



WEDNESDAY SLIDE CONFERENCE 2026-2027

Conference #7

7 October 2026

CASE I:

Signalment:

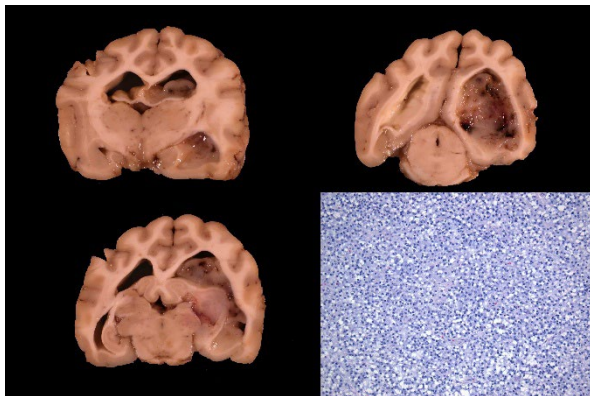
8-year-old, castrated, male, Boxer dog (*Canis lupus familiaris*)

History:

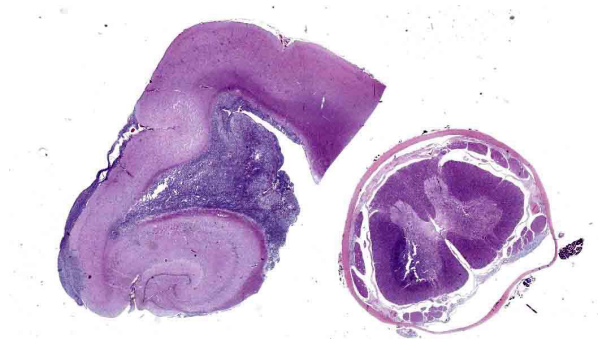
This Boxer dog presented with 1.5-week history of mild ataxia and chronic weight loss and 1 day of seizures and drastic mental decline despite anticonvulsant therapy. He was dull, mentally inappropriate, and non-ambulatory tetraplegic, with persistent partial facial seizures. The owners elected euthanasia.

Gross Pathology:

Filling and obstructing the left lateral ventricle was a gray, gelatinous, semi-firm mass, extending caudally from the level of the hippocampus to the occipital lobe. A similar, 2 mm,



1-1. Brain, dog: Filling and obstructing the left lateral ventricle was a gray, gelatinous, semi-firm mass, extending caudally from the level of the hippocampus to the occipital



1-2. Brain and spinal cord, dog: a section of cerebrum through the left lateral ventricle to include the hippocampus, and a section of lumbar spinal cord is submitted for examination. An encapsulated, infiltrative mass is present within the left lateral ventricle and the meninges overlying the neocortex. A similar mass is present surrounding the spinal nerves in the subarachnoid space of the section of lumbar spinal cord. (HE 9X)

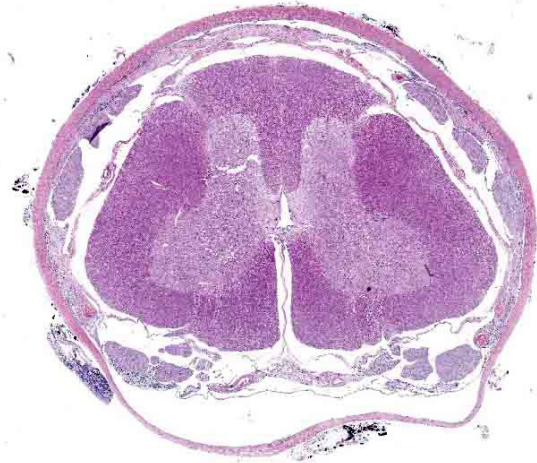
flattened, round, gray, gelatinous mass was also noted along the ventral aspect of the medulla oblongata. The cerebellum was caudally displaced with coning.

Laboratory Results:

None available

Histopathic Description:

Spinal cord: Infiltrating and expanding the leptomeninges, surrounding spinal nerve roots, is an intradural, extramedullary, unencapsulated, poorly demarcated, densely cellular neoplasm composed of sheets of closely packed polygonal cells, forming a honeycomb pattern, on a scant fibrovascular stroma.



1-3. Spinal cord, dog: This section of cervical spinal cord was provided to conference participants. (HE 13X)

Neoplastic cells have fairly distinct cell borders and moderate amounts of lightly staining cytoplasm or perinuclear halo. Nuclei are round, centrally located, and hyperchromatic with an indistinct nucleolus. Anisocytosis and anisokaryosis is moderate. There are 0 mitotic figures in 10 hpf. There is minimal change in the adjacent parenchyma.

Immunohistochemistry:

Immunohistochemistry for Olig2 showed diffuse, strong, intranuclear immunoreactivity, indicating oligodendroglial differentiation. Immunohistochemistry for GFAP revealed strong cytoplasmic expression within parenchymal astrocytes, but no expression was seen in the neoplastic population (not shown).

Contributor's Morphologic Diagnoses:

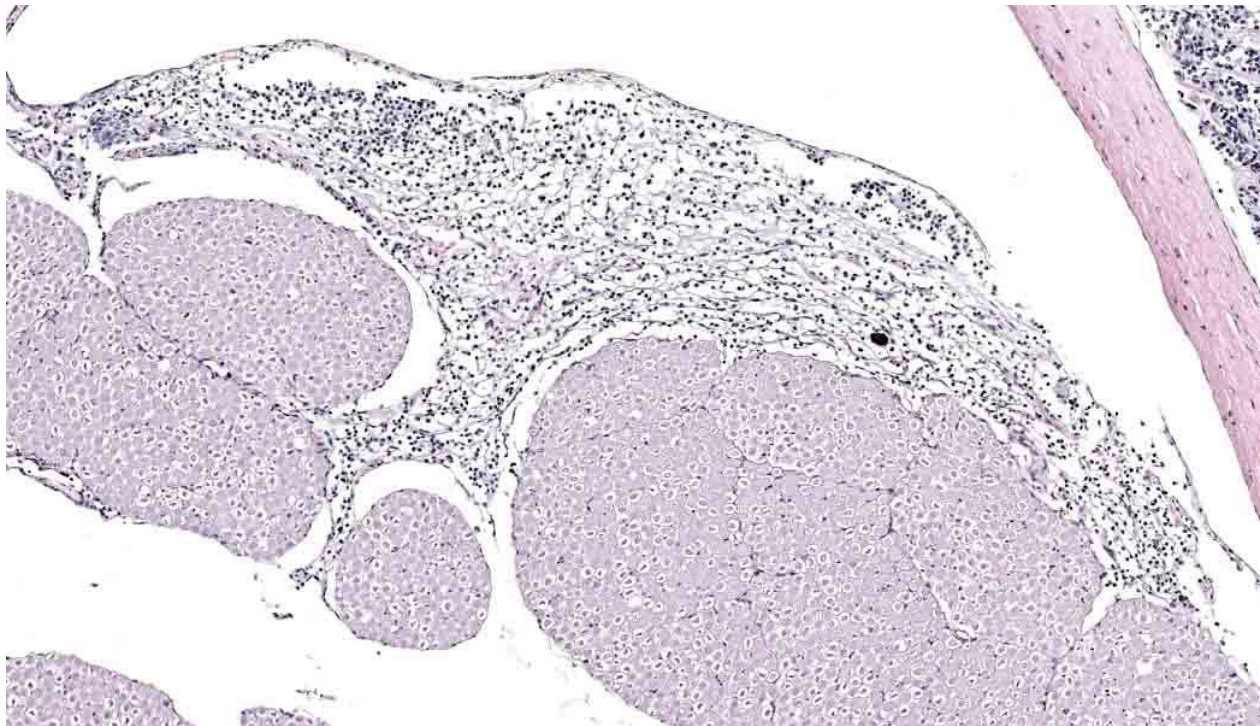
Spinal cord: Oligodendroglioma

Contributor's Comment:

Oligodendrogliomas are common intracranial neoplasms in mature dogs with brachycephalic breeds overrepresented^{2,4,6}. These neoplasms typically present as a solitary, gelatinous, gray-tan mass within white matter of the frontal and temporal lobes of the cerebrum,

occurring less frequently in the brainstem and spinal cord. Histologically, neoplastic oligodendroglial cells contain a lightly staining cytoplasm forming sheets of closely packed cells. Due to delayed post-fixation artifact, neoplastic cells may exhibit a non-staining appearance, or perinuclear halo, creating the classic fried egg or honeycomb pattern. Olig-2 is a reliably expressed immunohistochemical marker for non-neoplastic and neoplastic oligodendroglial cells, often used in combination with GFAP to rule out astrocytoma and E-cadherin/CD34 for meningiomas^{2,5}. Other immunohistochemical markers have been utilized in veterinary literature with mixed results including CNPase, doublecortin and vimentin^{2,5,7}. This neoplasm uncommonly breaks through the ependyma and disperses in the central nervous system (CNS) by migrating via cerebrospinal fluid and seeding structures along its path^{5,7-9}. This "metastatic" capability of oligodendrogliomas into distant sites within the CNS is a well characterized, but rarely reported, manifestation in humans. This presentation of oligodendrogliomas in dogs is similarly rare, and its recognition and characterization has emerged only recently in veterinary literature.

Atypical presentations of canine oligodendrogliomas that have been described include the following: 1) multiple, concurrent oligodendrogliomas at distant sites within the central nervous system, 2) primary intraventricular oligodendroglioma, and 3) diffuse leptomeningeal oligodendrogliomatosis^{1,3,5,7}. Brachycephalic breeds are overrepresented in all of these cases and their clinical signs reflect the volume and regions of the central nervous system that is affected. There have been three case reports of canine cerebral oligodendrogliomas arising within the ventricular system with minimal parenchymal involvement, and



1-4. Spinal cord, dog: Spinal roots are surrounded by an infiltrative neoplasm composed of glial cells with abundant cleared cytoplasm with a “chicken-wire” appearance (HE 135X)

two reports of multifocal neoplastic infiltration with leptomeningeal extension. Oligodendrogliomatosis manifests as diffuse, intradural, extramedullary neoplastic infiltration with no primary parenchymal mass. Four cases of canine oligodendrogliomatosis have been reported.

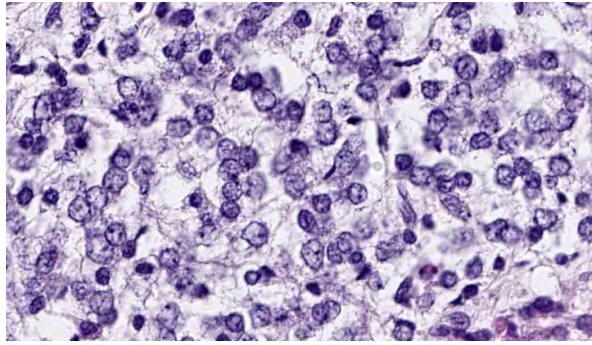
Conference participants received only a section of spinal cord with the neoplasm. Without knowledge of a primary neoplasm noted within the cerebrum, the intradural, extramedullary location of oligodendroglioma in this case may elicit a discussion and diagnosis of primary leptomeningeal oligodendrogliomatosis. However, oligodendroglioma in this dog was also noted within the brain, completely obstructing the left lateral ventricle, and histologically revealed continuous extension into the 4th ventricle as well as infiltrating the subarachnoid space along the ventral aspect of the medulla oblongata, and continuing as an intradural, extramedullary neoplasm throughout

the entire spinal cord tract. Although there was minimal periventricular involvement in the brain by neoplastic cells, suggesting a predominantly intraventricular growth pattern, the tumor most likely metastasized from the large primary mass in the brain into the leptomeninges of the spinal cord. Recognition of these atypical manifestations of oligodendrogliomas suggests that examination of the spinal cord should routinely be performed to determine the full extent of canine oligodendrogliomal spread, as this may be an underrecognized disease manifestation.

Contributing Institution:

Cummings School of Veterinary Medicine at Tufts University

<http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/>



1-5. Cerebrum, dog. Neoplastic cells have monomorphic open-faced nuclei which often palisade. (This image was taken from the ventricle, where the cellular preservation is better). (HE, 1390X)

JPC Diagnoses:

Lumbar spinal cord, subarachnoid space: Oligodendrogliomatosis.

JPC Comment:

This week's moderator was LTC Curtis Cline, a highly experienced Army veterinary pathologist and graduate of the JPC's training program (and previous WSC coordinator as a second-year resident). Following completion of his residency, he served in the Training Office at the JPC, and followed that with a tour at the US Army Medical Research Institute of Infectious Diseases, where he worked in biocontainment as well as being assigned to the 1st Area Medical Laboratory. He is currently assigned as the Chief of Comparative Pathology at the US Army Medical Research Institute of Chemical Defense.

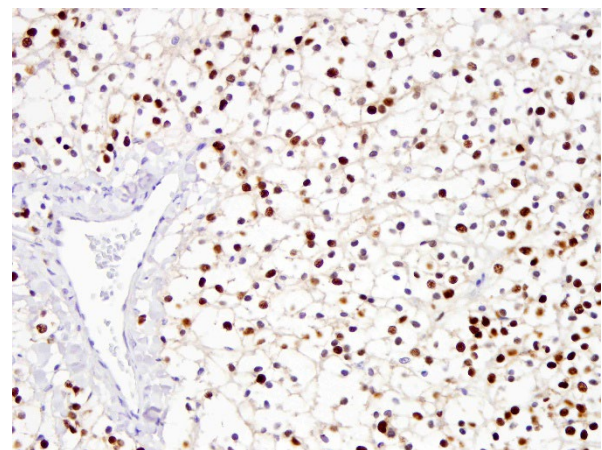
The contributor provides an excellent discussion of the different manifestations of how oligodendroglioma spreads in the dog. Since this case was submitted in 2016, the veterinary literature has added several excellent contributions to the classification and diagnosis of oligodendrogliomas in the dog.¹⁰⁻¹²

In 2018, the Comparative Brain Tumor Consortium published a revised classification scheme for the diagnosis of canine gliomas¹⁰,

which not only serves to simplify the classification of astrocytomas and oligodendrogliomas in canine patients but also helps to bring their classification more in line with the human literature, facilitating future incorporation of clinical outcomes and genomic data. This new classification scheme reduces astrocytomas and oligodendrogliomas to low- versus high-grade tumors, and this article presents the criteria for reclassification of each.¹⁰

Critical histologic criteria for the classification of oligodendrogliomas as high-grade versus low-grade are reduced to:

- necrosis (single-cell necrosis not considered),
- microvascular proliferation and pseudopalisading,
- the overt presence of features of malignancy (pleomorphism), and other evidence of significant cellular atypia
- mitotic count per 2.37 mm² field.¹⁰

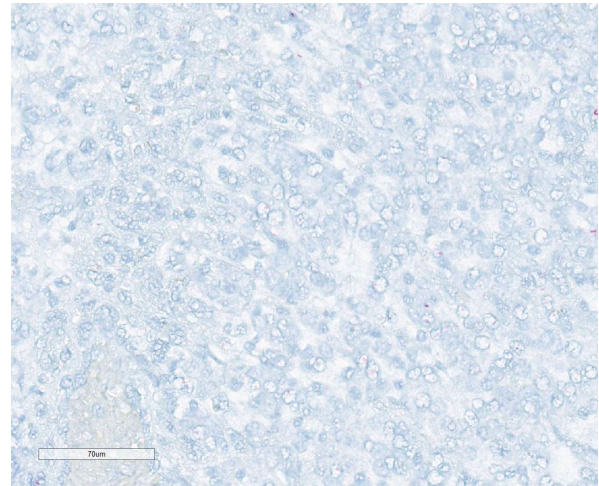


1-6. Ventricle, dog. Neoplastic cells demonstrate strong nuclear immunoreactivity for OLIG2 (anti OLIG2, 400X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, <http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/>)

This neoplasm, both in the cerebral and spinal cord locations, has a very traditional "oligodendroglioma-like" appearance, with widely spaced cells, abundant clear cytoplasm, and monomorphic bland nuclei, giving it a "chicken wire" appearance. In the ventricular location, in spite of the low mitotic rate, the neoplasm demonstrates evidence of microvascular proliferation, a key criterion for the diagnosis for high-grade oligodendroglioma. According to this schema¹⁰, a high grade tumor is warranted in this case.

The contributor provides a concise review of immunohistochemical markers used to differentiate gliomas. A 2024 article by Rissi et al. provides an outstanding review of these markers and other markers used to differentiate primary and secondary nervous system neoplasms in the dog and cat. Oligodendrocytes typically stain strongly positive with the nuclear transcription factor OLIG2.¹¹

A confounding issue in using immunohistochemistry to diagnose primitive central nervous system tumors is the common origin of neurons, astrocytes, and glial cells from a similar primitive neuroglial precursor. OLIG2 is an excellent immunomarker to differentiate neoplastic glial cells from primitive neural elements. However, OLIG2 (despite a name that would lead one to assume it is specific for oligodendroglia) will simultaneously stain the nuclei of both astrocytes and oligodendrocytes due to their common ancestry. For this reason, it should always be paired with glial fibrillary acidic protein, which stains astrocyte cytoplasm and processes but not those of their oligodendroglial counterparts.¹⁰⁻¹² (In most cases of glioma, even mixed gliomas), careful evaluation of the histology of these tumors on H&E sections, coupled with Olig-2 and GFAP, will provide a diagnosis and appropriate grade in most cases.



1-7. Ventricle, dog. Neoplastic cells are immunonegative for glial fibrillary acidic protein (anti-GFAP, 316X).

It should also be noted that time to fixation, as well as time in fixation, may affect these markers. OLIG2 appears more resistant to the antigen cross-linking associated with longer fixation periods, while other markers, including GFAP, CNPase, and Ki-67, should be interpreted cautiously. A lack of immunoreactivity should not be interpreted as a negative test for these markers without careful consideration.

Other immunomarkers that may be more useful in diagnosing oligodendrogliomas include CNPase, which stains the cytoplasm of oligodendrogliomas. MAP2 immunoreactivity may be tricky in some cases, as, like Olig-2, this marker may stain both astrocytic neoplastic cells within astrocytomas and oligodendrogliomas; in oligodendrogliomas, immunolabeling is predominantly perinuclear and cytoplasmic, whereas in astrocytomas, the marker predominantly stains the cytoplasmic processes.

Other less commonly used immunomarkers include nuclear transcription factors SOX2 and SOX10, platelet-derived growth factor alpha, and neural/glial antigen 2. However,

more studies are required to establish more rigid interpretive guidelines and their utility in differentiating gliomas.¹¹

The JPC diagnosis of “oligodendrogliomatosis” is based on the fact that the slide which was provided to the conference participants (and that described by the contributor above) contained only a section of lumbar spinal cord. The contributor did kindly provide a section of diencephalon with the tumor within the ventricle and meninges to answer questions about the origin of the tumor, following the expected discussion as to whether the spinal cord lesion represented a metastatic oligodendroglioma or a focus of leptomeningeal oligodendrogliomatosis. Based on the slide provided to participants, the origin of this lesion in the lumbar spinal cord cannot be definitively identified; hence we prefer a diagnosis of “oligodendrogliomatosis”. Ultimately, with all sections and the gross images of this lesion considered, a diagnosis of metastatic oligodendroglioma becomes appropriate.

Finally, for neuropathologists and budding pathologists, the Davis Thompson Foundation published the sixth edition of its series on the Surgical Pathology of Tumors of Domestic Animals in 2026, covering tumors of the nervous system.¹² Authored by members of the Comparative Pathology Tumor Board, this volume aligns with the newest literature.¹⁰⁻¹¹ It provides an outstanding, easy-to-read overview of primary and secondary neoplasms of the central and peripheral nervous system, their diagnostic features, biologic behavior, and appropriate immunohistochemistry. It is well worth the investment.

References:

1. Canal S, Bernardini M, Pavone S, et al (2013). Primary diffuse leptomeningeal

gliomatosis in 2 dogs. *Can Vet J*, 54: 1075-9.

2. Johnson GC, Coates JR, and Wininger F. (2014) Diagnostic immunohistochemistry of canine and feline intracalvarial tumors in the age of brain biopsies. *Vet Pathol*, 51: 146-160.
3. Koch MW, Sanchez MD, Long S, et al (2011). Multifocal oligodendroglioma in three dogs. *JAAHA*, 47: e77-85.
4. Koestner A and Higgins RJ. “Tumors of the nervous system.” Meuten DJ, *Tumors in Domestic Animals*, 4th Ed. 2002.
5. Kovi RC, Wunschmann A, Armien AG, et al (2013). Spinal meningeal oligodendrogliomatosis in two Boxer dogs. *Vet Pathol*, 50(5): 761-4.
6. Maxie MG and Youssef S. “Nervous system.” Jubb, Kennedy, and Palmer’s *Pathology of Domestic Animals*, 5th Ed. 2007.
7. Rissi DR, Levine JM, Eden KB, et al (2015). Cerebral oligodendroglioma mimicking intraventricular neoplasia in three dogs. *JVDI*, 27(3): 396-400.
8. Subramanian A, Harris A, Piggott K, et al (2002). Metastasis to and from the central nervous system – the ‘relatively protected site.’ Review. *Lancet*, 3: 498-507.
9. Wesseling P, van den Bent M, and Perry A. (2015). Oligodendroglioma: pathology, molecular mechanisms and markers. *Acta Neuropathol*, 129: 809-827.
10. Koehler JW et al. A Revised Diagnostic Classification of Canine Glioma: Towards Validation of the Canine Glioma Patient as a Naturally Occurring Preclinical Model for Human Glioma. *J Neuropathol Exp Neurol*. 2018 Nov 1;77(11):1039-1054.

11. Rissi DR, Miller AD, Demeter EA, Church ME, Koehler JW. Diagnostic immunohistochemistry of primary and secondary central nervous system neoplasms of dogs and cats. *J Vet Diagn Invest.* 2024 Mar;36(2):153-168. doi: 10.1177/10406387231221858. Epub 2024 Jan 17. PMID: 38234003; PMCID: PMC10929637.
12. Rissi D, editor. *Surgical Pathology of Tumors of Domestic Animals, vol 6: Tumors of the Nervous System.* Davis Thompson Foundation Publishing, 2026

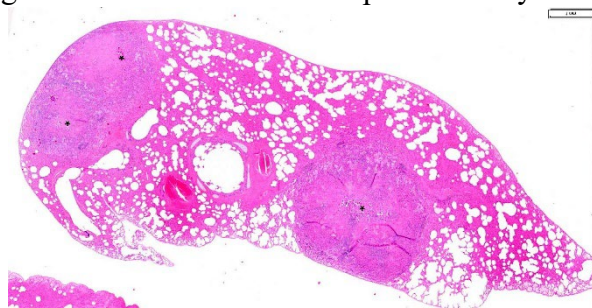
CASE II:

Signalment:

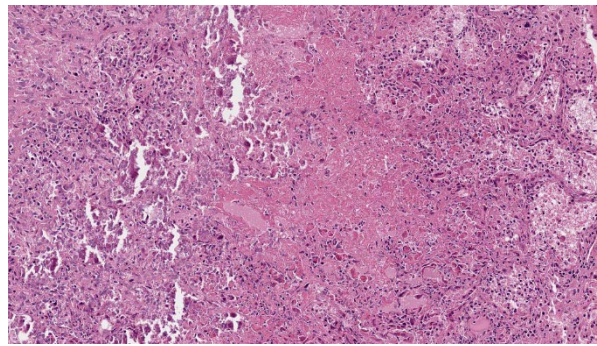
One-month-old, male, European Shorthair cat (*Felis catus*)

History:

The animal was handed over to a local animal shelter with multiple open, oozing skin wounds on several parts of the body. Treatment with amoxicillin showed no improvement. PCRs on feline herpes- and caliciviruses were negative. As the disease progressed, cutaneous nodules with purulent exudation began to occur. The cat died spontaneously and



2-1. Lung, cat: Effacing normal architecture there are multiple consolidated areas. (HE, 7X) (Photo courtesy of: Department of Pathology, University of Veterinary Medicine, Hannover, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/>)



2-2. Lung, cat: Areas of consolidation are composed of areas of lytic necrosis and mineralization which at their periphery are infiltrated by large numbers of macrophages. (HE, 281X) (Photo courtesy of: Department of Pathology, University of Veterinary Medicine, Hannover, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/>)

at the same time littermates started to show similar skin lesions.

Gross Pathology:

At necropsy, multiple, extensive, erosive to predominantly ulcerative, partly proliferative skin lesions on the frontal, ventral, and lateral surfaces of the trunk and limbs as well as the inner surfaces of the nostrils and auricles were present. The superficial cervical and popliteal lymph nodes were moderately enlarged. The lung parenchyma showed multifocal firm white-gray foci.

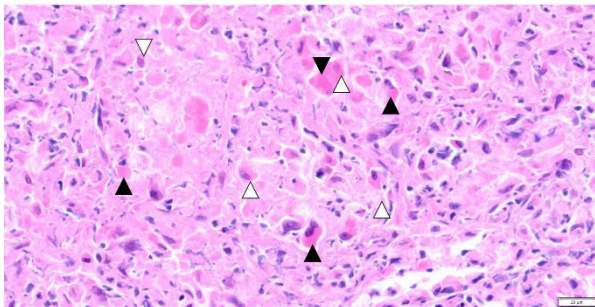
Laboratory Results:

A PCR on affected skin and lung tissue samples for Orthopoxvirus was positive. Immunohistochemical staining of lung tissue and skin for Orthopoxvirus antigen (mouse monoclonal α Orthopox 5B1, German Armed Forces, Munich) was positive.

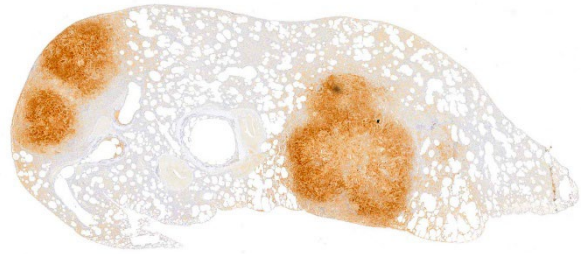
Microscopic Description:

In the lung there are multifocal to coalescing consolidated areas with loss of the organotypical architecture. These areas measure up to 2.8 mm in diameter and are composed of karyorrhectic, -lytic and -pyknotic cells and homogeneous eosinophilic cellular debris (lytic

necrosis). Necrotic foci contain moderate to large numbers of predominantly degenerated neutrophils, fewer macrophages, lymphocytes, plasma cells and occasional multinucleated giant cells. Central areas of the necrotic foci multifocally show mild mineralization of cellular debris (Fig. 2-2). Numerous macrophages and degenerated cells within the affected areas contain up to 10 µm large, cytoplasmic, bright eosinophilic viral inclusion bodies (A-type inclusions, Downie bodies, Fig. 2-3). Occasionally small eosinophilic, perinuclear inclusion bodies were visible (B-type inclusions, Guarnieris bodies, Fig. 2-3). Adjacent alveolar spaces contain moderate numbers of neutrophils and macrophages admixed with fibrin and homogeneous proteinaceous fluid. Bronchiolar lumina are filled multifocally with moderate amounts of cellular debris and degenerated immune cells. The epithelium of bronchi, bronchioles, and terminal bronchioles show varying degrees of necrosis and squamous metaplasia.



2-3. Lung, cat: Numerous macrophages and degenerated cells within the affected areas contain up to 10 µm large, cytoplasmic, bright eosinophilic viral inclusion bodies (A-type inclusions, Downie bodies, black arrowheads). Occasionally small eosinophilic, perinuclear inclusion bodies were visible (B-type inclusions, Guarnieris bodies, White arrowheads). (HE, 400X) (Photo courtesy of: Department of Pathology, University of Veterinary Medicine, Hannover, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/>)



2-4. Lung, cat: Immunohistochemical staining of lung tissue for orthopoxvirus antigen (mouse monoclonal αOrthopox 5B1, German Armed Forces, Munich) is positive. (anti-αOrthopox 5B1, 7X) (Photo courtesy of: Department of Pathology, University of Veterinary Medicine, Hannover, <http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/>)

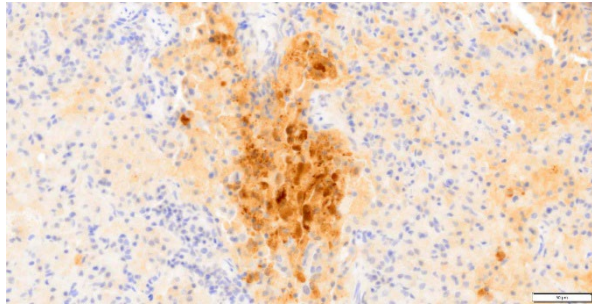
Contributor's Morphologic Diagnoses:

Lung, pneumonia, necrosuppurative, lymphohistiocytic and plasmacytic, multifocal to coalescing, severe, subacute with numerous eosinophilic, cytoplasmic viral inclusion bodies, feline.

Contributor's Comment:

Orthopoxvirus (OPV) infections in domestic cats manifest as cutaneous and generalized forms of disease.^{1-5,12,13} Gross lesions, histopathological and immunohistochemical (Fig. 2-4 and 2-5) as well as molecular findings are consistent with a generalized OPV infection as described previously by Schulze et al. (2007).¹³ As in the case presented here the affected animal exhibited generalized skin lesions and severe viral pneumonia.¹³

OPV infections of domestic cats, first described in 1978, are mainly caused by members of the genus Cowpox virus (OPXV) and are usually restricted to the skin.^{2,10,13} Initial infection is thought to occur during hunting or subsequent feeding on rodent reservoir species such as rats, bank voles and wood



2-5. Lung, cat: Immunohistochemical staining of lung tissue and skin for vaccinia virus antigen was positive. (anti-VACV, 7X) (Photo courtesy of: National Institute of Allergy and Infectious Disease)

mice.^{10,13,14} Affected animals develop a primary skin lesion usually in exposed areas such as head, neck, forelimbs, or paws¹⁰ Typical lesions develop from pink or red colored macules to papules and fluid filled vesicles that further develop to pustules. The typical pox virus lesion is thereafter formed upon eruption of the pustule by a raised hemorrhagic ulcer.^{1,7} The epitheliotropic virus replicates in infected cells initiating leukocyte infiltration and epidermal hyperplasia resulting in lesion formation.¹ Secondary cutaneous lesions develop during the viremic phase 1-3 weeks post infection, characterized by multiple ulcerating, rarely vesicular papules. Amelioration of lesions, usually occurring after 2-3 weeks, is thereafter characterized by thick gray crusts. Most animals recover within 5 weeks.¹⁰

Unlike other DNA viruses, OPVs replicate in the cytoplasm of infected cells. For several OPVs, two types of inclusion bodies can be differentiated.⁹ B-type inclusions, also called “Guarnieris bodies,” represent juxtanuclear viral factories responsible for poxvirus DNA replication, intermediate and late gene transcription, protein synthesis, and assembly of virions. All poxviruses form B-type inclusions and manifest as small, round to oval, slightly basophilic perinuclear inclusions.^{6,9,8,11} A-

type inclusions, also known as “Downie bodies,” are only formed by certain strains of the species Cowpox virus, Ectromelia virus, Fowlpox virus, Canarypox virus, Raccoonpox virus, and Volepox virus. They are made up of large quantities of mature virions embedded in a cytoplasmic matrix of A-type inclusion protein. They are thought to increase virion stability after cell lysis.^{6,8} Histologically, they are characterized by one or multiple large, acidophilic cytoplasmic inclusions.¹¹

Generalized infection with pneumonia represents a rare disease manifestation in domestic felids and is mainly seen in immunocompromised individuals or in association with systemic corticosteroid treatment.^{4,10,12,13} Previous reports include underlying infections with feline leukaemia virus, feline immunodeficiency virus (FIV), feline panleukopenia virus and feline herpesvirus.^{2,10} While OPV-induced pneumonia in juvenile cats seems mostly to occur in combination with widespread skin lesions, infection in older domestic cats or wild felids can occur in absence of typical secondary skin lesions.^{2,4,10,13}

Although human infections are overall rare, multiple transmission events from cats or rats to their handlers have been reported.^{5,13-15} Human infection is characterized by typical cutaneous lesions like those described above. As in cats, lesions are mainly restricted to the hands and face of infected individuals, but generalized, even fatal, disease can develop in immunocompromised persons.^{5,14}

Contributing Institution:

Department of Pathology,
University of Veterinary Medicine, Hannover
Buenteweg 17,
30559 Hannover, Germany.

<http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/>

JPC Diagnoses:

Lung: Pneumonia, necrotizing, subacute, multifocal, marked with syncytia and numerous intracytoplasmic viral inclusions.

JPC Comment:

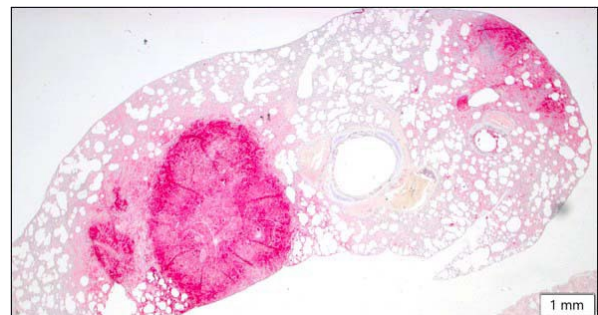
The contributor provides an excellent, in-depth review of cowpox infection in felids, the species that, in the last decade, has become the most prominent source of zoonotic infections, particularly in Europe, where this infection is considered endemic. Rodents, rather than cattle, are the reservoir host for this poorly named virus and are the obvious source of feline infections (and likely many infections in zoo animals and few infections in cattle and humans). Cowpox infection has also been documented in large felids, anteaters, mongooses, rhinoceroses, alpacas, okapis, elephants, and cotton-top tamarins.¹⁶ Cowpox isolates from these various species have been grouped into numerous genotypes which vary significantly in their pathogenicity.¹⁶

As mentioned by the contributor, orthopoxviruses include cowpox, variola virus (the causative agent of smallpox), and vaccinia virus, which has been used for immunization since the 19th century, when Edmund Jenner first used it to vaccinate against smallpox. The actual identity of the virus used by Jenner (or perhaps his mentor, Dr. James Fuster, another bit of historical controversy) is unclear, a several possibilities exist for the true identity of the pocks on the hands of milkmaid Sarah Nelms, as there is no way that Jenner could definitively identify the causative agent. At that time, the pocks could also result from a range of other diseases, to include bovine papular stomatitis, pseudo-cowpox or para-vaccinia (“milker's nodules”), herpes mammillitis

and papillomatosis, not to mention variola virus itself (the causative agent of smallpox.)¹⁷ It is likely that Jenner believed that he was actually using the agent of horsepox, a disease that was occasionally transferred from horses to cattle by farm workers. Interestingly, horsepox is a disease that does not exist today (the last natural case was seen in 1976 in Mongolia), but over the centuries, it likely evolved into what we call “vaccinia virus” today.¹⁷

While not “extinct” like horsepox, clinical cowpox in cattle is now extremely rare, but occurs sporadically in enzootic areas (Europe and Russia). Cowpox virus produces lesions on the teats and the contiguous parts of the udder of cows, and is spread through herds by the process of milking.¹⁶

Vaccinia virus, in addition to being the orthopoxvirus most closely related to cowpox, is also the most closely studied orthopoxvirus. Because orthopoxvirus structural proteins are highly conserved, immunization with VACV provides cross-protection against variola and other orthopoxviruses, such as monkeypox. (This close antigenic relationship is crudely demonstrated in Fig. 2-6, in which an anti-vac-



2-6. Lung, cat. Areas of inflammation stain strongly positive for variolavirus, a closely related virus to cowpox. (Anti-VACV, 7X)

cinia immunomarker was applied to the section in this case). But the utility of vaccinia virus extends well beyond its antigenic similarity and its role for hundreds of years as a vaccination proxy for smallpox. As significant disease is rarely seen with vaccinia inoculation (life-threatening infections are limited to approximately 1-2 individuals per million), it is also widely used in recombinant vaccines in humans carrying immunizing antigens of influenza, hepatitis B, rabies, herpes virus, HIV, among others. It has also been used to develop an oral vaccine against rabies for wildlife.

A few comments on the morphologic diagnosis in this case are likely appropriate. Based solely on histologic evaluation of the slide, it is very difficult to classify this particular pneumonia. The pathogenesis of the cowpox lesion is typical of an interstitial pneumonia, however, the multinodular appearance of the neoplasm in the submitted sections and the lack of diffuse interstitial thickening within the excellent sampling of this lung made diagnosis of an interstitial pneumonia difficult. In addition, the largest nodule in these sections surrounds a large airway. Because of the difficulties that classification presents in this particular H&E section, JPC staff has chosen discretion over valor and simply classified this case as "pneumonia".

Another interesting feature of this disease which made it into the morphologic diagnosis, is the presence of virally infected syncytia. This is an uncommon feature of most poxviral infections, but is well documented in cowpox. A previous case of cutaneous cowpox in a cat ([WSC 2018, Conf 11, Case 2](#)) also demonstrates syncytial cell formation.

References:

1. Bruneau RC, Tazi L, Rothenburg S. Cowpox Viruses: A zoo full of viral diversity and lurking threats. *Biomolecules*. 2023;13.

2. Caswell JL, Williams KJ. Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2 (6th Edition)*. W.B. Saunders; 2016:465–591.
3. Herder V, Wohlsein P, Grunwald D, et al. Poxvirus infection in a cat with presumptive human transmission. *Vet Dermatol*. 2011;**22**:220–224.
4. Hinrichs U, van de Poel H, van den Ingh TS. Necrotizing pneumonia in a cat caused by an orthopox virus. *J Comp Pathol*. 1999;**121**:191–196.
5. Hobi S, Mueller RS, Hill M, et al. Neurogenic inflammation and colliquative lymphadenitis with persistent orthopox virus DNA detection in a human case of cowpox virus infection transmitted by a domestic cat. *Br J Dermatol*. 2015;**173**:535–539.
6. Howard AR, Moss B. Formation of orthopoxvirus cytoplasmic A-type inclusion bodies and embedding of virions are dynamic processes requiring microtubules. *J Virol*. 2012;**86**:5905–5914.
7. James WD, Berger TG, Elston DM, Odom RB. *Andrews' diseases of the skin : clinical dermatology*, 10th ed. Philadelphia: Saunders Elsevier; 2006.
8. Kastenmayer RJ, Maruri-Avidal L, Americo JL, Earl PL, Weisberg AS, Moss B. Elimination of A-type inclusion formation enhances cowpox virus replication in mice: implications for orthopoxvirus evolution. *Virol*. 2014;**452-453**:59–66.
9. Leite JA, da Fonseca FG, de Souza Trindade G, et al. A-type inclusion bodies: a factor influencing cowpox virus lesion pathogenesis. *Arch Virol*. 2011;**156**:617–628.

10. Mauldin EA, Peters-Kennedy J. Integumentary System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Vol 1 (6th Ed.)*. W. B. Saunders; 2016:509–736.
11. Pfeffer M, Meyer H. Poxvirus diagnostics. In: Mercer AA, Schmidt A, Weber O, eds. *Poxviruses*. Basel: Birkhäuser Basel; 2007:355–373.
12. Schöniger S, Chan DL, Hollinshead M, et al. Cowpox virus pneumonia in a domestic cat in Great Britain. *Vet Rec*. 2007;**160**:522–523.
13. Schulze C, Alex M, Schirrmeier H, et al. Generalized fatal cowpox virus infection in a cat with transmission to a human contact case. *Zoonoses Publ Health*. 2007;**54**: 31–37.
14. Stolz W, Götz A, Thomas P, et al. Characteristic but unfamiliar--the cowpox infection, transmitted by a domestic cat. *Dermatol*. 1996;**193**:140–143.
15. Wolfs TF, Wagenaar JA, Niesters HG, et al. Rat-to-human transmission of Cowpox infection. *Emerg Infect Dis*. 2002;**8**:1495–1496.
16. McLachlan J, ed. Poxviruses. In: Fenner's Veterinary Virology. Academic Press, 2016, pp. 157-174.
17. Huygelen C. Jenner's koepokentstof in het licht van de moderne vaccinologie [Jenner's cowpox vaccine in light of current vaccinology]. *Verh K Acad Geneesk Belg*. 1996;**58**(5):479-536; discussion 537-8. Dutch. PMID: 9027132.
18. Jacobs BL, Langland JO, Kibler KV, Denzler KL, White SD, Holechek SA, Wong S, Huynh T, Baskin CR. Vaccinia virus vaccines: past, present and future. *Antiviral Res*. 2009 Oct;**84**(1):1-13. doi: 10.1016/j.antiviral.2009.06.006. Epub 2009 Jun 26. PMID: 19563829; PMCID: PMC2742674.
19. Vanderplasschen A, Pastoret PP. The uses of poxviruses as vectors. *Curr Gene Ther*. 2003 Dec;**3**(6):583-95. doi: 10.2174/1566523034578168. PMID: 14683453.

CASE III:

Signalment:

2 years-old, Nelore, *Bos Indicus*.

History:

In the last 2 years several cattle in this farm developed a wasting syndrome and locomotor difficulties with kneeling posture.

Gross Pathology:

Affected cow showed cachexia. The arteries, mainly the aorta, had diffusely rigid walls and were variably thickened. Their walls were expanded by extensive, coalescing, irregular, variably sized, gritty (mineralized) intramural plaques and nodules. These changes were most notable from the luminal (endothelial)

surface. The aortic semilunar valves were moderately thickened, irregular, and lacked their normal elasticity due to mineralization.



3-1. Skeletal muscle, ox. Skeletal muscles demonstrate opaque and roughened plaques of mineralization and fibrosis. (Photo courtesy of: Universidade Federal de Mato Grosso, Laboratório de Patologia Veterinária, <https://www.ufmt.br/ufmt/site/>)



3-2. Heart with aorta, ox: The aortic wall is thickened and rigid due to subendothelial mineralization, which extends to the aortic valve. T (Photo courtesy of: Universidade Federal de Mato Grosso, Laboratório de Patologia Veterinária, <https://www.ufmt.br/ufmt/site/>)

The lung lobes failed to collapse and showed inelastic areas with a pumice stone-like appearance. Other lesions included kidneys with whitish areas in the cortex and medulla, calcified tendons and ligaments, Skeletal muscles had opaque and rough plaques of mineralization

Laboratory Results:

None available

Microscopic Description:

At areas of thickening the aorta wall, there are extensive mineral deposits in the intima which extends to the media disrupting the elastic lamina. Extensive multifocal mineral deposits thicken the perimysium of skeletal muscle. In these areas, the vessels show mineralization of the intima.

Contributor's Morphologic Diagnoses:

Aorta, tunica intima and media: mineralization, diffuse and severe

Skeletal muscle: perimysium: mineralization, multifocal and severe

Contributor's Comment:

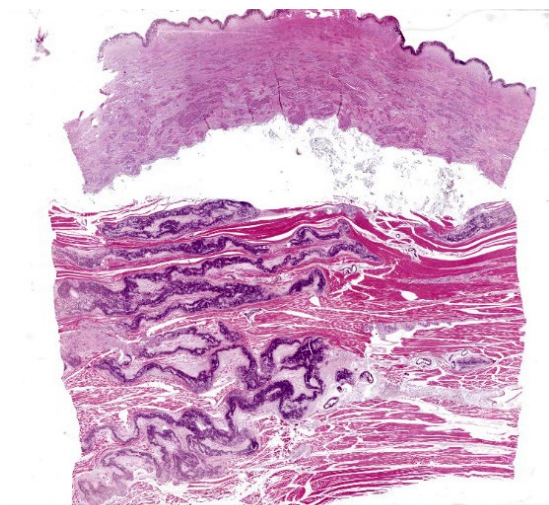
Enzootic calcinosis refers to a group of diseases characterized by extensive mineral deposition in soft tissues, induced by ingestion of calcinogenic compounds present in certain toxic plants. Of the calcinogenic plants known to cause this condition, *Solanum glaucophyllum* (*S. malacoxylon*), a perennial shrub native to South America, is one of the most extensively studied. Enzootic calcinosis induced by this plant causes considerable economic losses to the livestock industry. Other plants are well-recognized causes of Enzootic calcinosis worldwide, including *Solanum torvum*, *S. esuriale*, *S. verbascifolium*, *Nierembergia veitchii*, *N. rivularis*, *Cestrum diurnum*, *Trisetum flavescens*, *Stenotaphrum secundatum*. All of these plants are within the *Solanaceae* family⁴⁻¹³, except for *T. flavescens*² and possibly *S. secundatum*¹, which is a grass in the family *Poaceae*. Animals in several parts of the world develop calcinosis from consuming specific toxic plants. The disease has been found in many countries and are responsible for great financial loss in livestock. Cattle, sheep, goats, horses and pigs develop calcinosis and the disease receives different denomination depending on the country (Table 1)⁶. As can be seen, in spite of the knowledge developed, some cases of calcinosis remain without a known cause in Israel, India, Nigeria⁶ and Brazil⁵. The slides submitted to this case study are from a bovine that was grazing in a Cerrado area where *S. glaucophyllum* and *N. veitchii* do not occurs⁵.

Calcinogenic plants contain one or more steroidal glycosides that are biologically active analogues of vitamin D₃. Hydrolytic enzymes in intestinal, ruminal or other tissues or in the bacterial flora can cleave the sugar residue

Table 1 - Geographic localization, affected animal species and plant involved in calcinosis calcinosis in domestic animals

Geographic localization	Animal affected	Plant involved	Sickness name
Argentina	Cattle, sheep	<i>S. malacoxylon</i>	Enteque seco
	Pigs, horses	<i>S. malacoxylon</i>	Spontaneous <i>S. malacoxylon</i> poisoning
Brazil	Cattle	<i>S. malacoxylon</i>	Espichamento
	Sheep	<i>N. veitchii</i>	Calcinosis
	Cattle/Sheep/Goat	Unidentified	Enzootic calcinosis
Uruguay	Cattle	<i>S. malacoxylon</i>	Calcinosis
	Sheep	<i>N. rivularis</i>	
USA (Florida)	Cattle, horses	<i>C. diurnum</i>	<i>C. diurnum</i> poisoning
USA (Hawaii)	Cattle, goats	<i>C. diurnum</i> and <i>S. torvum</i>	Naalehu disease
Jamaica	Cattle, goats	<i>S. secundatum</i>	Manchester wasting disease
Cuba	Cattle	<i>C. diurnum</i>	<i>C. diurnum</i> intoxication
New Guinea	Cattle	<i>S. torvum</i>	Calcinosis
Australia	Sheep	<i>S. esuriale</i>	Humpy back
South Africa	Sheep	<i>S. verbascifolium</i>	Calcinosis
Israel	Sheep, goats	Unidentified	Vitamin D intoxication
India	Sheep	Unidentified	Plant intoxication
Nigeria	Buffalo	Unidentified	Calcinosis circumscripta
Austria	Cattle	<i>T. flavescens</i>	Weidekrankheit
	Horse	<i>T. flavescens</i>	Kalzinose
Germany	Cattle	<i>T. flavescens</i>	Enzootische kalzinose
Switzerland	Cattle, goats	<i>T. flavescens</i>	Enzootische kalzinose

Adapted from Mello 2003

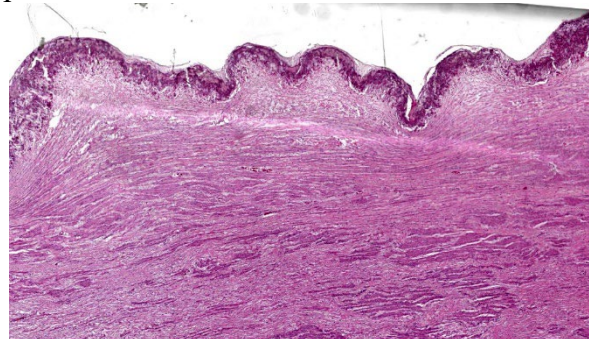


3-3. Aorta and skeletal muscle. At subgross magnification, the subendothelial region of the aorta (top) is expanded by mineral. There are ser-piginous bands of mineral and fibrosis within the section of skeletal muscle (bottom). (HE, 10X)

fragment, in most cases $1,25(\text{OH})_2\text{D}_3$. This excess of vitamin D hormone stimulates a calcium-binding protein synthesis and calcium and phosphate absorption, producing hypercalcemia and/or hyperphosphatemia. The hypercalcemic state depress PTH secretion and stimulates calcitonin secretion from the C cells of the thyroid. The formation of endogenous $1,25(\text{OH})_2\text{D}_3$ is inhibited because of the hypoparathyroidism, hypercalcemia, and/or hyperphosphatemia. The hypercalcitonism coupled with the hypercalcemia and hyperphosphatemia results in osteopetrosis. The excessively absorbed mineral cannot be physiologically accommodated, and the deposition in soft tissues results in calcinosis. It is suggested that $1,25(\text{OH})_2\text{D}_3$ induces cellular differentiation and the synthesis of a calcifiable matrix. Calcium salts are deposited in soft tissues, including the aorta, heart, kidney, tendons and ligaments^{3,6,10,12,13}.

Calcinogenic plants intoxication produces rapid wasting and marked elevations of the calcium and phosphate levels in the blood.

The patterns of these elevations of blood electrolytes as well as the soft tissue mineralization are comparable to those produced by intoxication by vitamin D. The typical wasting syndrome is seen clinically when animals are forced to eat large quantities of the leaves. The animals develop kyphosis with contraction of the tendons and ligaments, resulting in an inability to completely to straighten their front limbs and to a complete kneeling posture.. Contraction of the abdominal aponeurosis gives a unique tucked-up line to the abdomen, resulting in affected animals having a race-horse-like appearance. Many animals develop severe vascular and pulmonary disease as a result of irregular or occasional exposure to the plant because of mineralization and ossification of interalveolar septa in the lung adult animals are most commonly affected, usually producing progressive debility. The earliest signs are those of stiffness and wasting. There is subtle progression to permanent lameness resulting from contraction of the tendons. At this stage, affected animals often are excitable, tire easily if made to exercise, and may show signs of acute cardiac insufficiency. If they are removed from affected areas, the stiffness, and deformity are not lessened, but the wasting is stopped, and the animals gain weight if good pasture is available^{1,2,4,5,7-9,11}.



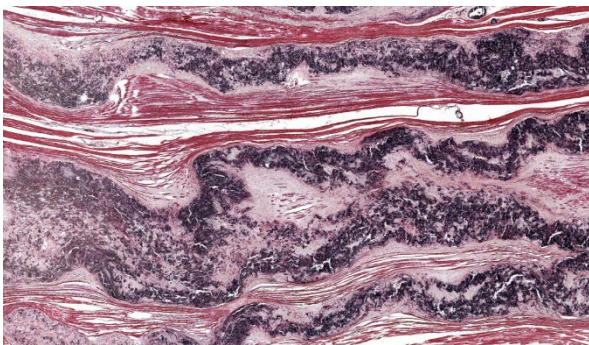
3-4. Aorta, ox: High magnification of the subendothelial mineral (top) with marked fibrosis of the arterial wall, which surrounds and separates atrophic smooth muscle cells. (HE, 56X)

Necropsy examination reveals widespread mineralization of tissues, particularly the aorta, heart, and lungs, often with areas of ossification. Frequently major arteries including the thoracic aorta, and pulmonary arteries has mineralized irregular plates in the endothelial surface that were inelastic, firm, and thickened. Soft tissue calcification can also be observed on Skeletal muscles, heart, lungs, and kidneys^{1,2,4,5,7-9,11}.

The main histological findings include calcium deposits in soft tissues like degenerated and mineralized elastic fibers of intima and media of the vessels, degeneration, necrosis and calcification of Skeletal and cardiac muscle fibers. The kidneys can show severe mineralization of the arteries and the interstitium^{2,4,5,7-9,11}.

Contributing Institution:

Universidade Federal de Mato Grosso,
Faculdade de Medicina Veterinária, Hospital
Veterinário, Laboratório de Patologia
Veterinária,
<https://www.ufmt.br/ufmt/site/>



3-5. Skeletal muscle, ox: High magnification of linear foci of fibrosis and mineralization with atrophy of the adjacent myocytes (HE, 589X)

JPC Diagnoses:

1. Skeletal muscle: Mineralization, chronic, multifocal to coalescing, severe, with fibrosis and myocyte degeneration, necrosis, atrophy, and loss.
2. Aorta: Mineralization, chronic, multifocal to coalescing, severe, with myxomatous degeneration, fibrosis, and smooth muscle hypertrophy, hyperplasia, and disarray.

JPC Comment:

The contributor provides an outstanding review of enzootic calcinosis due to Vitamin D analog-containing plants.

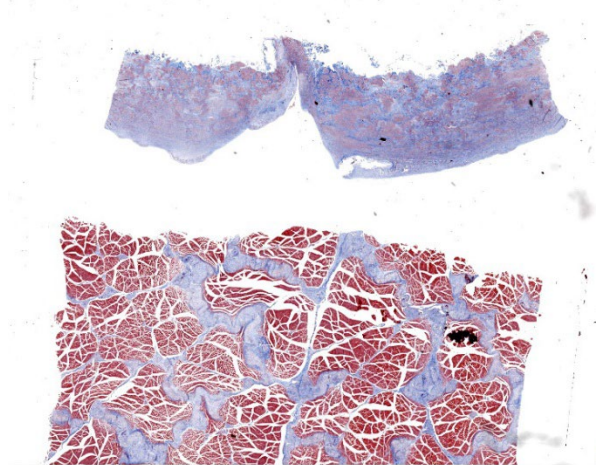
A review of other causes of soft tissue mineralization is likely useful at this point. There are five basic mechanisms of soft tissue mineralization: dystrophic, metastatic, idiopathic, iatrogenic, and calciphylaxis.

Dystrophic calcification is the most common form of tissue mineralization. Dystrophic calcification results from mineral deposition in injured and dead cells. This form of mineralization results from both intracellular and extracellular events. Cellular damage leads to calcium influx into the cell, where it is initially sequestered within mitochondria. Another event preceding dystrophic mineralization is the release of phosphate-binding proteins within the cell.¹⁴ Cellular damage progresses; irreversible mitochondrial damage results in the release of calcium within the cytoplasm, which, bound with phosphate, results in the formation of crystalline mineral. Extracellular factors which influence the rate and extent of dystrophic mineralization include ischemia and chronic inflammation. Inflammatory mediators such as tumor necrosis factor, interleu-

kin-1 beta, and IL-6 also contribute to dystrophic mineralization.¹⁴ Serum calcium and phosphorus levels are within normal limits.

Metastatic calcification is a systemic manifestation of abnormal calcium and phosphorus levels in the blood. When the calcium-phosphorus product exceeds 70 mg/dL, metastatic calcification will occur. Tissues where this process typically occurs are those that experience rapid changes in pH and have high levels of carbonic anhydrase. These include the kidney, lung, gastric mucosa, and subpleural intercostal musculature. In the kidney and lung, mineralization is concentrated in the basement membranes of vessels and the alveolar septa. In the gastric mucosa, the middle stripe of the gastric fundus is typically affected. This form of calcification may resolve with correction of the abnormal levels of calcium and phosphorus (if possible – a common cause is chronic renal failure, which is not truly correctable). Metastatic calcification of the lung (inappropriately named “uremic pneumonitis”) is likely the most life-threatening form of metastatic calcification, as it is exceedingly difficult to transfer oxygen across a mineralized alveolar septum and vascular basement membrane.

Idiopathic calcification, as the name suggests, results from deposition of calcium salts in the presence of normal calcium and phosphorus levels and in the absence of tissue damage. A common example of idiopathic calcification in dogs is calcinosis cutis, in which dermal collagen is mineralized in animals with hypercortisolism. Less common forms include various types of tumoral calcinosis, which is also most common in dogs.



3-6. Aorta and skeletal muscle: A Masson's trichrome demonstrates the fibrosis within the aortic wall and skeletal muscle, which accompanies the mineralization. (Masson's trichrome, 9X)

Iatrogenic calcification, as the name implies, is that which is often seen in animals being treated with calcium- or phosphorus-containing substances. One of the more common causes of this is seen in animals that are treated with intravenous administration of calcium gluconate used in the management of eclampsia, cardiac dysrhythmia due to hyperkalemia, and hypoparathyroidism.

Finally, today's case is an excellent example of the fifth cause of tissue mineralization, calciphylaxis. Calciphylaxis is the term used for systemic calcification in animals that are sensitized by ingestion or administration of so-called calciphylaxis-inducing agents (to include vitamin D analogs, as seen in this case, or parathyroid hormone) and subsequently exposed to a challenge substance such as trauma, iron, or aluminum.¹⁵

The changes noted in the aorta are difficult to sort out based solely on an HE section. A Movat's trichrome is extremely helpful in degenerative changes affecting arteries, and is a

must in this case. As this stain provides differential staining of smooth muscle, collagen, and elastin, it is excellent for differentiating and characterizing these common components of degenerating arteries. On the H&E section in this case, areas of proliferating ground substance and collagen are extremely difficult to differentiate, and application of Movat's pentachrome allowed for trainees to properly identify degenerative changes in the aorta.

References:

1. Arnold RM, Fincham IH. Manchester wasting disease: A calcinosis caused by a pasture grass (*Stenotaphrum secundatum*) in Jamaica. *Trop Anim hlth prod.* 1997;29:174-176.
2. Braum U, Diener M, Camenzind D, Flückiger M, Thoma R. Enzootic Calcinosis in Goats Caused by Golden oat Grass (*Trisetum flavensces*). *Vet rec.* 2000;146:161-162.
3. Burrows GE, Tyrl RJ. *Toxic plants of north America*. Ames, IA: Iowa State University Press;2001.
4. Doberainer J, Tokarnia CH, Costa JBD, Campos JLE, Dayrell MS. “Espichamento”, intoxicação de bovinos por *Solanum malacoxylon*, no Pantanal de Mato Grosso. *Pesq agropec bras, Sec Vet.* 1971;6:91-117.
5. Guedes KMR, Colodel EM, Castro et al. Calcinose enzoótica em ruminantes do Brasil Central. *Pesq vet bras.* 2011;31(8):643-648.
6. Mello JRB. Calcinosis- Calcinogenic Plants. *Toxicon.* 2003;41:1-12.
7. Odriozola ER, Rodríguez AM, Micheloud JF et al. Enzootic calcinosis in horses grazing *Solanum glaucophyllum* in Argentina. *J vet diagn invest.* 2018;30(2):286-289.
8. Riet-Correa F, Schild AL, Méndez MC, Wasserman R, Krook L. Enzootic caucinos in sheep caused by the ingestion of *Nierembergia veitchii* (Solanaceae). *Pesq vet bras.* 1987;7(3):85-95.
9. Rissi DR, Rech RR, Pierezan F, Kommers GD, Barros CSL. Intoxicação em ovinos por *Nierembergia veitchii*: Observações em quatro surtos. *Cienc rural.* 2007;37(5):1393-1398.
10. Rosol TJ, Grone A. Endocrine glands. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 3, Philadelphia, PA: Elsevier; 2016:319.
11. Santos CG, Pereira R, Etcheberry G et al. Enzootic calcinosis caused by *Nierembergia rivularis* in sheep. *J Vet Diagn Invest.* 2012;24(2):423-6.
12. Tokarnia CH, Brito MF, Barbosa JD, Peixoto PV, Döbereiner J. *Plantas tóxicas do Brasil*. 2. ed. Rio de Janeiro: Helianthus;2012.
13. Wasserman RH. Active vitamin D-like substances in *Solanum malacoxylon* and other calcinogenic plants. *Nutr Rev.* 1975;33(1):1-5.
14. Le C, Bedocs PM. Calcinosis Cutis. [Updated 2023 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK448127/>
15. Anfinson KP, Piercy RJ, Massey C, Smith KC, Kenny PJ, Garden OA. Severe systemic calciphylaxis in a young cat. *J Vet Intern Med.* 2014 Jul-Aug;28(4):1325-30. doi: 10.1111/jvim.12378. Epub 2014 Jun 5. PMID: 24903891; PMCID: PMC4857947.

CASE IV:

Signalment:

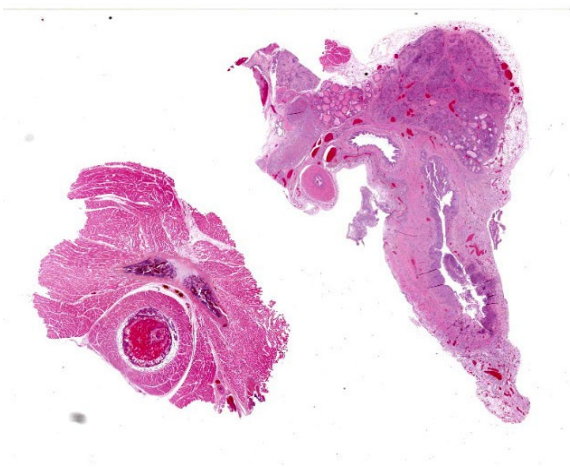
Adult, male, Sprague-Dawley (CD) rat (*Rattus norvegicus*)

History:

A week after arrival from the vendor, the rat had EEG telemetry implantation surgery. The rat was found by the research team quiet, alert, and responsive; cold to the touch; and slightly dehydrated approximately two weeks post-surgery. A veterinary evaluation revealed a decreased body temperature (32°C). Heat support & subcutaneous fluids were provided. The rat was found dead in cage later that day. A necropsy was performed due to the unexpected death.

Gross Pathology:

This 364 g, male CD rat is in optimum body condition with adequate subcutaneous and visceral adipose, 3/5 BCS, a mildly distended abdomen, eyes mildly sunken, mild dehydration, and in fresh condition. The top of the head was shaved with partial regrowth and a healed linear midline incision. There was illegible writing on the dorsal aspect of the tail.



4-1. Urinary bladder and penile root, rat: A section of urinary bladder and adherent prostate (top right) and pelvic urethra and pubic bone is submitted for examination. (HE, 7X)

A telemetry device was present in the left flank with wires running cranially through the subcutis to the head and there was fibrous tissue in the subcutis surrounding the telemetry device. There was a foul odor on opening the abdominal cavity. Within the abdominal cavity there was approximately 5-6 mL of cloudy yellow fluid and small amounts of fibrin and few adhesions between the mesentery and viscera (primarily the kidneys and urinary bladder) throughout the abdomen. On the outer/lateral surface of both kidneys there were multifocal 0.5-1.5 mm white-yellow foci. On the caudal and medial surface there were fibrous adhesions. On cut section, the pelvis of the left kidney was mildly dilated and there was a small amount of white purulent material present. The urinary bladder was empty, and the serosal surface was irregular/rough with multifocal areas of dark red discoloration and few foci of adherent fibrin. There was a firm adhesion between the mesentery and the apex of the bladder and one small indented irregular area that may represent a site of rupture. The spleen was diffusely dark and mildly enlarged. The oral cavity was empty. Within the stomach there was an abundant amount of normal ingesta, in the small intestine and cecum there was a moderate amount of normal digesta, and within the colon there were rare formed fecal pellets. There was mild congestion along the transverse and longitudinal cerebral fissures. There were four small EEG screws placed through the dorsal skull with acrylic on surface of screw. All other organs observed – NSGL.

Laboratory Results:

Culture results from abdominal fluid indicate strong growth of *Proteus mirabilis*.

Cytologic analysis of the fluid from the abdomen showed many degenerate and nondegenerate neutrophils, few macrophages, few

rhomboid crystals, small amounts of debris, and moderate numbers of intracellular (within neutrophils) and extracellular coccobacilli on a thick, pale eosinophilic background.

Microscopic Description:

Urinary bladder and prostate: There is ulceration of the bladder mucosa, marked thickening of the serosal surface with fibrosis, and marked fibrosis surrounding the prostate glands. There is submucosal infiltration of neutrophilic inflammation in the ulcerated area of the bladder and filling the bladder lumen. Neutrophils fill the lumen of one gland in the prostate and extend into the surrounding stroma.

Pelvic urethra (note pubic bone): The lumen is ectatic and expanded and filled by a dark eosinophilic coagulum. The coagulum is tightly associated with the attenuated epithelium; there are a few enmeshed neutrophils in the coagulum and there is edema in the submucosa.

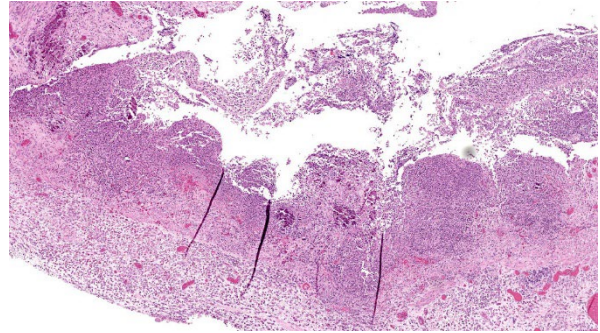
Contributor's Morphologic Diagnoses:

1. Kidney, right and left: Pyelonephritis, neutrophilic, acute on chronic, multifocal to focally extensive, marked with tubular necrosis (infarction), hemorrhage, pelvic erosion/ulceration and fibrosis, many gram-negative colonies of large rod-shaped bacteria and mineralization.

2. Ureter, left: Erosion, acute, multifocal, mild.

3. Urinary bladder: Cystitis, transmural, neutrophilic and mononuclear, acute on chronic, diffuse, marked with ulceration, fibrosis and mineralization, and moderate numbers of individual and small colonies of gram-negative rods, both intra and extra cellular, predominantly in the exudative debris in the lumen and fewer, predominantly extracellular gram-positive cocci.

4. Prostate: Prostatitis, pyogranulomatous and mononuclear, chronic, focally extensive, marked with severe fibrosis and atrophy, and moderate numbers of individual and small col-



4-2. Urinary bladder, rat: There is transmural necrosis, suppurative inflammation, and mineralization within the wall of the urinary bladder. (HE, 83X)

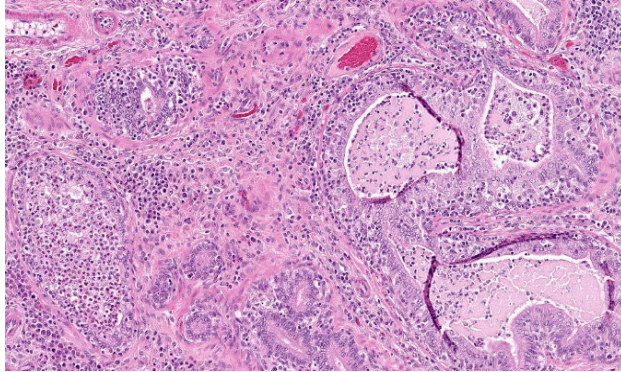
onies of gram-negative rods, both intra and extra cellular, predominantly in the exudative debris in the lumen and fewer, predominantly extracellular gram-positive cocci..

5. Pelvic urethra: Coagulum, adherent, sub-acute, diffuse, marked with fully occlusive, with urethral ectasia, bacterial colonies, low numbers of neutrophils and epithelial attenuation.

6. Penis: Mucosal hyperplasia, chronic, multifocal, moderate with luminal coagulum.

Contributor's Comment:

The cause of death in this rat is related to urethral obstruction followed by ascending urinary tract infection, pyelonephritis and urinary bladder rupture. The coagulum fully obstructed and expanded the urethral lumen, and in many locations appeared adhered to the mucosa. It was present across multiple transverse sections spanning both the pelvic and penile urethra, indicating its extensive nature. The severe degree of infection in the kidneys and



4-3. Prostate gland, rat. Here is diffuse suppurative inflammation within the glands and adjacent stroma of the prostate gland. (HE, 286X)

prostate, along with fibrosis in the renal pelvis, prostate and urinary bladder, indicates chronicity, suggesting this was an ongoing and severe condition which spread throughout the urogenital system. Infection in the kidneys was bilateral and characterized by the presence of many bands of neutrophils radiating throughout the cortex as well as abundant tubular necrosis and many colonies of gram-negative rod-shaped bacteria.

Proteus mirabilis (Order: Enterobacterales; Family: Morganellaceae) is a gram-negative bacillus bacteria often associated with urinary tract infection in human patients with long term urethral catheterization. Rats and mice have been used to study this agent as a cause of urinary tract infections.¹ The urinary bladder likely ruptured secondary to mechanical pressure from obstruction, but ongoing infection may have weakened the urinary bladder wall. Urinary obstruction in male rats secondary to urethral coagulum from secondary sex gland secretions is not an uncommon occurrence but is more commonly documented in mice. It does not always progress to urinary bladder rupture and ascending infection, depending on the completeness of obstruction and therapeutic intervention. In some cases,

obstruction may resolve spontaneously, particularly if partial, but is often fatal if the urethra is fully obstructed. Certain anesthetic protocols, drugs, and decreased ability to groom have been documented as risk factors². A second bacteria (gram-positive) was identified within the urinary bladder and prostate via tissue Gram stain, but this agent was not cultured from the abdominal fluid sample.

Proteus mirabilis is found in soil, water, and sewage, but is also a commensal in human and animal gastrointestinal tracts. It is well known for its urease activity, swarming motility across culture plates in a “bull’s eye pattern”, and biofilm formation. The enzymatic urease production breaks down urea into ammonia which alkalizes urine and promotes urinary calculi formation (typically struvite or apatite). The swimming and swarming motility is flagella mediated. Fimbriae and other adhesins play a significant role in catheter biofilm formation. Other virulence factors include various cytotoxins such as hemolysin³.

Contributing Institution:

Comparative Pathology Department, Research Support Division, U.S. Army Medical Research Institute of Chemical Defense
<https://usamricd.health.mil/>

JPC Diagnoses:

1. Urinary bladder: Cystitis, necrotizing and suppurative, chronic, transmural, severe, with mineralization.
2. Prostate: Prostatitis, necrotizing and suppurative, chronic, multifocal to coalescing, severe.
3. Peritoneum: Peritonitis, necrotizing and suppurative, chronic, focally extensive, severe.

4. Pelvic urethra: Seminal plug, chronic, with diffuse mild neutrophilic urethritis.

JPC Comment:

The contributor provides a concise review of urethral obstruction and *Proteus mirabilis* infection in laboratory rodents. A more detailed review of some of the aspects of *Proteus* spp. ability to cause disease is likely in order.

As the contributor mentioned, the main portal of entry for *Proteus mirabilis* is the urinary tract, as it is a well-known cause of UTIs and catheter-associated urinary tract infections in humans. It is typically the initial colonist of newly introduced urinary catheters, rapidly forming biofilms which are often polymicrobial. *Providencia stuartii* is a common microbial partner in catheter-associated urinary tract infections.

Proteus mirabilis benefits from its ability to change its mobility based on the medium in which it finds itself. In a liquid environment, the bacteria “swim”, while on a solid surface, such as the transitional epithelium or the urinary tract, or a urinary catheter, the bacilli “swarm”, forming large mobile rafts of bacilli.⁴ This ability, discovered in 1885 by the German pathologist Gustav Hauser, led him to name the bacterium *Proteus*, after the shape-shifting demigod of Greek mythology.⁴ Both methods of movement are facilitated by flagella; “swimmer” bacilli possess several peritrichous flagella, but the evolution to a swarmer-type bacterium on agar plates results in the development of literally hundreds to thousands of flagella.¹

Flagella production is “first and foremost” when it comes to the virulence factors produced by *Proteus mirabilis*. “Foremost” in that non-flagella-producing strains are non-patho-

genic, and “first” in that the additional virulence factors are produced only after the development of flagella and the assumption of the swarmer morphology.

P. mirabilis also possesses several other virulence factors, including fimbriae, urease, ZapA, iron and zinc scavengers, and multiple types of fimbriae, which make it a potent pathogen of the urinary tract. The bacilli’s ability to adhere to epithelium and the surfaces of catheters is mediated by up to 17 different fimbriae.¹

Urease is a nickel-based metalloproteinase that splits urea into ammonia and carbon dioxide, which increases pH.^{1,3} The presence of other uropathogenic co-infective bacteria enhances the production of urease by *P. mirabilis*. Urease increases the potential for bacterial proliferation and bacteremia, enhances the destructive effects of the inflammatory response, and facilitates urolith formation. Stone formation is likely an evolutionary adaptation of the bacterium, and it may sequester itself and proliferate within stones, evading the host’s immune response.⁵ Another unfortunate expression of urolithiasis is the fact that 50% of humans with long-term catheterization (>28 days) will experience catheter obstruction by crystalline deposits.³



4-4. Pelvic urethra, rat: A brightly eosinophilic coagulum occludes the pelvic urethral lumen.

Another interesting virulence factor of *P. mirabilis* is an IgA-degrading proteinase known as ZapA, which degrades IgA, the main mucosal-associated immunity factor of the mucosal tract. This metalloproteinase is produced during the bacterium's transition from "swimmer" to "swarmer" as it colonizes the solid surface of the urinary tract, where IgA is located.⁵

Another important flagella-driven activity of *P. mirabilis* is its ability to invade cells. Murine studies have demonstrated that within 30 minutes of inoculation, bacteria are present within urothelial cells. They may also infiltrate and live intracellularly within renal tubular epithelium in ascending infections (although this facet is less well developed than in other urinary pathogens, such as uropathogenic *E. coli*, which establishes intracellular "communities" of bacilli).³ This intracellular niche not only protects them from the body's immune system, but may also provide a resident population that contributes to infection chronicity.³ In addition to flagella, *P. mirabilis* utilizes the AipA autotransporter for internalization into bladder cells. If simple internalization is not enough, *P. mirabilis* can lyse bladder epithelial cells using a combination of the *Proteus* toxic agglutinin (Pta) and a hemolysin.³

The submitted slide in this case was a busy descriptive exercise for conference participants, which included two separate tissues with different pathogenesis. The conference moderator, who was also the contributor of this case, did not submit the kidney, which had a very significant pyelonephritis. 6.

The presence of a seminal plug in the pelvic urethra and its relationship to the cystitis, prostatitis, and pyelonephritis is unclear. Conference participants ultimately agreed that the

plug was most likely an agonal event, as the chronicity of the inflammatory response in the lower and upper urinary tract likely exceeds the expected lifespan of an animal with urethral obstruction.

References:

1. Jessica N Schaffer, Melanie M Pearson. *Proteus mirabilis* and Urinary Tract Infections. *Microbiol Spectr.* 2015 Oct;3(5):10.1128/microbiolspec.
2. Sara Wells, Chris Trower, Tertius A Hough, Michelle Stewart, Michael T Cheeseman. Urethral obstruction by seminal coagulum is associated with medetomidine-ketamine anesthesia in male mice on C57BL/6J and mixed genetic backgrounds. *J Am Assoc Lab Anim Sci.* 2009 May;48(3):296-9.
3. Chelsie E. Armbruster, Harry L. T. Mobley, Melanie M. Pearson. Pathogenesis of *Proteus mirabilis* Infection. *EcoSal Plus* 2018: 10.1128.
4. Jones B, Young R, Mahenthiralingam E, Stickler D. Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. *Infect Immun.* 2004; 72 (7):3491-3950.
5. Coker C, Poore CA, Li X, Mobley HLT. Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes Infect.* 2000; 2(12):1497-1505.