Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 7

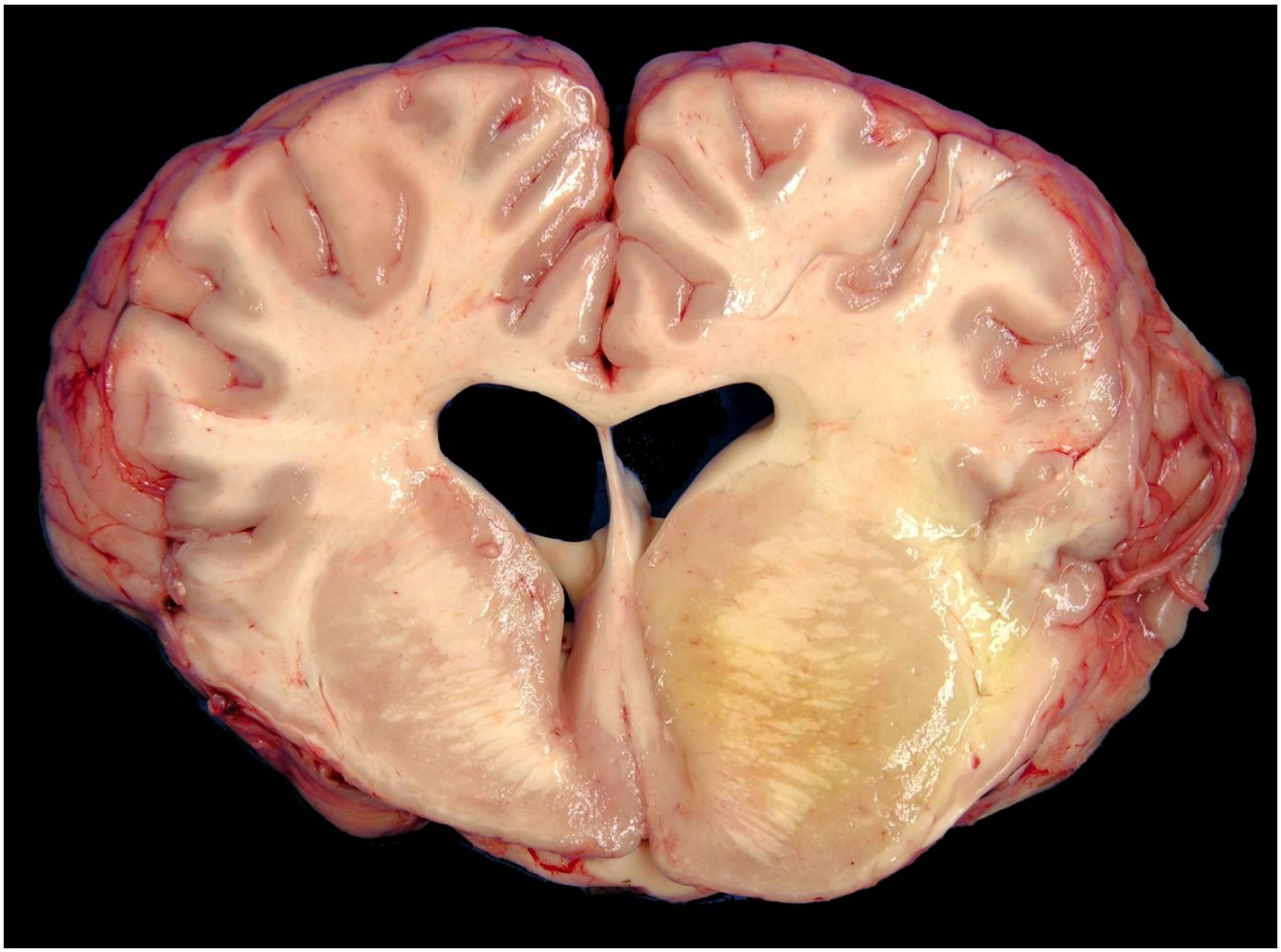
9 November 2015

CASE I: 2014 #1 (JPC 4066676).

Signalment: 4-year-old, castrated male, crioulo horse (*Equus caballus)*.

In this herd, affected animals displayed a 14-day course of ataxia, incoordination, and wobbling affecting the hindlimbs. (Photo courtesy of: Faculdade de Veterinária – UFRGS, Setor de Patologia Veterinária Av. Bento Gonçalves 909091540-000 Porto Alegre RS, Brazil.)

History: During the summer of 2003, approximately 200 horses on 3 bordering farms, as well as 10 mares from a fourth farm that were sent to one of the 3 farms for breeding, were maintained near the banks of a river. Farm employees reported that capybaras (*Hydrochoerus hydrochaeris*) were affected by a similar disease and died at the time horses were affected but we were unable to confirm this information. However, we could confirm that several horses in one of the farms had been treated with a common needle early in the outbreak. Approximately one month after their arrival in the farm, many of the horses presented with marked weight loss, muscle atrophy, and incoordination. Within 2 to 3 months, and extending through the rest of the year and into 2004, approximately 100 horses died. We did evaluate clinically and necropsied 15 of these horses, 22 of which presented a chronic wasting form of the disease. The horse of this report is one of a group in which the onset of disease was insidious and



There is asymmetrical necrosis and gelatinous edema within the thalamus. (Photo courtesy of: Faculdade de Veterinária – UFRGS, Setor de Patologia Veterinária, Av. Bento Gonçalves 909091540-000 Porto Alegre RS, Brazil. http://www.ufrgs.br/patologia/)

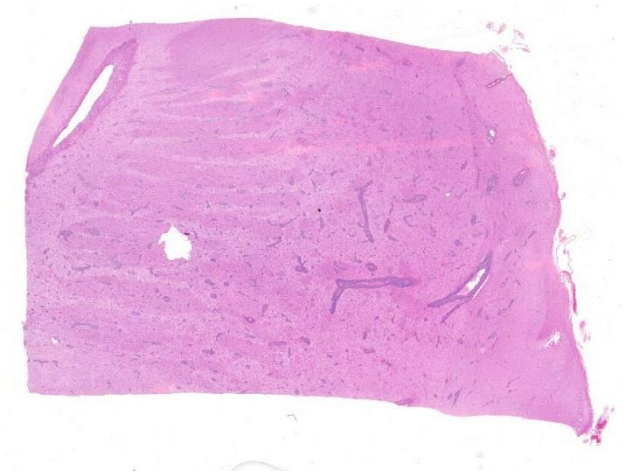
characterized by progressive weight loss (despite voracious appetite), lethargy, incoordination, gait instability (wobbling) involving the pelvic limbs (mal das cadeiras = disease of the hip), atrophy of heavy muscles of the hindquarters, difficulty in rising, muscle weakness, pallor of mucous membranes, subcutaneous edema of the ventral portions of the trunk and limbs. Additionally to above described signs that ran a course of 14 days, encephalic neurologic clinical signs including marked ataxia (Fig. 1), blindness, circling, hyperexcitability, somnolence, proprioceptive deficits, head tilt, and paddling movements were observed and ran a course of 20 days.

Gross Pathology: There were marked hindquarter muscle atrophy, splenomegaly, and lymphadenomegaly. Gross lesions in the brain included asymmetric swelling of the cerebral hemispheres with flattening of gyri. The white matter of the parietal, temporal, and frontal lobes was unilaterally yellow, gelatinous, and friable due to severe edema and malacia. Similar lesions were observed in the basal nuclei, thalamus, and mesencephalon.

Laboratory Results: Hematologic findings included normocytic normochromic anemia, with leukocytosis due to lymphocytosis. Erythrophagocytosis and protozoa identified as *T. evansi* were seen in the peripheral blood. High titers against *T. evansi* (optical density, 1,422) were detected in the serum of this horse.

Histopathologic Description: Histologically, lymphoid organs had marked follicular hyperplasia, erythrophagocytosis, and hemosi-

derosis. In the liver there was moderate lymphoplasmacytic periportal hepatitis, Kupffer cell hypertrophy, and hemosiderosis.



Thalamus, horse. Even at very low magnification, blood vessels are highlighted by prominent perivascular cuffs. (HE, 5X)

The brain lesions affected mainly the white matter and were characterized by moderate to severe perivascular lymphoplasmacytic meningoencephalitis, edema and necrosis. Lymphocytes and plasma cells, often with intracytoplasmic Russell bodies (Mott cells), greatly expanded the Virchow-Robin spaces and extended into the surrounding neuropil. Lesions in the spinal cord tended to wane from cranial to caudal. Organisms of *T. evansi* were not detected in the HE stained sections of brain, but numerous *T. evansi* organisms or fragments of these organisms were detected in formalin-fixed, paraffin-embedded sections of the brain by immunohistochemistry through anavidin-biotin-peroxidase complex immunoperoxidase method using rabbit anti-*T. evansi*, diluted 1:1000 as the primary antibody (the immunohistochemistry tests were performed at the Prairie Diagnostic Service, University of Saskatchewan, Western College of Veterinary Medicine). The parasites were observed in the perivascular spaces an in the neuropil.

Contributor's Morphologic Diagnosis:
Lymphoplasmacytic encephalitis, moderate to severe, 4-year-old, castrated male, crioulo, *Equus caballus*, horse.

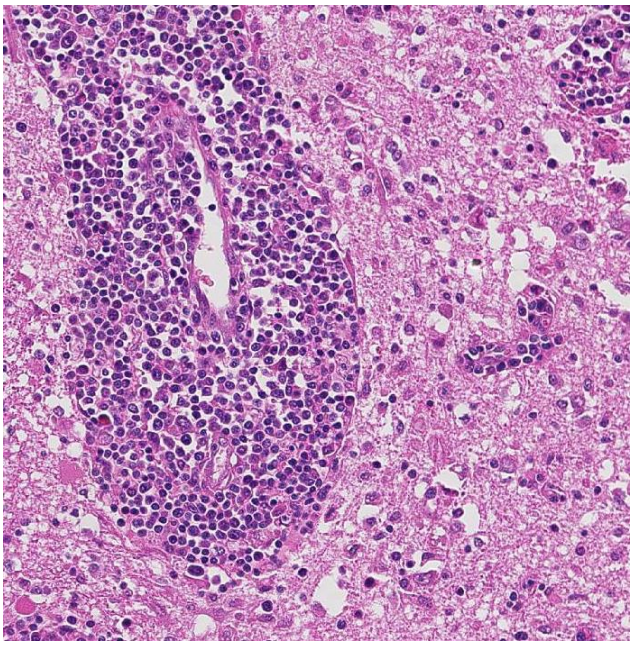
Etiologic diagnosis: Protozoal encephalitis

Etiology: *Trypanosoma evansi*

Name of the condition: Trypanosomiasis

Contributor's Comment: *Trypanosoma evansi* is a flagellate protozoan parasite that causes disease in several mammalian species. The hind limb weakness of equine trypanosomiasis gave rise to denomination "mal das cadeiras" or "mal de caderas" respectively in in Brazil and in many of Spanish speaking countries of South America. Other denominations for the same disease are murrina (Panama) and surra (Asia). In South America, capybaras (*Hydrochoerus hydrochaeris*) coatis (*Nasua nasua*) small marsupials (e.g., *Monodelphis* spp.), and armadillos (*Dasypus* spp.) are possible reservoirs for *T. evansi*. Unlike the African *Trypanosoma* species that cause nagana in animals and ''sleeping sickness'' in human beings, *T. evansi* does not require development stages within its the vector, and is mechanically transmitted through the bite of insects, especially tabanids and stomoxids, by the South American vampire bat (*Desmodus rotundus*) and, possibly, by ticks.[3,6]

Trypanosomiasis in horses is characterized by intermittent fever, anemia, progressive



Thalamus, horse. Virchow-Robins space is expanded by up to 15 layers of lymphocytes with fewer histiocytes and rare plasma cells (HE, 144X).

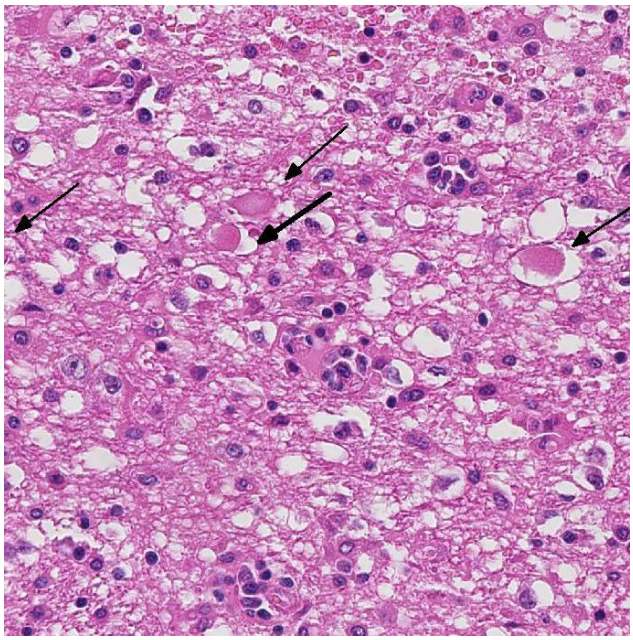
weakness, loss of body condition, and unstable gait.[21] Neurologic signs have been described occasionally in horses in the terminal phase of natural infection by *T. evansi*.[21] Trypanosomiasis by *T. evansi* is enzootic in horses from the Midwestern Brazil, mainly the Pantanal region.[3,4,6,9,22, 23]

T. evansi induces a wasting disease with a protracted clinical course[28] associated with anemia and instability of pelvic limbs in horses, camels, and dogs.[4] Natural infection by *T. evansi* rarely is recognized as a cause of encephalitis in horses.[21] Neurologic signs have been reported in cattle[27] and hog deer[26] naturally infected by *T. evansi*; however, the histopathologic changes in the brain of these species were not fully described. Mild lymphoplasmacytic meningo-encephalitis was reported in horses, donkeys, dogs, goats,[2] coatis (*Nasua nasua*),[9] and buffalo[25] experimentally infected by *T. evansi*. The horse of this report and the other necropsied horses in the current outbreak had a severe lympho-plasmacytic meningoencephalitis with marked edema and variable necrosis. The lesions in the brain of these horses resembled those described in horses infected by *Trypanosoma brucei brucei*,[14,17] which causes a disease known as nagana, and those described for human trypano-somiasis caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. [13,16]

T. evansi antigen was detected by immunohistochemistry in the brain of 8 of 9 horses affected in the outbreak, including the brain of the horse of this report. The protozoa were detected within blood vessels, in perivascular spaces, and in the brain parenchyma. This suggests that trypanosomes invaded the brain parenchyma and were responsible for the lesions observed here. The pathogenesis of cerebral necrosis was not elucidated since thrombosis was absent. Neither the mechanism by which trypanosomes entered the neuroparenchyma was determined. The introduction of *T. evansi* into a naïve population, as was the case here, could explain the development of fatal trypanosomiasis with severe encephalitis. The reason for the sudden appearance of the disease in this region of Brazil, where it was previously unreported, is unknown, but it could have been introduced by transportation of infected horses or migration of capybaras from regions where trypanosomiasis is enzootic in this country. A factor that may have contributed to the development of severe encephalitis was that these affected horses were treated with subtherapeutic doses of diminazene aceturate and other antitrypanosomal drugs.[19,20] Several studies have demonstrated that the use of subtherapeutic doses of diminazene aceturate may prolong survival of horses experimentally infected by *T. brucei* spp. however this faulty procedure results in subsequent invasion of the central nervous system by the organisms which induce necrotizing encephalitis.[8,10,11,15,18,24] Diminazene aceturate clears trypanosomes from tissues except those localized on the central nervous system,[19] because the drug does not cross the blood-brain barrier[11,17] allowing trypanosomes harbored in the central nervous system to survive antitrypanosomal therapy; a change in these organisms surface glycoproteins favor recurrent parasitemias by variants of the organism.[20]

How trypanosomes penetrate the blood-brain barrier is not clear, but several mechanisms have been proposed: (i) entrance through sites where the blood-brain barrier is incomplete, such as sensory ganglia and circumventricular organs;[16,] (ii) increase in vascular permeability due deposition of immune complexes in the choroid plexus; and (iii) opening of intercellular tight junctions of the ependymal lining of the ventricular system by toxins released by the parasite.[12] It is believed that invasion of the central nervous system by trypanosomes occurs where the blood-brain barrier has been disrupted, either directly by the parasites or by release of chemical mediators, such as cytokines and proteases.[7,13,15] An interaction between the trypanosomes and the host response could promote tissue damage and facilitate the entry of parasites into the central nervous system.[13] This scenario is supported by the histopathologic changes and immunohistochemistry observe in the horse of this report. Trypanosomiasis due to

T. evansi should be considered in the differential diagnosis of encephalitis in horses in regions where the disease is enzootic.



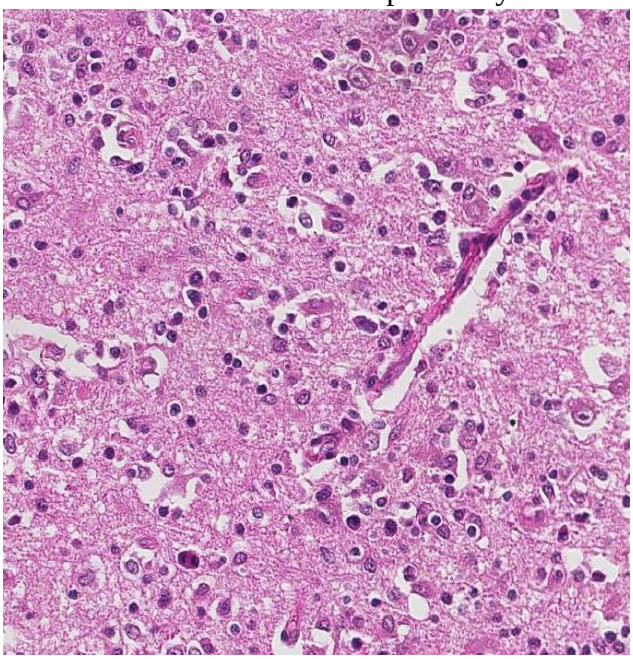
Thalamus, horse. Within affected white matter, there are frequent swollen axons (spheroids) (arrows). (HE, 168X)

The list of differentials should include equine herpesvirus type 1 myeloencephalopathy, Eastern equine encephalitis, Western equine encephalitis, Venezuelan equine encephalitis, equine protozoal myeloencephalitis, West Nile virus infection, and rabies. The nature and distribution of lesions in the central nervous system of horses with naturally occurring *T. evansi* infection should help to distinguish trypanosomiasis from viral or other protozoal infections.

JPC Diagnosis: Telencephalon: Meningoencephalitis, lymphoplasmacytic and histiocytic, diffuse, severe with vasculitis, spongiosis and gliosis.

Conference Comment: Conference participants were struck by the marked degree of perivascular cuffing and described the presence of edema, lymphocytes, and macrophages (Gitter cells) expanding Virchow-Robin spaces. They also described lymphocytic infiltration, gliosis, gemistocytes and neuronal degeneration and necrosis in the surrounding neuropil. The meninges were also expanded by a lymphoplasmacytic infiltrate and edema. Some sections contained areas of vascular damage with the presence of cellular debris, protein and hemorrhage in vessel walls and therefore some participants also described vasculitis. Multifocally, there are hypertrophied endothelial cells in the less affected vessels.

T. evansi is the causative agent of "surra," which originated in Africa, but is also present across the Middle East, Asia, in Central and South America and has also been reported in Russia. It is thought to derive from genetic modification (a deletion) in *T. brucei*. It has a wide host range, indeed the widest among salvarian trypanosomes, including camels, dogs, horses, deer, llamas, cattle, sheep, goats, cats, pigs, and elephants among others. Disease is particularly devastating in camels and horses, often proving fatal in the absence of treatment. Clinical signs can vary dramatically between species and even between individuals of the same species. Infection in camels can occur acutely as in horses, but often has a more protracted course with signs such as intermittent fever, weakness, anorexia, weight loss, abortion, anemia, edema and petechial and ecchymotic hemorrhages. All age groups are affected and the course of the disease can be up to 2-3 years. The



Thalamus, horse. There is marked gliosis in affected areas within the grey matter. (HE, 168X).

urine of infected camels apparently has a specific odor, which may allow diagnosis of the disease. Dogs are also highly susceptible to infection and death may result at 1-4 weeks post infection. i

Trypanosomes have developed mechanisms which allow them to both evade the immune system and cause immunosuppression. This includes antigenic variation in main membrane surface glycoproteins, variant surface glycoprotein (VSG), which forces the host to constantly redevelop their humoral response. The immunosuppressive effects of *T. evansi* infection are not fully understood but may involve modulation of macrophage activity, decreased responsiveness of lymphocytes, and/or changes in the CD4:CD8 lymphocyte ratio; they may even be capable of eliminating memory B cells. The complex immunosuppressive or immunomodulatory mechanisms of *T. evansi* have created significant barriers in developing effective vaccines and maintaining effective treatments.[5]

Contributing Institution:
Setor de Patologia Veterinária, UFRGS
http://www.ufrgs.br/patologia/

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CASE II: E 4992/14 (JPC 4067274).

Signalment: 5-year-old gelding horse (*Equus ferus caballus*).

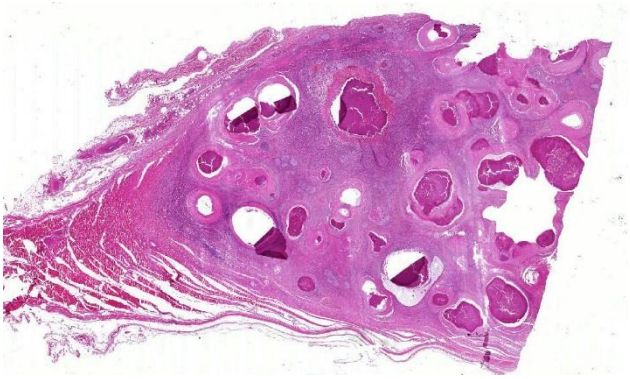
History: A solitary, non-painful, superficial mass in the skin over the left scapula was recognized by the owner. Only minimal growth was apparent during the last six months.

Gross Pathology: One sample measuring 7.1 x 5.5 x 1.7 cm with a well demarcated mass of 5.2 x 4.5 x 1.7 cm was submitted for histopathological examination.

Laboratory Results: None

Histopathologic Description: Subcutis (per contributor, connective tissue and skeletal muscle): Expanding the subcutis and infiltrating, separating and surrounding collagen bundles and skeletal muscle fibers is a mostly unencapsulated, poorly circumscribed mass. It is composed of small nests of round cells with distinct cell borders and moderate amounts of amphophilic cytoplasm that occasionally contains small basophilic granules (mast cells). Nuclei are centrally located, mostly round with finely stippled chromatin and indistinct nucleoli. Mitotic rate is low with less than one mitotic figure in ten high power fields (400x). Mast cell islands are separated by abundant fibrovascular stroma with thick bundles of collagen and high numbers of variable sized eosinophilic granulomas. Granulomas are characterized by central accumulation of partly degenerated eosinophils and peripherally bordered by few macrophages, variable numbers of fibroblasts / fibrocytes, abundant collagen fibers and few lymphocytes and plasma cells. Occasionally central debris is mineralized (deposition of fine granular basophilic material) and collagen bundles are hyalinized (collagenolysis). Intermingled between mast cell islands and granulomas are high numbers of viable eosinophils and fewer extravasated erythrocytes. Rarely, vessel walls are severely thickened by edema and few migrating eosinophils.

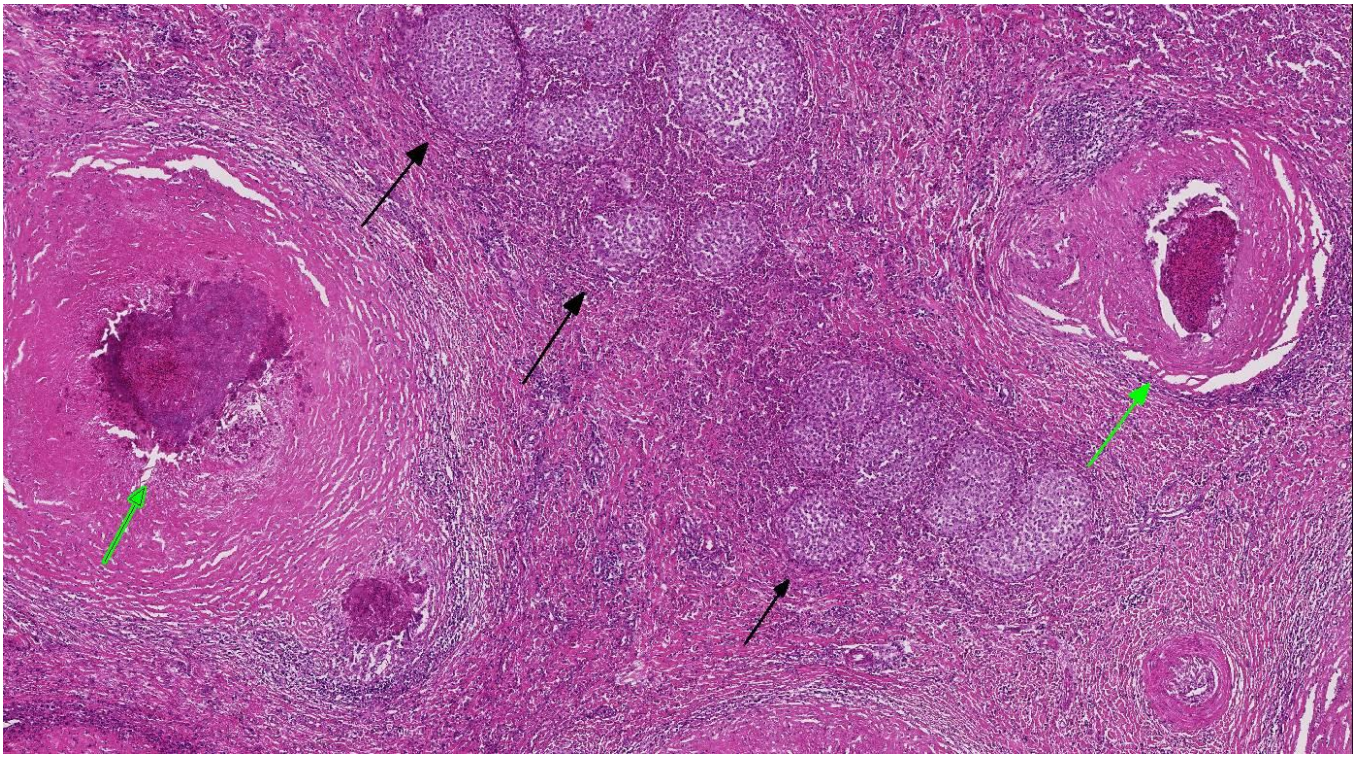
Contributor's Morphologic Diagnosis: Subcutis (per contributor): Mast cell tumor



Skin, horse. A section of subcutis with attached underlying skeletal muscle is submitted for examination. (HE, 6X).

Contributor's Comment: Mast cell tumors are common dermal neoplasms especially of dogs and also cats, but occur in all domestic species including horses, ruminants, pigs, and ferrets. Depending on affected site and species the biological behavior is variable. Besides the skin, mast cell tumors occasionally arise in parenchymal organs like the spleen, liver, intestine, or as mast cell leucosis affecting the bone marrow. Additionally, metastasis from a cutaneous site may manifest in regional lymph nodes or generalize to diverse locations.

Mast cells are involved in numerous inflammatory conditions, especially type I hypersensitivity. After binding of IgE to membrane receptors and cross-linking of immunoglobulins on these receptors by specific antigens, degranulation of stored primary mediators and de novo synthesis of secondary mediators occurs. Granules contain, among others, primary mediators like biogenic amines (histamine, adenosine and serotonin), chemotactic factors (eosinophil chemotactic factor and neutrophil chemotactic factor) and multiple enzymes. Secondary mediators include leukotrienes, prostaglandins, platelet activating factor and several cytokines (TNF-alpha, IL-1, 3, 4, 5 and 6). In summary, these mediators generate edema, mucus secretion, smooth muscle spasm, and the recruitment of additional leukocytes, especially eosinophils.



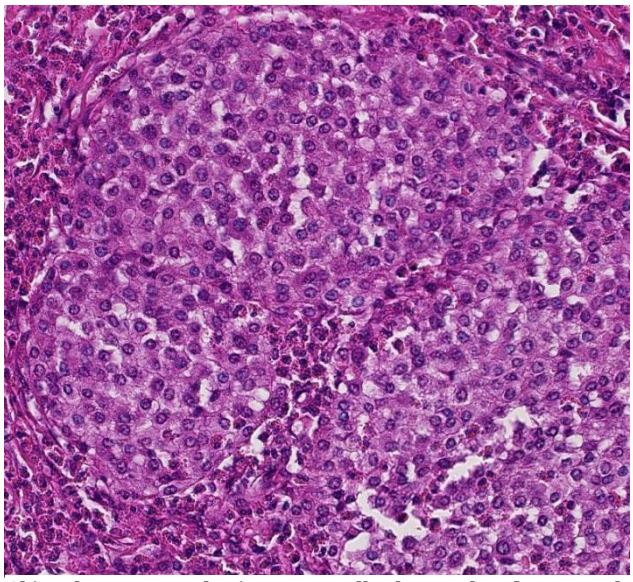
Skin, horse. The neoplasm is composed of nests of neoplastic round cells (black arrows) and eosinophilic granulomas (green arrows) scattered throughout a dense collagenous stroma. (HE, 25X)

In the dog, mast cell tumors are common, accounting for 15 – 20 % of all skin tumors, being additional the most frequent malignant or potentially malignant cutaneous neoplasia.[2] Well differentiated neoplastic cutaneous mast cells typically form diffuse loose sheets or densely packed cords with intermingled mature eosinophils. They are never encapsulated or even well demarcated. Ulceration may occur in large tumors after traumatization due to pruritus triggered by mediators degranulated from mast cells. The proto-oncogene c-KIT has been discussed as a possible prognostic factor in canine cutaneous mast cell tumors with mutations or aberrant expression of c-KIT turned out to be poor predictors.[13]

In horses, mast cell tumors are relatively rare, covering only 3 – 7% of cutaneous and muco-cutaneous neoplasms.[11] Commonly affected horses are male without an apparent breed predilection and the mean age is 7 - 11 years with a range from 1 to 30 years.[1, 5,6] In a recent large study Arabians were affected more frequently.[1] Tumors typically present as solitary immovable nodules on the head, trunk, neck, and limbs, where they are often found close to joints.[11] The tumors may be hyperpigmented, alopecic or ulcerated.[3] Nevertheless, most tumors are neither painful nor pruritic.[3] Usually, mast cell tumors in the horse behave benign and excision is curative as recurrence is uncommon.[5]

Histologically, cutaneous mast cell tumors in horses often differ from their counterparts in the dog. Foci of necrosis, severe eosinophilic infiltration with subsequent collagenolysis, reactive fibrosis and dystrophic mineralization are often present. In this respect mast cell tumors may resemble equine collagenolytic granulomas or verminous dermatitis due to onchocerciasis or habronemiasis.[2] Typically, neoplastic mast cells are well-differentiated and arranged in multifocal small nests or coalescing nodules.[11] Malignant tumors are rare, presenting with loss of cellular differentiation and high mitotic index.[8] Local but not visceral metastasis has been documented.[8,9] In contrast to canine mast cell tumors, neither changed c-KIT staining pattern nor histologic features were associated with an unfavorable clinical course.[1]

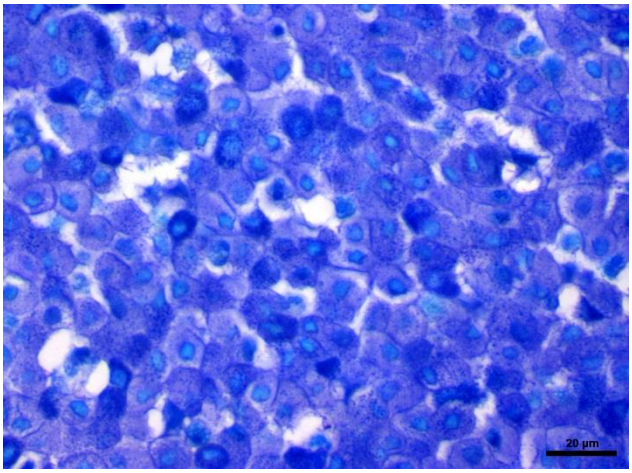
JPC Diagnosis: Subcutis and skeletal muscle: Mast cell tumor.



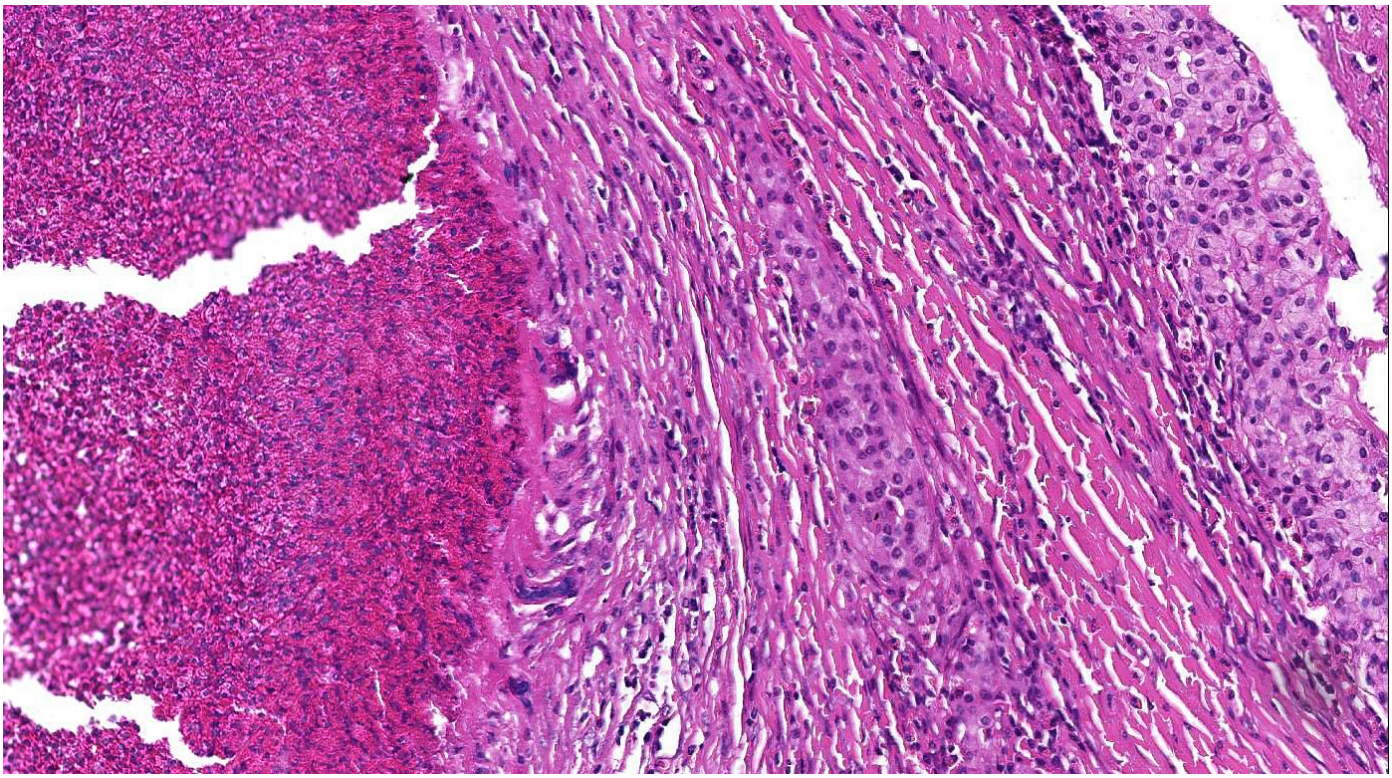
Skin, horse. Neoplastic mast cells have abundant poorly granular cytoplasm with centrally-placed nuclei (HE, 196).

Conference Comment: This was a challenging slide for many conference participants with the histologic description largely focusing on the presence of eosinophilic granulomas. Other differential diagnosis considered for this lesion included equine nodular collagenolytic granuloma, cutaneous habronemiasis, cutaneous pythiosis and multisystemic eosinophilic epitheliotropic disease. At sub gross the eosinophilic granulomas were the most prominent feature, and due to the paucity and/or inconspicuous nature of the equine mast cell granules the cells were easily overlooked and/or misinterpreted.

In canine mast cell tumors, the proto-oncogene *c-kit* encodes for the transmembrane receptor tyrosine kinase (RTK) KIT, which normally plays a role in transcription of genes that control mast cell growth and survival, but also plays a role in tumor development in some tumors. A percentage of canine mast cell tumors demonstrate mutations in *c-kit*, including internal tandem duplication (ITD) mutations in exon 11, which result in activation of KIT in the absence of ligand binding, as well as point mutations in *c-kit* extracellular domains at exons 8 and 9. Specific mutations in the gene that encodes the KIT RTK results in constitutive activation of the RTK (via tyrosine phosphorylation) and cell proliferation in the absence of growth factor stimulation. In general tumors with ITD mutations in *c-kit* exon 11 are known to behave more aggressively.[7] However, the presence of these specific mutations also results in a more favorable response to treatment with tyrosine kinase inhibitors (TKIs) as compared to mast cell tumors without these specific mutations. Additionally, it has also been demonstrated that *c-kit* mutations are conserved between the primary tumor and lymph node metastasis, indicating that both can be used for mutational testing to select patients that will respond to favorably to TKIs.[4] Mutations in KIT genes may also result in changes in cellular proteins involved in motility and the cytoskeleton.[10] To our knowledge, analogous studies on equine mast cell tumors and their response to TKI therapy have not been published at the time of this conference. Additionally, another study of canine mast cell tumors showed that in addition to KIT, cellular expression patterns of the RTK vascular endothelial growth factor receptor 2 (VEGFR2) may also be predictive of a poorer prognosis.[12]



Skin, horse. A Toluidine blue stain demonstrates few intracytoplasmic granules within equine mast cells. (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin, http://www.vetmed.fu-berlin.de/en/einrich-tungen/institute/we12/index.html))



Skin, horse. The eosinophilic granuloma at left is bounded by a layer of epithelioid and foreign body type giant cells and concentric lammelations of collagen, which entrap trabeculae of neoplastic mast cells at center and right. (HE, 100X)

Contributing Institution:
Department of Veterinary Pathology, Freie Universität Berlin, Germany,
http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html

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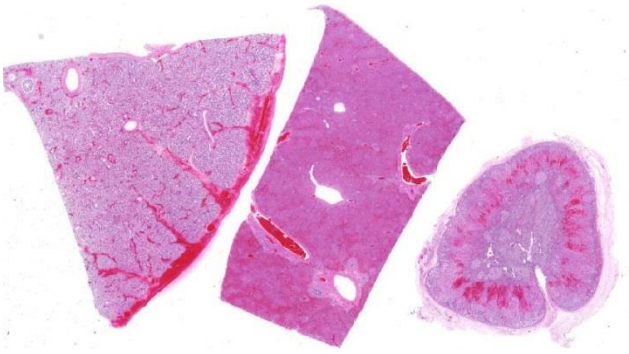
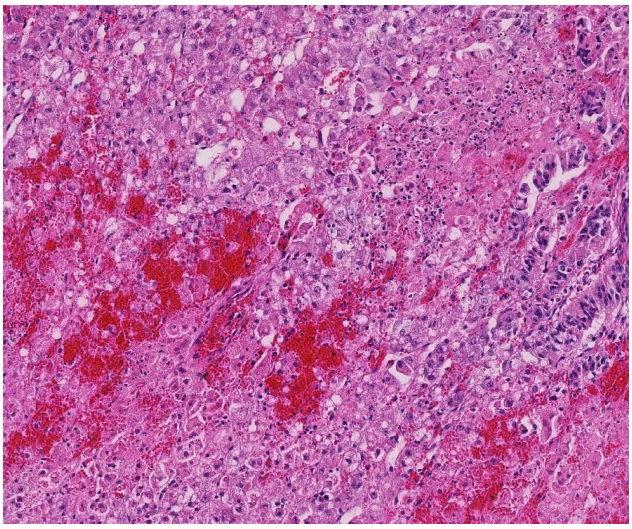
CASE III: E-6398-14 (JPC 4048653).

Signalment: 5 day-old, male, Quarterhorse (*Equus caballus*).



A section of lung, liver, and adrenal gland are submitted. (HE, 5X)

History: The foaling was not witnessed but there were no obvious signs of dystocia or distress. The colt was not seen drinking colostrum but was seen nursing the next morning. By 36 hrs of age, he became depressed, weak and stopped nursing. His condition progressively worsened and he was presented to the veterinary teaching hospital at 48 hrs of age. Bloodwork performed at that time revealed severe leukopenia, azotemia, hypoproteinemia, severe dehydration, hypoxemia, hypercapnia, and acidosis. After 48 hrs in hospital, the colt developed thrombocytopenia. Despite fluid therapy, oral mucous membranes and coronary bands became severely hyperemic and the extremities became cool. He then broke with severe watery, yellow diarrhea. He died early on the morning of his third day of hospitalization.



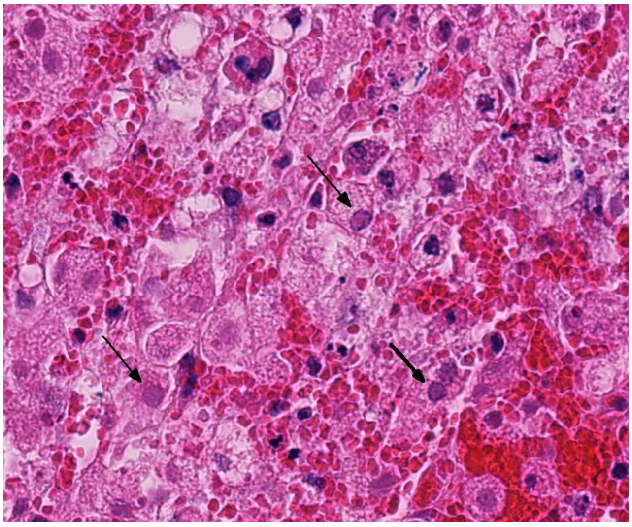
Adrenal gland, horse. There are confluent areas of necrosis and hemorrhage throughout the adrenal cortex. (HE, 92X)

Gross Pathology: The carcass was in fair body condition having small body fat stores. Oral mucous membranes, the conjunctiva and most soft tissues were faintly yellow. Approximately 1 L of cloudy, yellow brown fluid filled the thorax. Approximately 200 mls of similar fluid was noted in the pericardial sac. The thymus was pale and contained many petechia. The lungs were diffusely dark red, very heavy, wet and rubbery. Sections sunk when placed in formalin. There were multifocal to coalescing petechia and ecchymoses on the epi- and endocardial surfaces of the heart.

The abdomen contained approximately 500-700 mls of yellow-brown cloudy fluid. The liver was diffusely dark red-brown with slightly rounded edges with many, small, randomly scattered, pinpoint white foci scattered throughout. The

spleen was moderately enlarged, dark red-black and oozed blood on cut surface. Frequent tiny petechia were scattered on the serosa of the gastro-intestinal tract.

Laboratory Results: Antemortem blood cultures - *Actinobacillus equuli* and *Streptococcus sp*. were isolated.
Fecal culture (antemortem sample) - no *Salmonella* sp. isolated
Aerobic cultures of lung and liver (postmortem samples) - no microbial growth
RT-PCR on pooled liver and lung samples - positive for Equine herpesvirus-1



Adrenal gland, horse.Numerous cells within necrotic areas of the cortex contain intranuclear herpesviral inclusions (arrows). (HE, 280X)

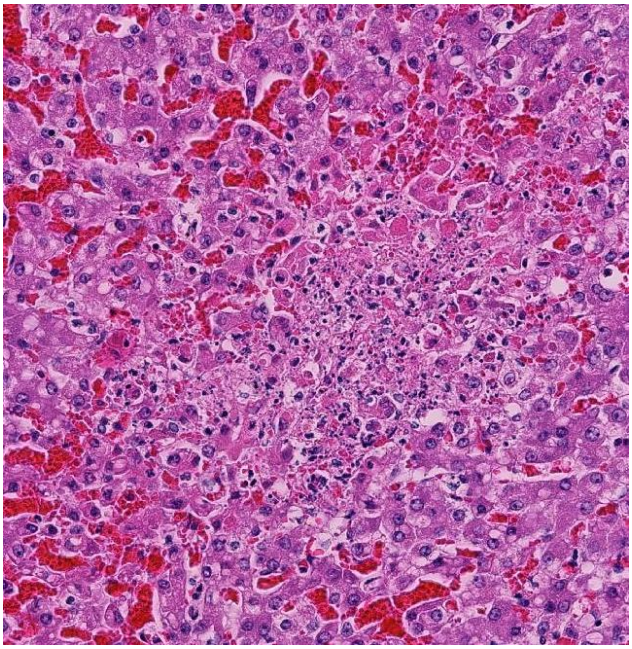
Histopathologic Description: Sections of lung were diffusely moderately congested. The adventitia surrounding medium to large caliber arteries were diffusely expanded with pale edema fluid and frequent foci of hemorrhage. Alveoli were diffusely distended with pale edema fluid, and sometimes contained small flakes of aspirated keratin and/or small amounts of pale proteinaceous debris. Interlobular septa and the pleura were often moderately to markedly expanded due to edema and variably sized, sometimes large areas of hemorrhage. Occasionally randomly scattered within the parenchyma were small pale foci of acute necrosis where alveoli contained small aggregates of fibrin, few neutrophils and sparse cell debris which partially obscured interalveolar septa. Rare cells in and around these foci (assumed to be alveolar pneumocytes) contained pale, pink, intranuclear inclusion bodies (INIB) which marginated chromatin.

Hepatic sinusoids were often mildly congested. Frequent, randomly scattered foci of lytic necrosis were present throughout the sections. Within these foci, hepatocytes were replaced by pale proteinaceous debris admixed with sparse karyorrhectic debris. Hepatocytes at the margins of these foci sometimes contained pale pink, intranuclear inclusions. Rare dense aggregates of bacteria were noted within sinusoids.

There were frequent, multifocal to coalescing areas of hemorrhage and lytic necrosis within the adrenal cortex. Adrenocortical cells at the periphery of areas of necrosis also often contain INIBs similar to those described above. Small foci of acute lytic necrosis were noted in the spleen and thymus.

Contributor's Morphologic Diagnosis:
1. Lung: Severe, diffuse, acute, pulmonary congestion and edema with frequent foci of hemorrhage and occasional, mild, multifocal (embolic), fibrinonecrotizing, pneumonia with rare intranuclear inclusion bodies (INIBs).
2. Liver: Moderate, acute, multifocal and random, necrotizing hepatitis with intrahepatocellular INIBs.
3. Adrenal gland: Severe, acute, multifocal to coalescing, necrotizing and hemorrhagic, adrenalitis with INIBs.

Contributor's Comment: Equine herpesvirus-1 (EHV-1) is a common cause of respiratory disease, neurologic disease, and abortion in horses. The virus is widespread with a worldwide distribution. Abortions may occur several weeks to months after exposure and usually occur in the last 3-4 months of gestation. There is generally no premonitory clinical signs in the dam with rapid expulsion of a fresh, minimally autolyzed fetus. Abortions may occur



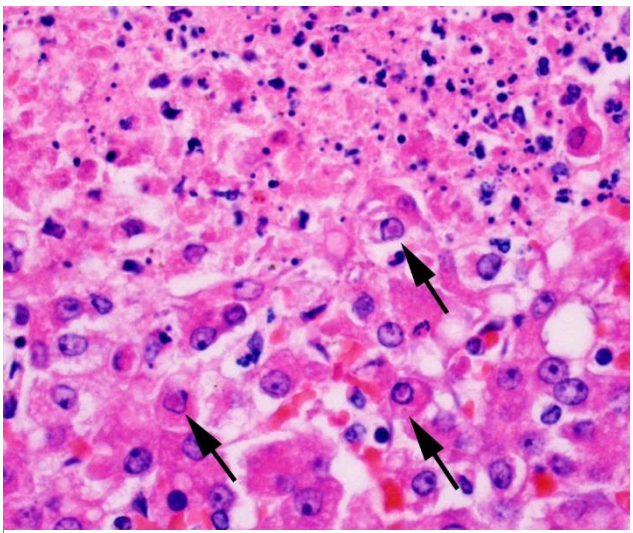
Liver, horse. There are randomly scattered areas of necrosis throughout the liver. (HE, 188X)

sporadically within a herd or as outbreaks typically affecting multiple mares over a short period of time.[3, 2]

Generalized neonatal disease, as demonstrated in this case, is also reported where foals infected in utero may be born alive. As in this case, infected foals generally die in the first few days of life due to acute interstitial pneumonia, pulmonary edema, and secondary septicemia. *Actinobacillus equuli* and *Streptococcus sp*. are commonly isolated agents. Interestingly, one reference[2] indicated that while areas of hepatic necrosis are common in aborted fetuses, such lesions are generally not present in neonatal foals succumbing to systemic EHV-1 infection. However, in this 5 -ay old foal, hepatic necrosis with INIBs was a prominent finding.

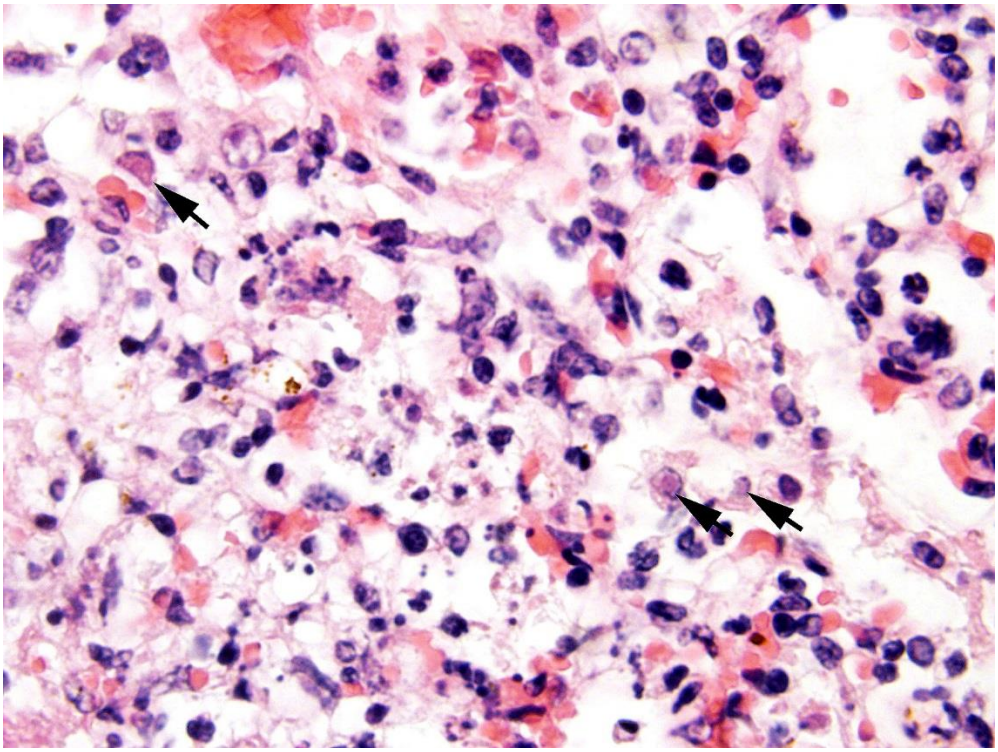
In natural disease conditions, EHV-1 infects epithelial cells in the upper respiratory tract which may cause mucosal damage, predisposing affected horses to infection with other respiratory pathogens (bacteria, fungi, etc). Subsequent infection of circulating leukocytes (typically monocytes and T cells) enables the virus to disseminate to other organs including the uterus and central nervous system. Infection of endothelial cells in the gravid uterus and CNS (in neurologic cases) may result in vasculitis and thrombosis. Acute and severe disruption of placental circulation is likely responsible for the sudden death and expulsion of fresh fetuses which is characteristic of EHV-1 abortion.[1]

Exposure to EHV-1 is very common in most horse populations. Horses, including foals and young horses, in large breeding/training facilities are often seropositive for EHV-1, with or without any signs of respiratory disease.[1] Infections are usually acquired via nasal secretions from the dam, other foals, pasture mates, and/or from contact with fomites. Latent infections are common; the virus may be harbored in the trigeminal ganglia or in lymphoid cells. Latently infected horses do not shed virus and are clinically normal. However, under certain conditions, there may be reactivation of the virus and recrudescence of infection. The virus can be reactivated experimentally in horses by administration of high doses of glucocorticoids. Stressful situations such as transport, sales, competitions, or mixing of horses, as well as immunocompromise due to concurrent disease, are thought to be factors leading to recrudescence of disease. In closed herds, abortions due to EHV-1 infection are often attributed to recrudescence of infection in individuals, which may or may not be accompanied by clinical disease, and who subsequently may shed virus and act as a source of infection to other exposed, susceptible horses.[1] There was frequent move-



Liver, horse: Hepatocytes on the periphery of the areas of necrosis contain intranuclear herpesviral inclusions. (HE, 300X).

ment of horses within the barn that this mare and foal originated from, so both late gestational infection and/or recrudescence of infection in the mare are possibilities in this case.



Lung, horse. Type 1 pneumocytes within areas of septal necrosis in the lung contain intranuclear herpesviral inclusions (arrows). (HE, 320X)

JPC Diagnosis:
Adrenal gland: Adrenalitis, necrotizing and hemorrhagic, multifocal to coalescing, severe with intranuclear viral inclusion bodies.
Liver: Hepatitis, necrotizing, multifocal and random, marked with intranuclear viral inclusion bodies.
Lung: Pneumonia, interstitial, necrotizing, diffuse, mild with necrotizing vasculitis and intranuclear viral inclusion bodies.

Conference Comment: The adrenal gland has linear areas of hemorrhage and lytic necrosis within the zona reticularis and zona fasciculata, with the presence of both degenerate (vacuolated) and necrotic adrenal cortical cells which contain characteristic alpha herpes viral inclusions. The lytic necrosis within the liver is random with the presence of fewer inclusion bodies in degenerate and necrotic hepatocytes, and few conference participants reported identifying rare syncytia within the section. The septal and subpleural hemorrhage within the section of lung is impressive. Some participants reported inclusion bodies within the endothelium, and the degree and nature of vascular damage in both the lung and liver is necrotizing, though more prominent in the lung. There is also septal necrosis and infiltration of inflammatory cells into the interstitium in the section of lung. Other differential diagnosis discussed for these lesions included equine adenovirus, equine arterivirus and African horse sickness; although the nature and distribution of lesions and characteristic alpha herpesviral inclusion bodies made for a less ambiguous diagnosis.

Equine herpesvirus infection within the central nervous system is less common than the respiratory and abortive manifestations of EHV-1, only occurring in a small percentage of infected horses. However, the neurologic form can be particularly devastating, ultimately resulting in a vascular origin myeloencephalitis, which begins with an upper respiratory infection as described above. The precise factors determining why some horses develop neurologic signs is not well understood, but may be related to infection with certain virus strains. In contrast to CNS herpes viral infections in some other domestic species (bovine – IBR, porcine – pseudorabies), EHV-1 is not neuronotropic, although neurons and astrocytes may become infected, and the CNS form of the disease is more commonly seen in adult horses. Circulating infected cells, primarily T lymphocytes and monocytes, spread the virus to endothelial cells

of small vessels of the CNS resulting in vasculitis, thrombosis and infarction of tissues supplied by those vessels. Lesions can occur throughout the CNS, including the spinal cord, but inclusion bodies within the CNS are generally not seen.[5] Gross lesions consist of randomly distributed areas of malacia with accompanying hemorrhage and edema,[4] reflecting the vascular nature of the lesion.

Contributing Institution:
Department of Pathology/Microbiology
Atlantic Veterinary College, University of Prince Edward Island
www. avc.upei.ca

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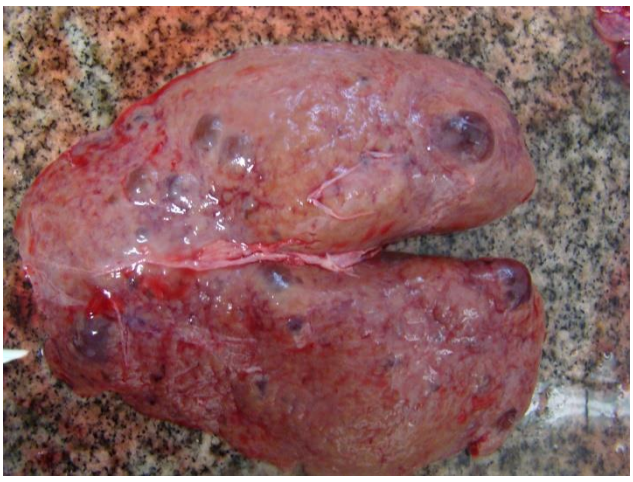
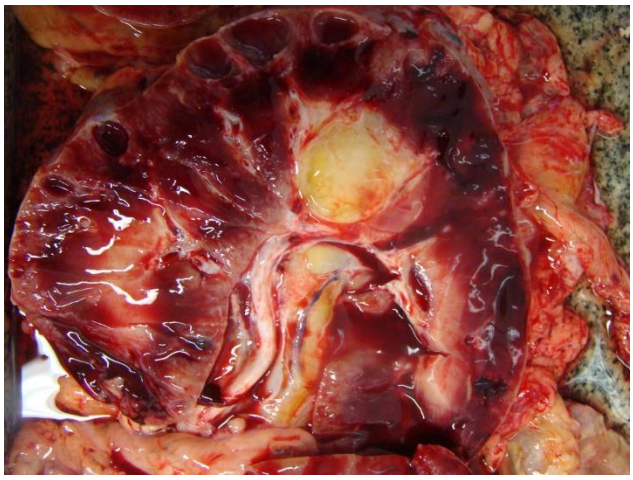
CASE IV: Case III (JPC 4001093).

Signalment: 17-year-old, female, intact Brasileiro de Hipismo equine (*Equus caballus*).

History: A 17-year-old, female, Brasileiro de Hipismo equine was presented to the Equine Hospital of the Faculty of Veterinary Medicine and Zootechny, University of São Paulo (FMVZ-USP), São Paulo, Brazil with the history of acute progressive weight loss, hyporexia and selective appetite in the last 15 days. Food intake had been declining progressively and presently the horse was anorexic. No difficulties in prehension or mastication had been noticed. At the veterinary hospital, the animal was treated with ringer lactate and 5% glucose fluid/ IV. Physical examination was unremarkable except for an increased rectal temperature (39.2°C) in the first day and at rectal examination the left kidney was enlarged. The clinical course followed to depression, emaciation (486 Kg), prostration, ataxia, labial and hindlimb edema, epistaxis and mildly pasty diarrhea. Samples of blood, serum and urine were collected for lab analysis. Renal failure was confirmed by marked increased blood urea nitrogen (BUN) and creatinine, and those levels were 400 mg/dL and 14.4 mg/dL, respectively. The hematology showed leukocytosis and neutrophilia (13.617/ mm3). Urine analysis revealed low normal specific



Kidney, horse. The right kidney was enlarged and tan with a irregular capsular surface and numerous cysts visible through the capsule. (Image courtesy of: Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia , Av. Prof. Dr. Orlando Marques de Paiva, 87, Cep – 05508-000)



Kidney, horse. On cut section, there are numerous subcapsular cysts and the calyx is markedly distended. ***(Image courtesy of: Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia , Av. Prof. Dr. Orlando Marques de Paiva, 87, Cep – 05508-000)***

gravity (1.015), pH 8.0, moderate amount of hemoglobin (++) and moderate amount of protein (++). Microscopic examination of the sediment allowed identification of a moderate increase in leukocytes.

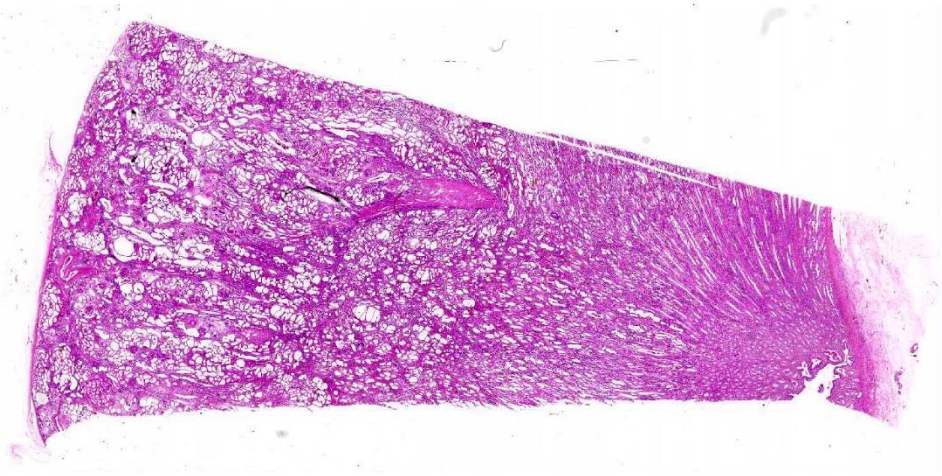
The percutaneous abdominal ultrasonography revealed an enlargement in both kidneys with multiple cystic cavities filled with anechoic fluid. A larger cyst, measuring 6.7 cm in diameter, was found in the left kidney. The right kidney was enlarged but smaller than the left and also displayed multiple cysts. The animal presented a rapid decrease in its general condition due to renal failure, and the lack of improvement to initial fluid therapy indicated a poor prognosis and euthanasia was advised after five days. The necropsy was performed a few hours later in the same day.

Gross Pathology: The animal was extremely thin, with ribs, lumbar vertebrae and pelvic bones easily visible. The pericardial sac, thoracic and abdominal cavity presented serous yellowish translucent free liquid of 100ml, 1L and 8L, respectively. There was a marked mesenteric and subcutaneous fat atrophy. The left kidney showed a marked increase in size, irregular brownish outer surface with multiple cystic cavities, measuring up to 2.0 cm in diameter and was filled by a yellowish turbid liquid, and no capsular adhesion. A large cyst was observed in the left cranial kidney pole, measuring about 6.5 cm in diameter and draining a reddish-yellow turbid liquid. Corticomedullary ratio was slightly increased and the kidney was irregular grayish tan. The consistency of the parenchyma was firm. The right kidney was also enlarged, presenting a brownish, irregular outer surface with multiple cystic cavities and without capsular adhesion. The corticomedullary ratio was increased, and there was a dilation of the renal pelvis and calyces, caused by the presence of yellowish firm sandy structures (calculi) measuring up to 2mm in diameter. The urinary bladder was moderately distended and contained translucent urine with fine sandy structures, and its mucosa was irregular, red and thickened. The aglandular gastric mucosa presented multiple ulcers, measuring up to 2.1 x 1.7cm, and there was a marked thickening (3.0 cm) of the glandular mucosa wall and dilated, congested and varicose vessels in the serous gastric membrane. The other organs were unremarkable.

Laboratory Results:

Hematology (ref. values):
RBC 6,7 x 106/mm³ (6.8 – 12,9 x 106/mm3)
Hematocrit 30 % (32-53%)
Hemoglobin 10.5 mg/ dL (11-19mg/dL)
MCV 44.78 fl
MCHC 35 %
WBC 15300 /mm³ (5400-14300 /mm³)
Neutrophils 89% (13.617) (2260-8580/ mm3)
Bands 0
Basophils 2% (306) (0-100/mm³)
Lymphocytes 7% (1071) (1500-7700/mm³)
Monocytes 0 (0-1000/mm³)
Eosinophils rare (0-800/ mm3)

Serum chemistry (ref. value):
Creatinine: 14,4 mg/dL (1.2-1.9 mg/dL)
BUN: 400 mg/dL (21.4-51.36 mg/dL)
Triglycerides: 807 mg/dL (22.9-46.0 mg/dL)
Cholesterol: 258.7 mg/dL (61-112 mg/dL)
Total protein: 6.4 mg/dL (5.2-7.9 g/dL)
Albumin: 2.9 mg/dL (2.65–3.69g/dL)
Ca e P: not measured



Kidney, horse. At low magnification, cortical tubules as well as Bowman's capsules are markedly dilated (HE, 4X)

Abdominal ultrasonography: showed hyperechoic, bilateral polycystic kidneys with the biggest cyst measuring 6.7 cm located in the left kidney cranial pole.

Urine culture (at necropsy): *Klebisella pneumoniae*

Pericardial fluid (at necropsy): pH 7.5, specific gravity 1014, protein 1.2g/ dL, 200 nucleated cells/ mm3, without bacteria.

Abdominal fluid (at necropsy): pH7.5, specific gravity 1012, protein 0,8g/ dL, 600 nucleated cells/ mm3, without bacteria.

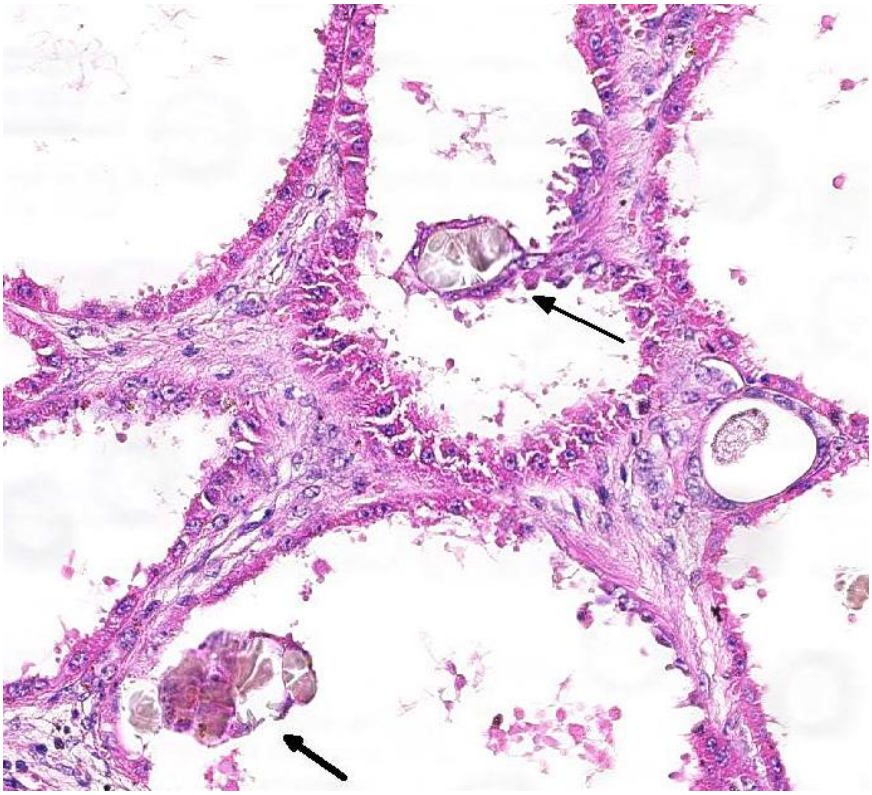
Histopathologic Description: The main alteration of the kidney histopathology is the presence of diffuse tubule and glomeruli cystic cavities of different sizes with a marked renal architectural loss. The cysts involve the corticomedullary tubules and most of them are in the cortex rather than in the medulla. Cysts are lined by flattened or cuboidal to columnar epithelium and some are divided by thin trabeculae or have papillae lumen projection. Few cysts have amorphous eosinophilic plugs (tubular proteinuria) in the lumen and were located in the cortex and medulla; some were associated with multifocal presence of crystals, and some showed hyaline plugs mix with degenerated leukocytes in the lumen. The glomeruli were generalized diffusely changed and the main features were basal membrane thickening and cellular proliferation, i.e. marked diffuse chronic membranoproliferative glomerulonephritis. There was glomerular crescent formation, glomerulosclerosis and several foci of glomerular obsolescence which can be characterized by shrunken, eosinophilic and hypocellular glomeruli. Occasionally, few glomeruli had cystic dilation and had glomerular tuft atrophy. These main features of cysts and glomeruli can be easily seen using Periodic Schiff Acid (PAS) and Masson's Trichrome stain (special stains were not sent). Mild tubular proteinuria (PAS-positive plugs) and moderate interstitial fibrosis were also clearly seen. There are also hemorrhagic foci and moderate suppurative interstitial nephritis. In addition, brownish granules consistent with hemosiderin pigmentation were observed in the tubular epithelium close to congestion areas and mild diffuse medial hypertrophy of small arteries could be seen. In some slide sections, small foci of mononuclear infiltrate were found in the collagenous capsule of a larger cyst (not present in all slides). Other findings consisted of moderate lymphoplasmacytic portal hepatitis associated with moderate vacuolization of hepatocytes and marked fibrosis, as showed by Masson's trichrome stain (not present in the slide). Marked medial hypertrophy and presence of small thrombi were also noticed in small arteries and capillaries of the spleen and liver (not present in the slide).

Contributor's Morphologic Diagnosis: Kidney: Tubular and glomeruli cysts, multiple, diffuse, marked associated with chronic membranoproliferative glomerulonephritis, glomerulosclerosis, and chronic interstitial nephritis with moderate interstitial fibrosis (polycystic kidney disease).

Contributor's Comment: Polycystic kidney disease (PKD) is described in several domestic and laboratory animal species as well as in humans. There were only few case reports published of PKD in exotic or wildlife species, including young springboks (*Antidorcas marsupialis*), young and adult slender lorises (*Loris lydekkerianus*), and adult Brazilian agoutis (*Dasyprocta leporina*).

Cystic diseases of the kidney include various conditions characterized by one or more grossly visible cystic cavities in the renal parenchyma. Cysts can arise during organogenesis, and may be associated with histological criteria of renal dysplasia. In general, PKD is described as either 1) a congenital form or 2) an adult form. The congenital form is known to occur in dogs, cats, horses, cattle, sheep, pigs, several laboratory animal species, and humans. In humans, this congenital form is based on an autosomal recessive trait caused by mutation on the PKHD1 gene encoding fibrocystin, which is a receptor protein. It is proof that this genetic mutation follows an autosomal recessive trait similar to the childhood form of PKD in humans is also responsible for polycystic kidney disease in Persian kittens, lambs, and West Highland White and Cairn terrier puppies, whereas the inheritance of congenital PKD in other species is unknown.

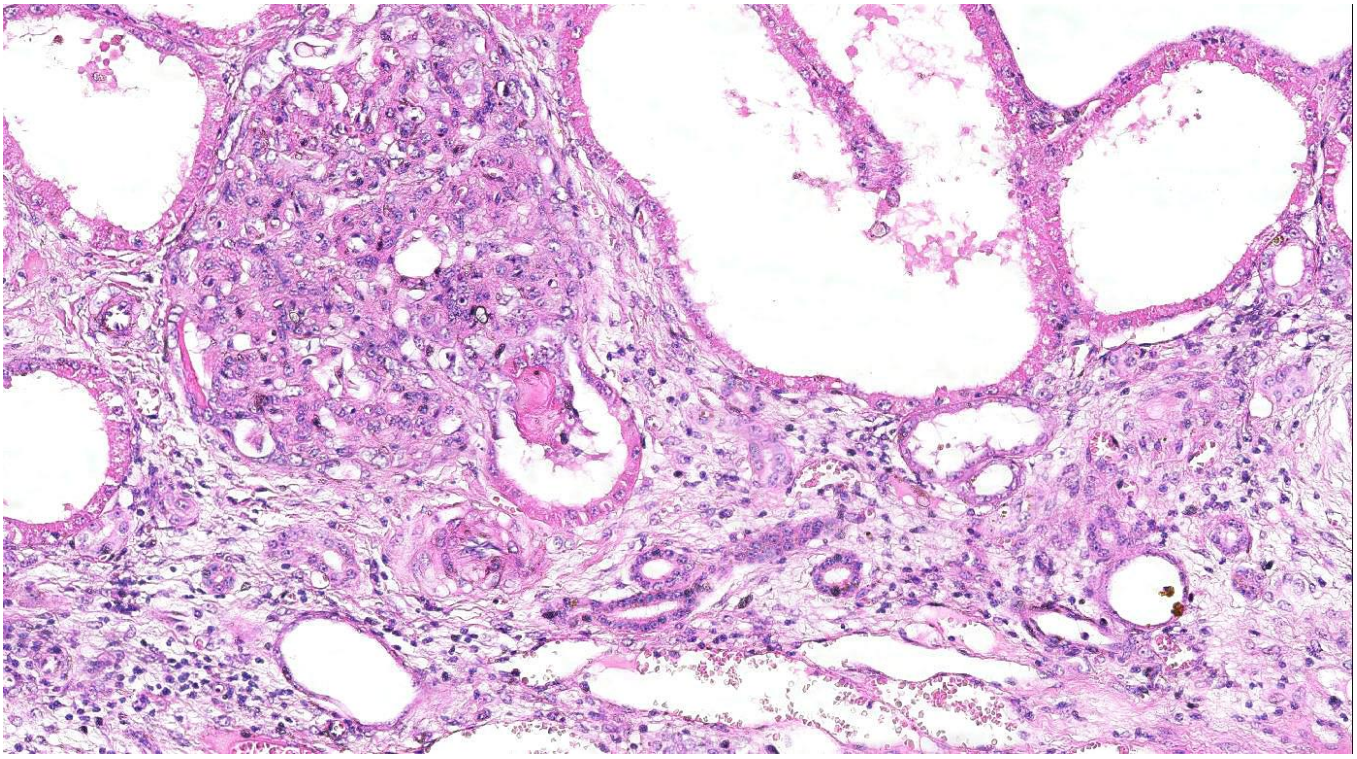
Cysts can develop in any part of the nephron, including the glomerular space, or in the collecting system. In glomerulocystic disease, they only develop in Bowman's space, but this exclusivity is exceptional. There is no evidence that cysts are caused by a failure of nephrons to unite with the collecting system. Analysis of their content indicates that they are part of functional nephrons, and that their activity is consistent with their location in the nephron. Three mechanisms, which are not mutually exclusive, may lead to the formation of renal cysts. The pathogenesis of cyst formation in PCKD is thought to represent a combination of altered cell growth, fluid secretion and altered composition of the extracellular matrix.



1Dilated tubules are lined by hyperplastic epithelium containing numerous protein droplets, and often contain sheave-like birefringent oxalate crystals. (HE, 45X)

Nevertheless, the gross and histopathological similarities of the lesions together with the progressive nature of lesions resulting in presentation in middle to old age is similar to these entities, implying a similar pathogenesis. As such, it would appear most likely that equine PCKD results from a random genetic defect resulting in downstream malformation of distal tubules/collecting ducts within the kidneys, ultimately culminating in effacement of normal renal architecture by progressively enlarging space occupying fluid-filled cysts.

Renal cysts can be subdivided into congenital or acquired lesions. Solitary renal cysts occur in many species, most commonly in pigs and cattle, and are essentially incidental findings. Renal cysts become clinically significant when multiple and bilateral; such cases are named



Glomeruli are decreased in number and markedly hypercellular with numerous synechiae. Surrounding tubules are markedly etatic, and the interstitium is markedly expanded with loosely arranged fibrous connective tissue. (HE, 180X)

polycystic kidneys. Polycystic kidney disease (PCKD) is best characterized in man with further subdivision into autosomal dominant and autosomal recessive forms.

Autosomal recessive PCKD is rare and usually is associated with disease in childhood that is often severe. Those patients who do survive may progress to the development of hepatic fibrosis. In veterinary species, a similar entity has been described in Cairn terriers, characterized by juvenile onset and multiple cysts in both kidneys and liver.

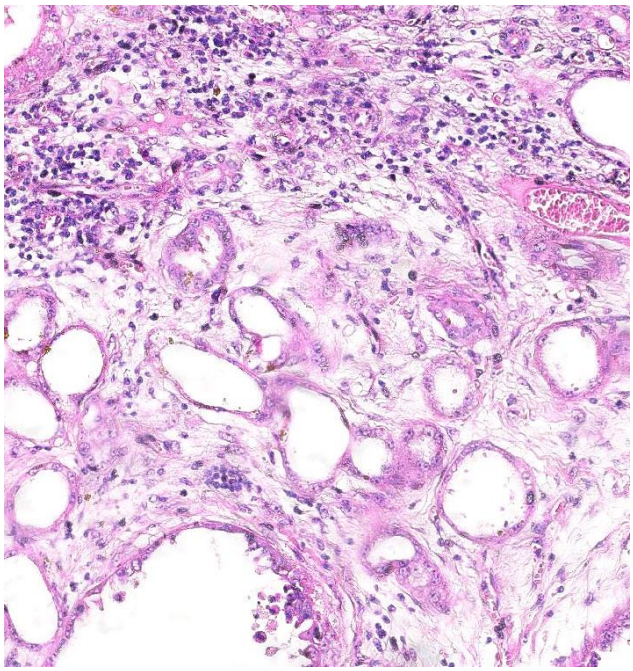
Polycystic kidneys are uncommon in horses; to our knowledge, there have been only five previous cases of PCKD in mature horses. In those cases, clinical signs (severe weight loss and anorexia) were similar to our case, although some author had related hematuria as the main clinical sign. Both kidneys were presented enlarged, containing multiple cystic structures that distorts the normal renal architecture are the classical gross findings.

Studies in man and Persian cats have been carried out in attempt to identify the segment of the nephron which is involved in cyst development. In man, the cysts have been identified as focal dilations of proximal or distal convoluted tubules of the nephron, with occasional involvement of glomeruli. The cysts result from initial epithelial proliferation which then becomes separated from the tubule lumen and is filled not only by glomerular filtrate but also by a dysfunction of epithelial fluid absorption and secretion.

With regard to hematological and biochemical parameters, major biochemical abnormalities include consistently increased urea and creatinine levels, as would be expected with a chronic renal disease resulting in progressive reduction of renal function. Anemia was reported in cases where hematuria was a clinical finding but not in the present one and in those who this clinical sing was absent; therefore, this abnormality may reflect blood loss rather than

diminution of erythropoietin production by the peritubular interstitial tissue of the kidney. Although secondary hyperparathyroidism due to chronic renal failure is a common sign, it was not found in the present case.

Despite the morphological similarity of the lesion seen in man and Persian cats, it is unlikely that a similar familial genetic defect is responsible for the disease in horses, given the paucity of reported cases and lack of evidence of familial association disease. Nevertheless, the gross and histopathological similarity between lesions together with the progressive nature of lesions resulting in presentation in middle to old age is similar to these entities, implying a similar pathogenesis. As such, it would appear most likely that equine PCKD results from a random genetic defect resulting in downstream malformation of distal tubules/collecting ducts within the kidneys, ultimately culminating in effacement of normal renal architecture by progressively enlarging space occupying fluid-filled cysts. Immunohistochemistry can be performed in order to clarify the segment of epithelium in cyst formation. A combination of vimentin and a specific anticytokeratin antibody direct against cytokeratin 18 showed to be useful to characterized collect tubule involvement.



Kidney, horse The renal interstitium is markedly expanded by loosely arranged collagen and moderate numbers of lymphocytes and plasma cells. Tubules are ectatic and lined by attenuated epithelium (HE 140X)

JPC Diagnosis: Kidney: Nephritis, interstitial, chronic, diffuse, severe, with cystic tubular dilation, membranoproliferative glomerulonephritis, synechia formation, neutrophilic tubulitis and marked interstitial fibrosis.

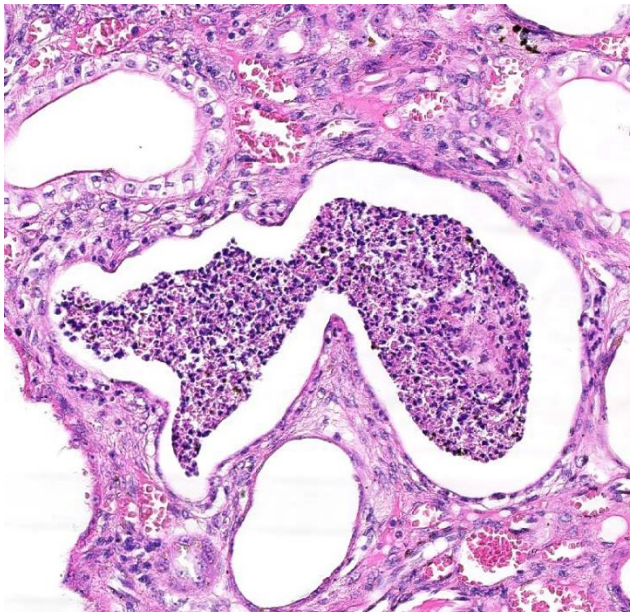
Conference Comment: Conference participants unanimously agreed that this case provided a superb descriptive challenge, with heterogeneous changes in nearly all sections of the nephron, but expressed varying interpretations as to the underlying pathogenesis and etiologic diagnosis. Most participants initially focused on the glomerular changes, including membranoproliferative glomerulonephritis, crescent formation, synechia and periglomerular fibrosis as described by the contributor. Participants agreed that the cortical tubules are markedly ectatic and/or cystic, and some participants felt strongly that there was significant tubular loss somewhat obscured by dilation of remaining tubules. Nearly all participants observed cellular casts, cellular debris, inflammatory cells and oxalate crystals within tubular lumina, with fibrosis and chronic inflammation in the adjacent interstitium. Some participants described shrunken, obsolescent glomerular tufts within a dilated urinary space; others interpreted the same finding as nodular proliferations of hyperplastic tubular epithelium projecting into the lumina of dilated tubules. Most participants interpreted the renal changes, including the markedly dilated tubules, as an acquired or secondary lesion vice a primary underlying congenital condition.

This case was studied by Dr. Rachel Cianciolo at the Ohio State University, who has extensive experience and a keen interest in veterinary nephropathology; she interprets the renal changes as representing two simultaneous ongoing processes: 1) obstructive nephropathy with tubular dilatation and hyperplastic renal pelvis epithelium, which may be related to the evidence of urolithiasis described grossly, and the presence of oxalate crystals in the tubules; and 2)

proliferative and sclerosing glomerulonephropathy, which may or may not be immune complex-mediated. She also observes that there is "protracted tubular epithelial degeneration and necrosis with secondary interstitial edema and inflammation that could be due to either of the above processes." She further elaborates that polycystic kidney disease, as it occurs in human medicine, specifically refers to hereditary syndromes, either autosomal dominant or autosomal recessive; polycystic kidney is most appropriately applied when the specific genetic mutation has been identified or there is support for a heritable process. In this case, the tubular dilation, degeneration and necrosis are likely related to urolithiasis / crystalluria, and which is aligned more closely with an "acquired cystic disease."

This case was additionally studied in consultation with the Departments of Genitourinary and Nephropathology at the Joint Pathology Center, whose medical pathologists are familiar with polycystic kidney disease as described in humans. They indicated the renal lesions in this case are not consistent with polycystic kidney disease, at least as it occurs in humans. Similar to conference participants, the medical pathologists identified dilated tubules with atrophic tubular epithelium, including within collection ducts, and a mesangioproliferative glomerulonephritis with decreased numbers of glomeruli. They characterize the lesion as chronic tubulointerstitial disease, and also describe arteriolar and arterial sclerosis. They also commented on the birefringent, smooth-surfaced crystals within tubules and within the interstitium and stated that, by light microscopy, the crystals are consistent with calcium oxalate. They also mentioned the presence of basophilic non-birefringent crystals vicinity the renal pelvis, consistent with calcium phosphate.

The underlying cause of the glomerular changes remains uncertain in this case. Typically, glomerulonephritis is described as an immune meditated disease and the proliferative (or mesangioproliferative) form occurs most commonly in horses. Glomeruli can also be non-specifically



Kidney, horse. Largely within the medulla, ectatic tubules contain moderate to large numbers of neutrophils (tubulitis) (HE, 30X)

involved in association with other renal conditions, such as tubulointerstitial disease,[4] which may have caused the glomerular changes in this case vice an immune mediated process. Other non-immune mediated causes of glomerular injury include hypertension and coagulation secondary to endothelial injury.[4]

That said, the glomerular lesions in this case were severe enough to result in crescent formation, a finding which generally occurs as a response to fibrin exudation into the urinary space, followed by invasion of mononuclear cells, proliferation of parietal epithelium, and production of collagen by fibroblasts.[4] Additionally, the presence of synechia, which occurs with loss of podocytes and adhesion of the glomerular basement membrane to the parietal epithelium, is another indicator of severe glomerular damage. Glomerulonephritis in horses is apparently not uncommon, but progression to renal failure is reported as rare. Infectious causes in horses include equine infectious anemia and *Streptococcus equi*. The contributor's necropsy observations of a urinary bladder with thickened, irregular red mucosa and reported urine culture of *Klebisella pneumonia* provide further plausible explanation for acquired renal tubular disease with simultaneous glomerular lesions.

Contributing Institution:
Faculdade de Medicina Veterinária e Zootecnia
Departamento de Patologia
University of São Paulo
São Paulo, Brazil

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