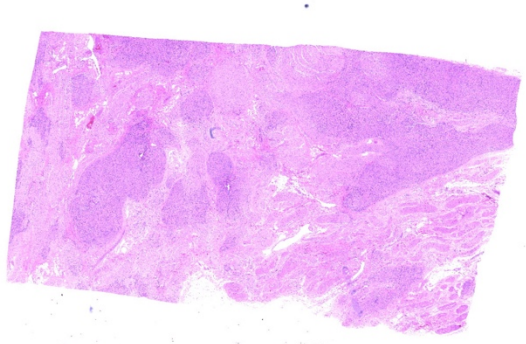
occasionally between neurons. Multifocally, there is a mild increase in satellite cells. Rare individual and small clusters of lymphocytes are present scattered within the ganglion.

Contributor's morphologic diagnosis:
Coeliac/ cranial mesenteric ganglia: Neuronal chromatolysis, degeneration and apoptosis, multifocal, severe

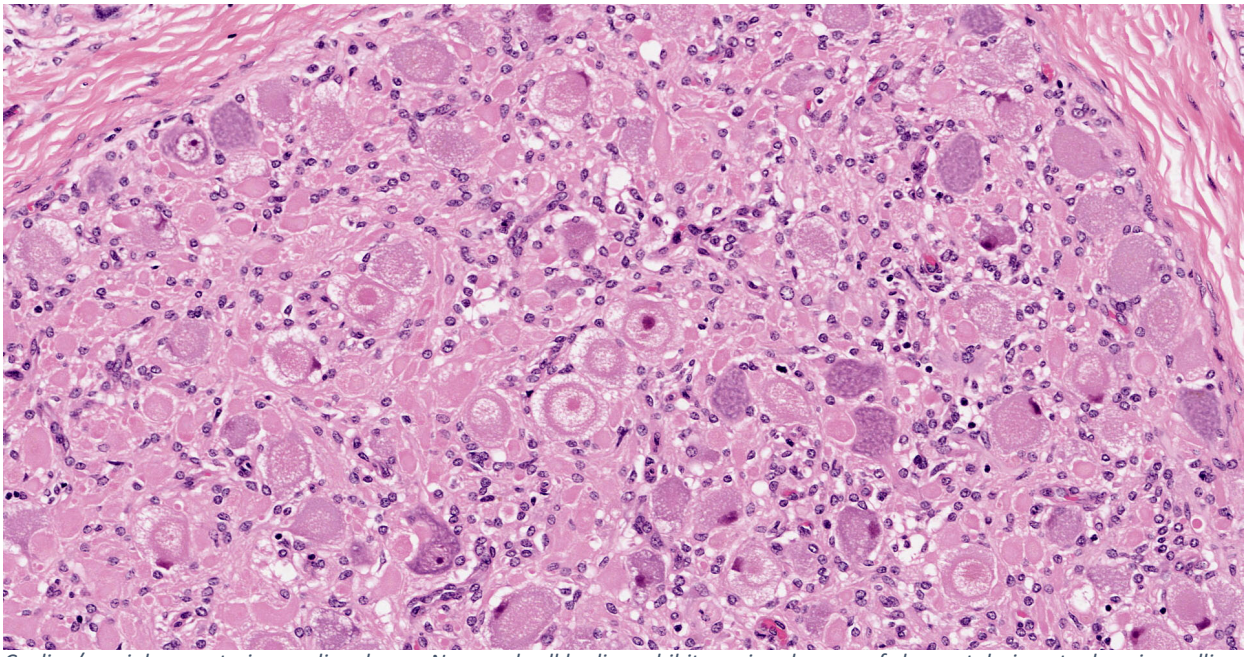
Contributor's comment:
The presence of classical gross and histopathological features confirms the clinical suspicion of equine dysautonomia (equine grass sickness) in this case. Equine dysautonomia has a high mortality rate, and can have a significant impact in at risk areas.[12] Acute, subacute, and chronic clinical presentations are recognized, and although these frequently overlap, this classification has utility in clinical decision making.[6] Equine dysautonomia occurs most commonly in the United Kingdom,[3] northern Europe, and South America (where it is referred to as "mal seco"),[14] and has a strong association with grazing, with a peak incidence in the northern hemisphere during the spring and early summer months. Risk factors associated with the disease include recent movement to a new pasture, other cases of the disease on the same premises, pasture disturbance and a high number of horses. Younger animals are more often affected with a peak incidence in 2-7 year old horses.[6] Equine dysautonomia is one example of a dysautonomia affecting domestic animals; others include the well characterized feline dysautonomia[12] (Key Gaskell syndrome) and those occurring in dogs, sheep, rabbits and hares.[1]

All dysautonomias in domestic animals are typified by injury to the postganglionic sympathetic and parasympathetic neurons of the peripheral autonomic and enteric nervous system,[8,12] resulting in failure of both sympathetic and parasympathetic innervation in several organ systems including the gastrointestinal tract.[1] In the horse, functional obstruction of the gastrointestinal tract due to intestinal dysmotility is a significant and life threatening consequence.[12] The result is gastric and small intestinal dilation, and impaction of the large intestine;[14] both were evident in this case.

Characteristic neuropathological findings are extensive chromatolysis and neurodegeneration, with subsequent loss of neurons and degeneration and loss of dependent axons in autonomic ganglia of the peripheral nervous system.[1] These changes were prominent in the section of coeliac/ cranial mesenteric ganglion submitted to the conference and also in the ganglia of the submucosal/ Meissner's and myenteric/ Auerbach's nerve plexuses of the ileum (not submitted to the conference), consistent with a previous report of the utility of this site in antemortem diagnosis of the disease.[9] Additionally, immunohistochemical staining of the synaptic vesicle membrane protein synaptophysin showed marked cytoplasmic



Coeliac/cranial mesenteric ganglion, horse. A section of ganglion is submitted for examination. (HE, 5X)



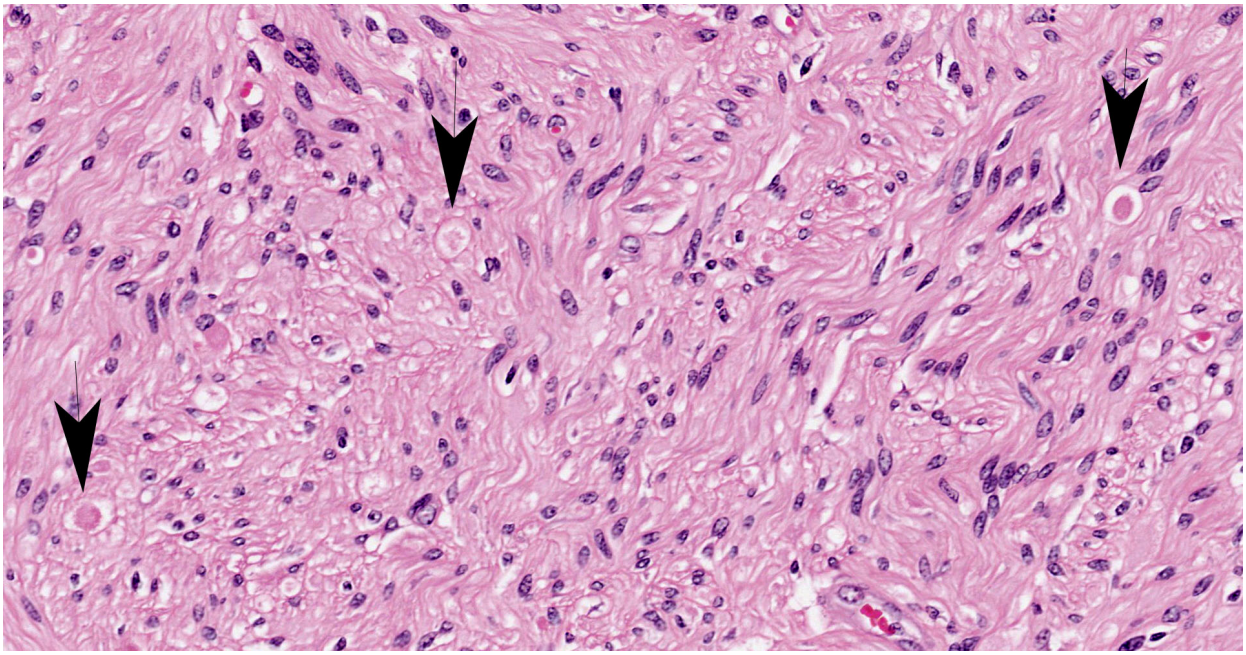
Coeliac/cranial mesenteric ganglion, horse. Neuronal cell bodies exhibit varying degrees of chromatolysis, cytoplasmic swelling, vacuolation, and nuclear peripheralization. (HE, 280X)

accumulation in neurons within both the coeliac/ cranial mesenteric ganglia and ileum. Intracytoplasmic synaptophysin accumulation, as demonstrated by immunohistochemistry, is recognized in dysautomonias of both horses and cats. Its use as a diagnostic marker has been demonstrated in horses and shows a staining pattern that is distinct from healthy control horses, colic cases and neuroparalytic botulism.[2,16]

A small increase in the numbers of non-neuronal satellite cells was observed in the section of coeliac/ cranial mesenteric ganglion examined. Reactive and proliferative changes in these elements occur with progression of the disease.[1] Additionally the presence of multifocal small and loose aggregations of T lymphocytes and few individualized B lymphocytes in primarily perivascular locations (confirmed by immunohistochemistry for CD3 and PAX5 respectively) is also an unexpected and seemingly non-specific finding in this case as inflammation is not anticipated associated with neuronal degeneration and loss in equine dysautonomia.

The etiology of equine dysautonomia is currently unknown and is a focus of research into the disease, with *Clostridium botulinum* neurotoxins C and/ or D originating in the gastrointestinal tract (toxico-intestinal infection) hypothesized to be a possible cause.[6,12] Low level serum antibodies to *C. botulinum* type C antigens is reported as a risk factor for the disease.[6] This hypothesis has recently been tested in a *C. botulinum* type C vaccination trial in the United Kingdom,[3,12] however the results are not available at the time of writing. An alternative etiology is the ingestion of a pasture derived mycotoxin,[6,12] and a diverse range of other possibilities have also been investigated[1] (for example heavy metal toxicity or plant derived toxins).

Accurate antemortem diagnosis is imperative to facilitate clinical decision-making; the most appropriate course of action is often prompt euthanasia, while limited number chronic cases do respond to medical management. Ileal biopsies, although highly sensitive and specific, require general anesthesia for abdominal surgery.[6] In an attempt to circumvent this issue the utility of rectal biopsy has been assessed, however sensitivity and specificity have been suboptimal, possibly due the small number of cases examined and/ or to the lesser numbers of neurons present in the rectal submucosa.[12] Most recently detection of β-amyloid precursor protein



Coeliac/cranial mesenteric ganglion, horse. Few swollen axons (spheroids) are present within nerve fibers. (HE, 380X)

(β-APP) in submucosal neurons of the rectal mucosa has been shown to be a sensitive and specific method of confirming equine dysautonomia,[4] and examination of the neurons of the subgemmal plexus of gustatory papillae for changes suggestive of neuronal injury has also shown promise for use as a antemortem diagnostic test.[7] Both offer the advantage of sample acquisition being relatively non-invasive.

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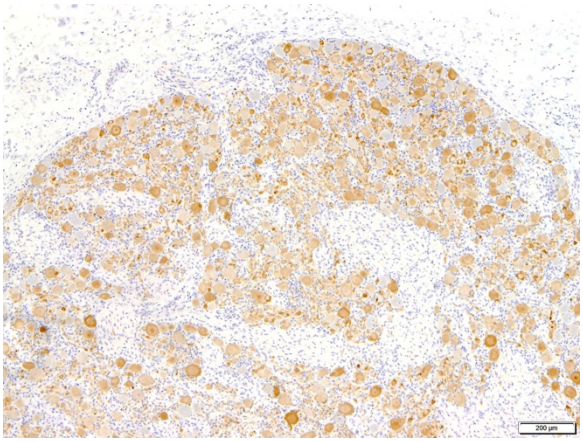
JPC diagnosis:
Ganglion: Neuronal degeneration, multifocal, moderate, with chromatolysis, mild satellitosis, and lymphocytic ganglionitis.

JPC comment:
There was spirited discussion among participants regarding this case. While the neuronal vacuolation in this case is significant and a prominent feature, vacuolation of ganglial cells has been found in both healthy and affected animals.[5] A short review of affected species that have been reported as being affected by dysautonomia include the horse, rabbit, cat, dog, hare, and human. While overo lethal white foal syndrome is a heritable trait and grass sickness appears to be acquired, the similar dysfunction of celiac/mesenteric ganglia results in similar clinical signs.

While there still exists a substantial knowledge gap regarding this disease, recent research may address certain specific aspects of predisposition, diagnosis, and treatment. A pedigree analysis of a population of Lipizzaner horses in Hungary showed a disproportionate number of horses affected by equine grass sickness were descendants of certain studs, with one having nearly 22% of offspring affected by the disease. While the true etiology of this disease is likely multifactorial, this suggests there may be a heritable component.[15]

Efforts to diagnose this disease antemortem have resulted in a preliminary scoring system, that incorporates epidemiologic data and clinical data. The most important factors identified in this



Coeliac/cranial mesenteric ganglion, horse. Neuronal cell bodies stain immunopositive for accumulated cytoplasmic synaptophysin. (anti-synaptophin, 100X)

framework include daily access to pasture and presenting from April to June, generalized or localized sweating, muscle tremors, and dysphagia. The score cutoff for this model results in a sensitivity of 100%, and a specificity of 53%. As additional risk factors are identified, the applicability of this model, as well as its specificity, may be improved.[13]

Cytology of scrapings from the cranial cervical ganglia of affected horses has resulted in a highly sensitive and specific method of diagnosis. Using a combination of May-Grunwald Giemsa (MGG), cresyl fast violet, ad hematoxylin and eosin, and using histologic sections of tissue as the good standard for diagnosis, independent pathologists agreed on diagnoses of equine dysautonomia. The primary limitation of cytology is the occasional specimen that contains too few neurons for evaluation.[11]

Though acute cases of equine dysautonomia are fatal, horses with chronic cases may survive with appropriate medical management. In chronic cases, the number of neurons found in the prevertebral and paravertebral ganglia, and in the enteric plexuses is lower than normal, but also lower than acute cases of dysautonomia. Additionally, small intestinal dilation, muscular hypertrophy, gastric mucosal hypertrophy and ulceration were common post-mortem findings in chronic cases. In most cases, the ileum was the most profoundly affected section of the gastrointestinal tract.[10]

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CASE 2: S19-3401-3 (4153161-00)

Signalment:
6-year-old, mare, Hackney, *Equus ferus caballus*

History:
The provided sample was from a subcutaneous mass on the neck. The horse has no travel history outside the state of Florida, USA.

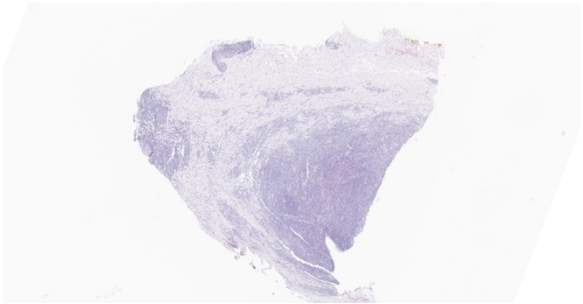
Gross Pathology:
Received is a roughly elliptical 1.7 x 1.2 x 0.6 cm, light tan to brown firm tissue.

Laboratory results:
Immunohistochemistry for *Leishmania* spp. is immunoreactive. PCR is negative.

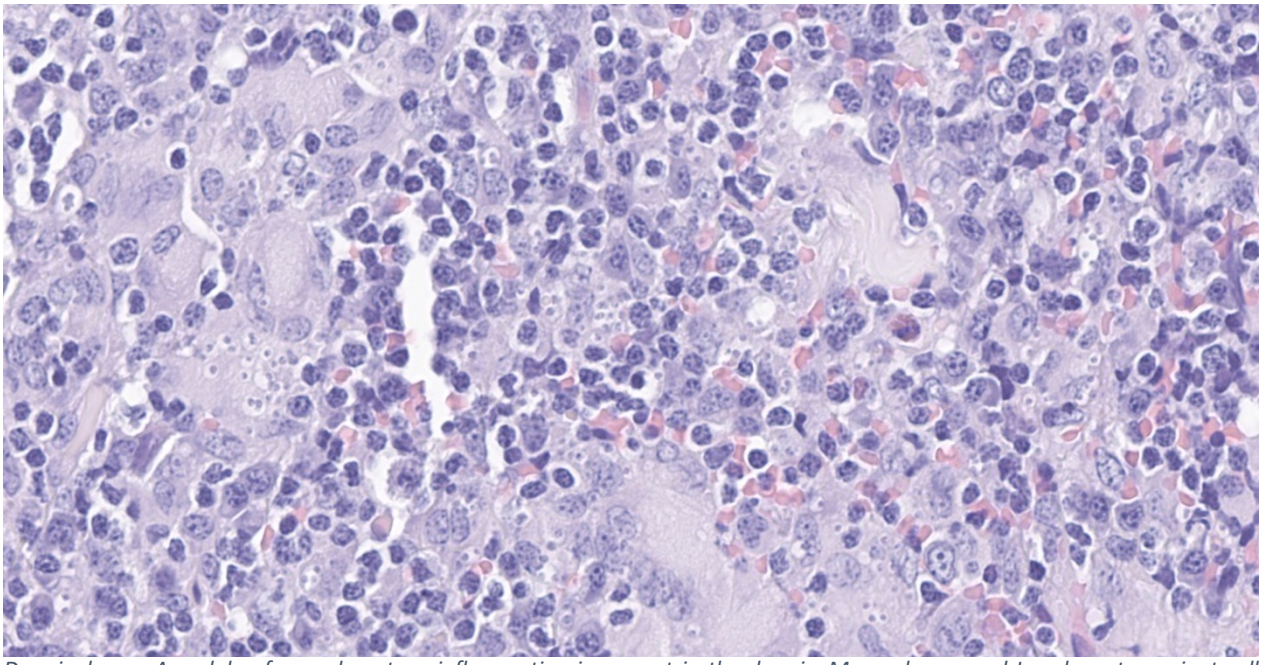
Microscopic description:
The fibrous connective tissue is moderately expanded by large numbers of macrophages and epithelioid macrophages admixed with moderate number of lymphocytes, plasma cells, and low to moderate numbers of multinucleated giant cells. Numerous macrophages contain one to eight pale eosinophilic, round, protozoal organisms (amastigotes) that are 2-3 µm in diameter, stain with a Giemsa histochemical stain, have an oval nucleus and a kinetoplast that is orientated perpendicular to the nucleus. Intrahistiocytic amastigotes are frequently surrounded by a thin optically clear halo (parasitophorous vacuole). There are multifocal areas within the larger nodule of inflammation of hypereosinophilic and degenerate collagen.

Contributor's morphologic diagnosis:
Dermatitis and panniculitis, granulomatous, lymphoplasmacytic, chronic, multifocal to coalescing, marked, with myriads of intrahistiocytic amastigotes and fewer extracellular amastigotes, subcutaneous mass from neck.

Contributor's comment:
Leishmaniasis is a zoonotic disease caused by *Leishmania spp.*, an obligate intracellular protozoa. *Leishmania spp*. are found on every continent except Australia and Antarctica. Leishmaniasis has three types of disease presentations: cutaneous, visceral, and mucocutaneous. The disease presentation depends on the species of *Leishmania* and the host immune responses.[10] Most of the *Leishmania* species (>85%) only cause cutaneous lesions. Therefore, cutaneous leishmaniasis is the most common presentation in humans. However, the prevalence of cutaneous leishmaniasis in domestic animals is largely unknown[9] and likely underdiagnosed as it is suspected the majority of infections have mild clinical disease and undergo



Dermis, horse. A section of sclerotic dermis without overlying epidermis is submitted for examination (HE, 5X)

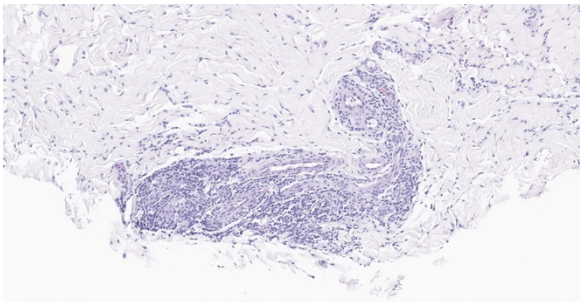


Dermis, horse. A nodule of granulomatous inflammation is present in the dermis. Macrophages and Langhans-type giant cell macrophages contain numerous protozoal amastigotes within their cytoplasm. (HE, 400X)

spontaneous regression.[7] To date, only two *Leishmania* species, *L. donovani* and *L. infantum* (also known as *L. chagasi*), are known to cause visceral leishmaniasis in mammals. In cases of visceral leishmaniasis, there is often concurrent cutaneous involvement. The spectrum of the severity of the cutaneous lesions in leishmaniasis reflects the dynamics of host immune responses. In general, a balanced Th1 response with proper IFN-γ dominant delayed type hypersensitivity promotes parasite killing and resolution of disease. However, an overly strong Th1 response could cause tissue damage and leads to severe, immune-mediated mucocutaneous manifestation. On the other end of the spectrum, an antibody dominant, or a Th2-skewed response that lacks IFN-γ, is associated with a high parasite load and diffuse, severe cutaneous disease.[14]

The sandfly is the known vector for *Leishmania spp.* The sandflies acquire cell-associated amastigotes from the blood meal of an infected host. The amastigotes transform to the promastigote stage in the gut and migrate to the proboscis. The promastigotes are then regurgitated into the skin when the sandfly bites a new host. Once in the skin, the promastigotes are quickly phagocytosed by macrophages. Within the phagolysosome the promastigotes are protected from degradation from the macrophages, mature into amastigotes, and start to multiply inside the macrophages. Eventually the macrophages burst from the amastigote burden releasing amastigotes into the surrounding tissue to then be phagocytosed by other macrophages. Different species of sandflies are responsible for different species of *Leishmania* parasite. There are at least 40 species of *Leishmania* parasites listed in the taxonomy database in NCBI (National Center for Biotechnology Information) website and more than 30 species of sandflies serve as vectors for *Leishmania* parasite. And different species of *Leishmania* parasite seem to rely on different species of mammals as primary reservoirs to maintain its transmission cycle. For example, dogs serve as a natural reservoir for *L. infantum.* Sloths, hyraxes, opossums and rodents are other documented reservoirs for various species of *Leishmania*.[3] In dogs, in addition to horizontal transmission by sandflies[12], vertical transmission of *L. infantum* from naturally infected, pregnant bitch to puppies has been proven[15] and is believed to contribute to the maintenance of the pathogen in endemic areas.

The histiocytic and lymphocytic dermatitis is a consistent feature for cutaneous leishmaniasis.



Dermis, horse. At the deep margin of the specimen, vessels are surrounded by 4-5 layers of lymphocytes and plasma cells. (HE, 158X)

However, the burden of the protozoa could be variable which reflects the dynamics of the interplay between the host and the pathogen. Furthermore, the incubation time of cutaneous leishmaniasis is highly variable from several month to seven years. Spontaneous regression and recurrence of cutaneous leishmaniasis have been reported in cats[15] and horses.[8]

In the United States, while most cases reflect travel patterns, cutaneous leishmaniasis is considered endemic in Texas and Oklahoma and North Dakota, with identification of autochthonous cutaneous leishmaniasis human cases and detection of female *Lutzomyia anthophora* sand flies at the residences of the case-patients.[5] In the similar geographical regions in Texas where human autochthonous cutaneous leishmaniasis were reported, feline autochthonous cutaneous leishmaniasis caused by the same Leishmania species (*L. Mexicana*) have also been reported sporadically.[16] Despite Florida not currently considered endemic for leishmaniasis in people, there has be a reported case of equine autochthonous cutaneous leishmaniasis case in Florida caused by *L. siamensis* in 2011.[11] *L. siamensis* is an emergent leishmania species that is not genetically close to the leishmania species in the New World and in the Old world. It was first reported in a visceral leishmaniasis patient with AIDS in Thailand and subsequently in at least four horses with cutaneous leishmaniasis in central Europe.[8] In addition, an autochthonous bovine cutaneous leishmaniasis case caused by *L. siamensis* was reported in Switzerland in 2010.[6] The presented case is the second recognized horse native to central Florida with no travel history outside Florida, suggesting Florida is endemic for cutaneous leishmaniasis. Leishmaniasis should be considered as an endemic disease in Florida and be included as a differential diagnosis of nodular skin disease in horses with no travel history.

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JPC diagnosis:
Dermis: Dermatitis, granulomatous, focally extensive, severe, with numerous intrahistiocytic amastigotes.

JPC comment:
The contributor provides an excellent review of *Leishmania* spp. One hallmark feature of this protozoan is the presence of a kinetoplast. This structure is a complex organization of circular DNA in a large mitochondrion. The circles of DNA contain many copies of mitochondrial DNA, composed of minicircles and maxicircles. Maxicircles are similar to those of mitochondrial DNA is other eukaryotes, but minicircles are involved in RNA editing of maxicircle encoded transcripts. *Leishmania tarentolae* has been used as a research model successfully, and sequencing of *L. infantum* and *L. baziliensis* has shown remarkable conservation of minicircle DNA sequences across species. However, there exists a high degree of heterogeneity and diversity of maxicircle DNA, providing another way to identify and classify individual species within the clade of kinetoplastids.[4]

The parasitophorous vacuole is an important structure in this disease, with contributions from the host cell as well as the parasite. The molecular composition of the host contributed components have partially been identified, while the contributions from the parasite are largely elusive. *Leishmania* spp parasitophorus vacuoles have many shared characteristics with phagolysosomes, including the lysosome-

associated proteins 1 and 2 (LAMP1, LAMP2) and ATPase. Also expressed and present are MHC class II, the glycoprotein gp91^{phox} portion of NADPH oxidase complex, macrosialin, and cathepsin proteases B, D, H, and L. Additional molecules have been identified, and research as to their function remains to be conducted.[17]

In the recent past, multi-locus enzyme electrophoresis (MLEE) was the reference technique for identifying *Leishmania* species, which often resulted in classification into species complexes. However, more recent molecular techniques based on either single gene sequences or multi-locus sequences, categorize in a definitive manner that does not support species complexes. Within major groups, markers such as MLSA, microsatellites or genome-wide SNPs are most appropriate. By leveraging molecular techniques, it allows for research on genetic drift and evolution of *Leishmania*, and has resulted in a clonal evolution hypothesis, with rare sexual recombination events.[13]

There is some variability in participant slides for this case, with some containing adnexal structures which were helpful for tissue identification. The moderator discussed the potential differential diagnosis of cutaneous histoplasmosis in this horse. However, there is a paucity of *Histoplasma capsulatum* var. *capsulatum* reported in horses in the literature, with horses more often affected by *Histoplasma capsulatum* var. *farciminosum* (equine Histoplasmosis). The latter disease is of a more nodular nature, and frequently results in ulceration, exudation, scarring, and spreads via lymphatics.

The lesion of leishmaniasis typically contains a significant plasmacytic component. In this case, while a few plasma cells were observed, many felt inclusion in the morphologic diagnosis was not warranted. Definitions of 'granulomatous' inflammation may include plasma cells as a component of chronic inflammatory cells,[2] or it may specifically refer to macrophages, epithelioid macrophages, and multinucleated giant cells.[1] In this case, we choose to include the small numbers of plasma cells in the term 'granulomatous.'

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CASE 3: S1601220 (4101207-00)

Signalment:
14-year-old, neutered male Quarter horse, *Equus caballus*

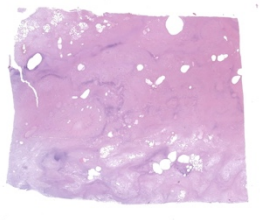
History:
The horse had a three-day history of progressive neurologic signs, fever ranging from 102 to 105°F, and icterus, and had been treated unsuccessfully with non-steroidal anti-inflammatory drugs, prior to death.

Gross Pathology:
Full necropsy was performed approximately 6 hours after death. The carcass was in good nutritional condition with adequate internal fat reserves. Ocular sclera, fat [subcutaneous, pericardial, and abdominal], and articular surfaces [hip and shoulder] were icteric. Ecchymotic to suffusive hemorrhages were present in the ribcage (pleural, subpleural and intramuscular), along the ventral aspect of the vertebral column, multifocally in musculature, epicardium, endocardium, on serosal surfaces of most viscera, and in cerebral cortex. Heart contained deep red-black blood. Abdominal cavity contained approximately 5 liters of serosanguineous fluid with abundant fibrin that coated serosal surfaces of viscera and the peritoneal lining of the diaphragm. The liver was markedly enlarged, and the right and quadrate lobes were orange brown and friable. The left liver lobe was firm and deep red-black with numerous subcapsular and parenchymal emphysematous bullae. Cut surface had large, irregular pale foci surrounded by a hemorrhagic to black rim.

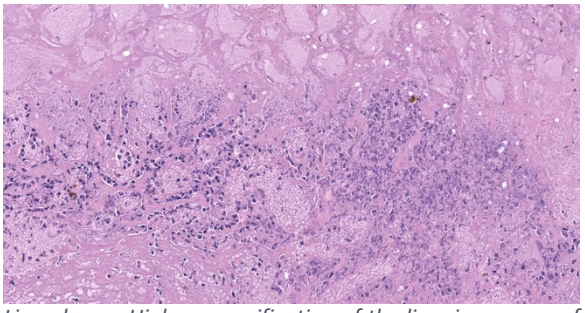
Meninges overlying brain and spinal cord were congested, and there were focal hemorrhages throughout the cerebral cortex. No lesions were seen in the rest of the carcass.

Laboratory results:
Liver impression smears processed by FAT were positive for *Clostridium novyi*, and negative for *C. chauvoei*, *C. septicum* and *C. sordelli*. Cultures were negative for *C. perfringens*, *C. difficile* and *C. sordellii*. Liver samples were positive for *C. novyi* type B *fliC* and *TcnA* genes, and negative for the *fliC* genes of the other clostridia tested.

Microscopic description:
Liver: The hepatic capsule is multifocally lined by fibrin and cellular debris, and there are multiple, variably sized subcapsular and parenchymal emphysematous bullae. There is multifocal to focally extensive coagulative necrosis particularly of centrilobular to midzonal hepatocytes with frequent extension to involve entire lobules, accompanied by intralesional hemorrhage, fibrin, cellular debris, and large aggregates of gram-positive bacterial rods. Some of the bacteria had subterminal spores. Large numbers of viable and degenerate neutrophils border necrotic foci and are present in surrounding sinusoids. There is bile stasis, focal hemorrhages, vascular fibrinoid necrosis and thrombosis.



Liver, horse. A single of liver is presented for examination. The liver has poor stain affinity, and linear aggregates of cellular debris. (HE, 5X)



Liver, horse. Higher magnification of the liver in an area of diffuse coagulative necrosis, dilated sinusoids, and a linear aggregate of necrotic neutrophils admixed with cellular debris. The process of degeneration increases from left to right in this image. (HE, 320X)

IHC: There was positive staining for *C. novyi* antigen.

Contributor's morphologic diagnosis:
Liver: Hepatitis, necrotizing, focally extensive to diffuse, severe, with cholestasis, emphysematous bullae, vascular fibrinoid necrosis and thrombosis, and intralesional gram-positive bacilli, etiology consistent with *Clostridium novyi*

Contributor's comment:
The presumptive diagnosis of infectious necrotic hepatitis [*Clostridium novyi* hepatitis] was made based on the typical gross and histologic findings, positive FA on liver impression smears, and positive staining for *C. novyi* antigen by IHC. Diagnosis was confirmed by PCR detection of the *fliC* and *TcnA* genes of *C. novyi* type B.[8]

Infectious necrotic hepatitis [black disease] is an acute lethal disease of ruminants and is rarely seen in horses and dogs.[1,3,5,9] *C. novyi* is a gram-positive, spore-forming, anaerobic bacillus, which is commonly found in soil and feces of animals.[9] It is classified into types A, B, and C based on the range of toxins produced.[8] *C. novyi* type A produces mainly the lethal, edema-inducing alpha toxin (TcnA), and the non-lethal phospholipase, gamma toxin; this bacterium is associated with gas gangrene. *C. novyi* type B produces TcnA in addition to the necrotizing and hemolytic beta toxin. *C. novyi* type C is considered to be non-toxigenic and non-pathogenic. *C. novyi* type D is commonly known as *C. haemolyticum* and mainly produces beta toxin, the main virulence factor for bacillary hemoglobinuria in cattle.

It is speculated but not proven that the spores of *C. novyi* type B are absorbed from the intestine and reach the liver via the portal circulation, after which there is systemic dissemination. Spores are phagocytized and remain latent in Kupffer cells of the liver, and in macrophages of the spleen and bone marrow.[3,4] Both infectious necrotic hepatitis [INH] and bacillary hemoglobinuria [BH] are thought to occur after liver injury causing necrosis and the associated anaerobic conditions that are required for germination of latent spores and the production of toxins.[5] Among causes of hepatic injury, migration of immature forms of *Fasciola hepatica* through the liver is considered the most important predisposing factor for both BH and INH in ruminants, and both diseases are more common in areas with high prevalence of fascioliasis.[3] No predisposing factors were identified in the equine case presented here.

Contributing Institution:
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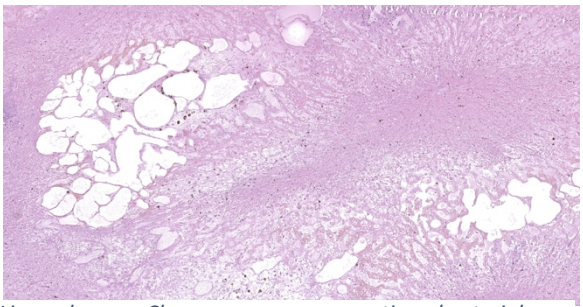
University of California Davis, San Bernardino Branch
105 W. Central Ave., San Bernardino, CA 92408
www.cahfs.ucdavis.edu

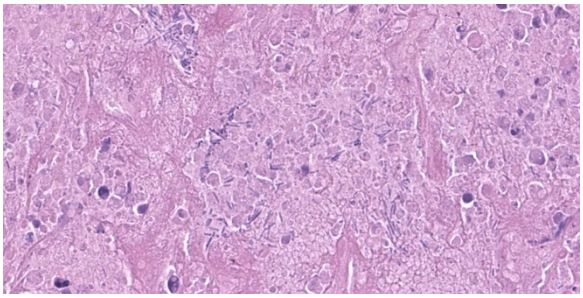
JPC diagnosis:
Liver: Hepatitis, necrotizing, diffuse, severe, with thrombosis, emphysema, and numerous spore-forming bacilli.



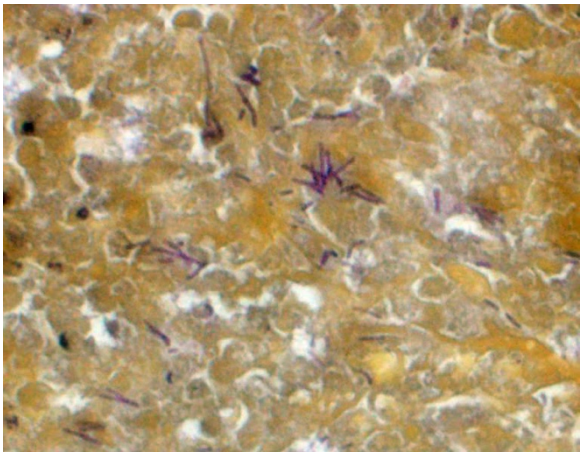
Liver, horse. Clear spaces representing bacterial gas production (emphysema) are scattered throughout the section. (HE, 71X)

JPC comment:
The contributor provides a concise summary and subsequently published this case report in 2018.[7] This is an uncommon disease in horses, and at the time of this writing, only seven cases of equine infectious necrotizing hepatitis have been reported. Sheep are most often affected by infectious necrotizing hepatitis, but as in this case, other species are affected as well. While the most important toxins were detailed by the contributor, the production of TcnA is related to phage infection of the bacterium.[6] When *Clostridium novyi* type A or type D were cured of phages NA1^{tox+} and NB1^{tox+}, respectively, they no longer produced alpha toxin. Re-infection resulted in the renewed ability to produce alpha toxin.[2] While this was published in 1976 and there is not a current effort to improve medical treatment by targeting bacteriophages, as technology improves, it may enjoy renewed research.

When latent spores experience a low oxygen microenvironment, they begin to elaborate TcnA and beta toxin. TcnA enters host cells by means of receptor mediated endocytosis using a currently unidentified membrane receptor. Acidification within the endocytic vesicle allows cleavage of the N-terminal domain and the toxin's movement into the cytosol. Rho- and/or Ras-GTPases from UDP-*N*-acetylglucosamine are glucosylated by the active form of TcnA. This results in disruption of the actin cytoskeleton, and minor disruption to the vimentin and microtubule systems. While beta toxin is also capable of causing hepatic necrosis, such small quantities are produced that it is unlikely to play a significant role in pathogenesis.[6]



Liver, horse. Numerous robust bacilli are scattered throughout the parenchyma. (HE, 450X)



Liver, horse. Numerous gram positive bacilli are distributed throughout the affected section of liver.

Other *Clostridium* spp also cause significant liver disease, in a wide variety of animals. As discussed, *C. haemolyticum* was previously known as *C. novyi* type D and is the cause of bacillary hemoglobinuria in ruminants (and rarely horses) through large quantities of beta toxin. *C. piliforme* is the only gram negative, obligate intracellular clostridia, and is the cause of Tyzzer's Disease in foals, laboratory rodents, and other lagomorphs.[6] Unfortunately, the virulence factors for *C. piliforme* have yet to be identified.

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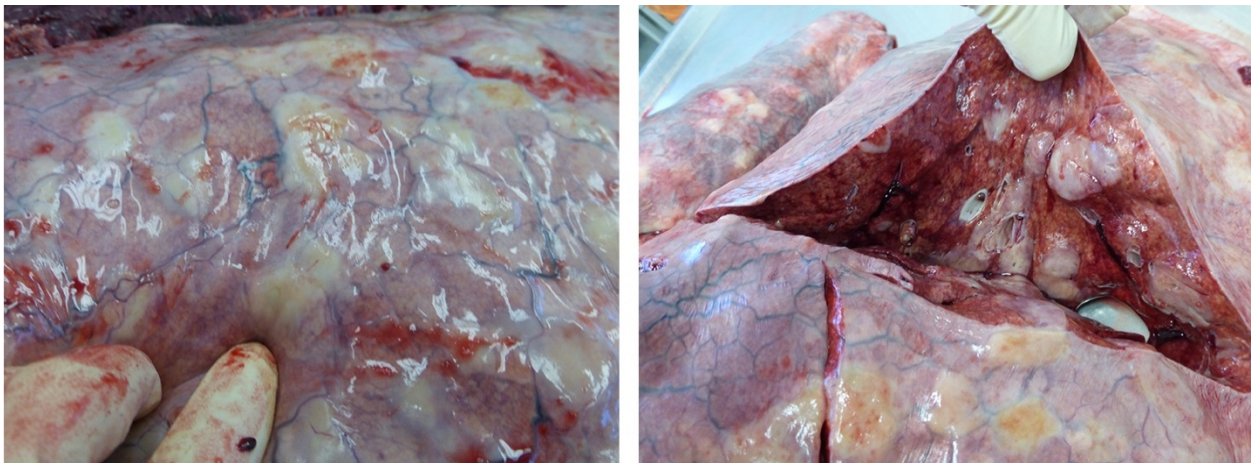
CASE 4: K18-1448 (4127786-00)

Signalment:
16 years old, gelding, Thoroughbred, *Equus caballus*, equine

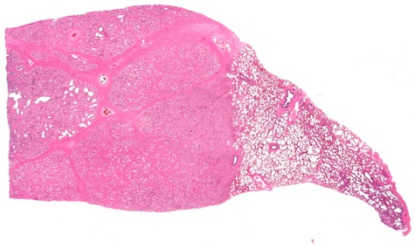
History:
Intermittent fever for five weeks, which improved with administration of tetracycline, but then fever returned, and the horse developed a cough. *Rhodococcus equi* pneumonia was confirmed, and results of polymerase chain reaction (PCR) testing for *Equid herpesvirus*-2 and EHV-5 on tracheal wash fluid were positive. Concurrent equine multinodular pulmonary fibrosis was suspected.

Gross Pathology:
The horse is in good body condition, has good adipose reserves, and is in good postmortem condition. The oral mucous membranes are pale. There is stable foam in the trachea. There are multifocal, diffuse, round, firm nodules throughout the pulmonary parenchyma affecting approximately 50% of the lungs. The stomach and small intestine are gas filled. There is soft, wet, green ingesta in the stomach. The cecum and colon contain solid feces. The spleen is enlarged and there are multifocal, diffuse, ecchymotic, capsular hemorrhages. There is yellow concentrated urine in the urinary bladder.

Laboratory results:
Mycoplasma culture negative
Bacterial culture negative
EHV-1 PCR negative
EHV-2 PCR positive
EHV-4 PCR negative
EHV-5 PCR positive
Equine Influenza virus H3N8 PCR negative



Lung, horse. Two sections from the joint, to include the thickened joint capsule and synovium are submitted for examination. (Photo courtesy of: Kord Animal Health Diagnostic Laboratory, PO Box 40627, Nashville, TN 37204-0627 https://www.tn.gov/agriculture/consumers/pets/animal-health-diagnostic-lab0.html)

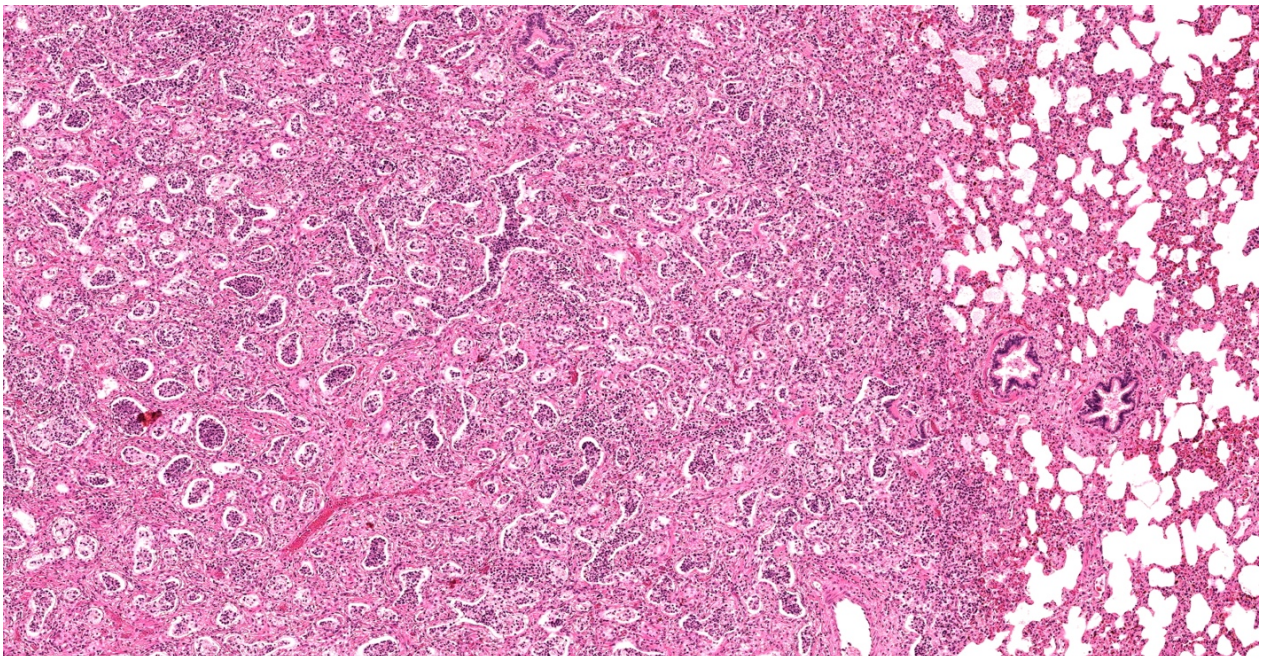


Lung, horse. A section of lung with a large fibrous nodule (left) is submitted for examination. (HE, 64X)

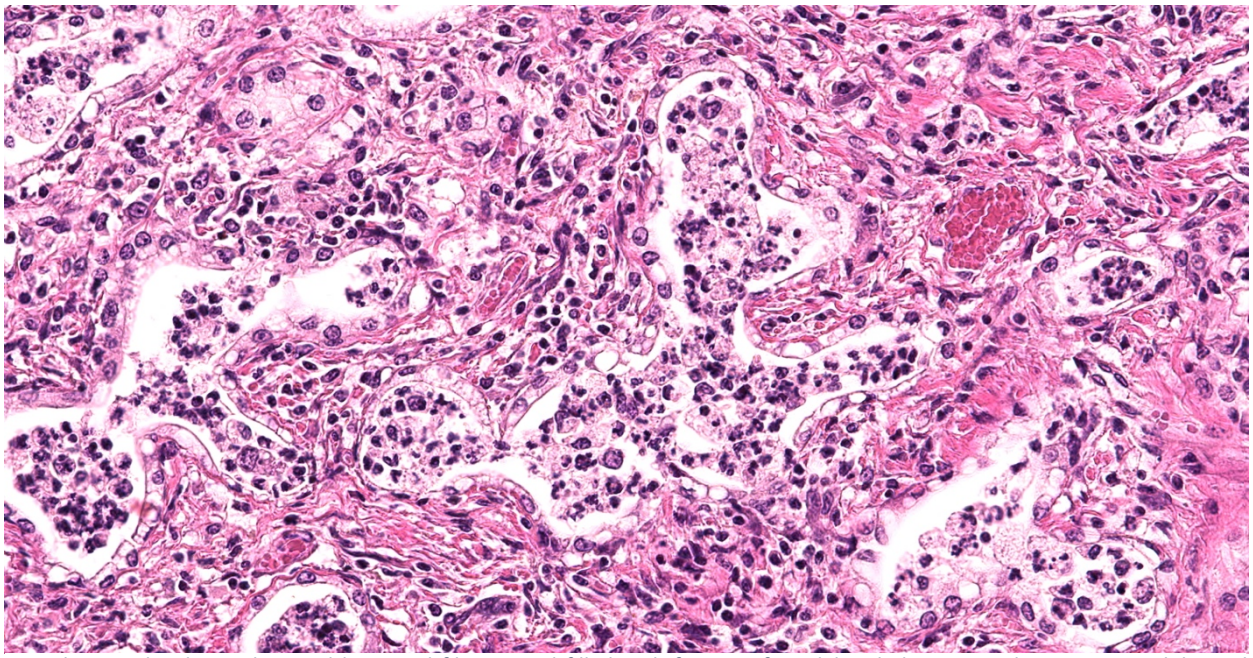
Microscopic description:
There are multifocal compressive nodules where alveolar septa are diffusely thickened by loose to mature fibrocollagenous stroma with infiltrates of scattered histiocytic cells, mononuclear cells, and neutrophils. The alveoli are often lined by cuboidal epithelium (type II pneumocyte hyperplasia) and contain variable numbers of neutrophils, histiocytic cells, sloughed epithelial cells, and/or mononuclear cells. A few alveoli contain necrotic cellular debris. Scattered intra-alveolar macrophages contain amphophilic, 4- to 6-micron, intranuclear inclusion bodies. Bronchi in the affected tissue are often lined by hyperplastic epithelium. In the less severely section of the parenchyma, there are scattered intra-alveolar hemorrhages and hemosiderin-laden macrophages. The pleura is thickened.

Contributor's morphologic diagnosis:
Multifocal interstitial pulmonary fibrosis with bronchointerstitial pneumonia and intrahistiocytic intranuclear viral inclusion bodies consistent with equine multinodular pulmonary fibrosis.

Contributor's comment:
Since 2007, the fibrotic interstitial lung disease of horses known as equine multinodular pulmonary fibrosis (EMPF) has been associated with infection by EHV-5.[3,7] *Equid herpesvirus* 5, family *Herpesviridae,* subfamily *Gammaherpesvirinae*, genus *Percavirsus*, is considered ubiquitous in horse populations.[3] Clinically affected horses are often concurrently infected with *Equid herpesvirus* 2 (EHV-2), which may suggest a synergist effect between the two viruses. The role of EHV-5 in the development of the disease is not known.[3] Results of the quantitative real-time PCR reaction indicates that virus load is highest in lung tissue, transtracheal wash fluid, and bronchoalveolar lavage fluid, but virus isolation is typically unsuccessful.[3] However, due to its ubiquitous



Lung, horse. The expanding nodule is composed of fibrotic alveolar septa and distorted alveoli filled with inflammatory cells. (HE, 128X)



Lung horse. Alveoli are distorted by septal fibrosis and filled with foamy, often debris-laden macrophages, neutrophils, and cellular debris. Septa are expanded by mature collagen and variable combinations and concentrations neutrophils, macrophages, lymphocytes and plasma cells. (HE, 381X)

nature, EHV-5 is often also found in horses unaffected by EMPF.[5]

In 2013, a group of researchers isolated EHV-5 from horses with EMPF, inoculated horses without EMPF, and reproduced the disease, but were unable to re-isolate the virus from the clinically affected test subjects. However, EHV-5 antigen was detected in affected lung tissue via immunohistochemistry.[8] By use of PCR, EHV-5 of the inoculating sequence was detected in peripheral blood mononuclear cells (PBMCs) only twice in the experiment, whereas EHV-5 of a non-inoculating sequence was detected three times.[8] There is marked genomic heterogeneity of EHV-5, which likely results in strains of variable virulence, explaining why horses can be infected with various strains over their lifetime and not necessarily develop disease.[2] Genomic heterogeneity is even greater in EHV-2.[2]

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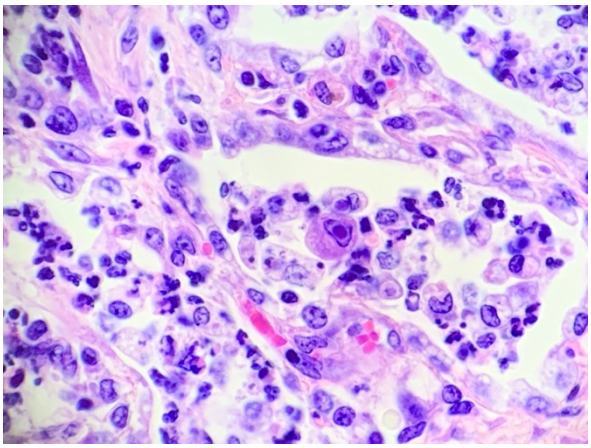
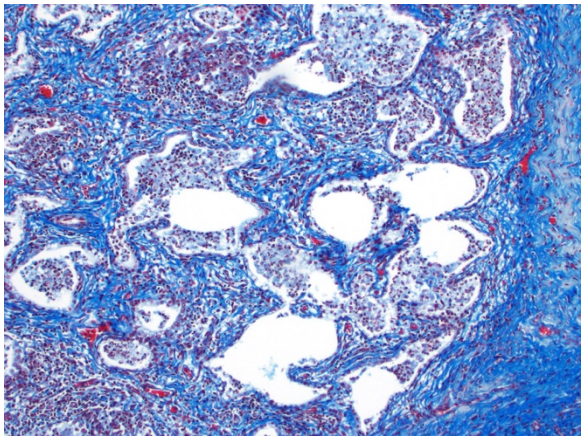
https://www.tn.gov/agriculture/consumers/pets/animal-health-diagnostic-lab0.html

JPC diagnosis:
Lung: Pneumonia, necrotizing and sclerosing, interstitial, focally extensive, severe, with marked alveolar remodeling, type II pneumocyte hyperplasia and rare intrahistiocytic intranuclear viral inclusions.

JPC comment:
Recent research on *Equid herpesvirus-5* has revealed small windows into its pathogenesis in



Lung, horse. Alveolar macrophages occasionally have 4-6 micrometer diameter, homogenous eosinophilic intranuclear viral inclusions.



Lung, horse: A Masson's trichrome stain demonstrates the amount of fibrous connective tissue expanding the collagenous septa as well as the cellularity which may not be evident on HE. (Masson's trichrome, 100X)

the horse. EHV5 preferentially infects alveolar cells in the lung but does not infect ciliated respiratory epithelium of the trachea or nasal airways. Additionally, similar to the human Epstein-Barr virus, another gammaherpesvirus, EHV5 induces a lytic infection of T and B lymphocytes, with peak viral antigen expression at 24- and 72-hours post-inoculation, respectively. The subsequent drops in levels of expression are hypothesized to indicate a possible latency mechanism in the host.[6]

While PCR and in situ hybridization has been previously used to identify EHV5 in affected horses, the first two reported cases in Japan also used an RNA-based in situ hybridization to identify viral mRNA, with positive signals identified in the cytoplasm and nuclei of alveolar macrophages in affected regions of the lung. This result suggests alveolar macrophages may play an important role in the pathogenesis of EMPF.[4]

The current treatment for EMPF is focused on limiting inflammation to prevent further fibrosis, specifically focusing on limiting the actions of EHV5. Corticosteroids and nonsteroidal anti-inflammatory drugs (NSAID) limit associated inflammation, and tetracycline therapy are chosen for their antifibrotic properties and their abilities to decreased metalloproteinase activity. The antiviral drug valacyclovir, which is metabolized to the active form acyclovir, is an inhibitor of viral DNA polymerase. A single case report of valacyclovir described a clinically healthy horse two years after treatment. However, in a recently conducted study in six horses with EMPF, valacyclovir did not effectively reduce the viral load as measured in peripheral blood, nasal secretions, or bronchoalveolar lavage fluid. Additional research may be warranted due to the low sample size, and that the clinical manifestation of disease is likely more complicated than strict viral kinetics.[1]

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