Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 13

4 January 2017

Cynthia M. Bell, MS, DVM, Diplomate ACVP
Director - Center for Comparative Oral and Maxillofacial Pathology
School of Veterinary Medicine, UW-Madison
Madison, WI 53706

CASE I: F1475435 (JPC 4084214).

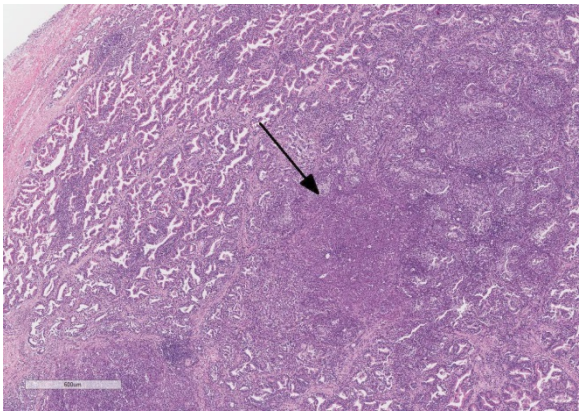
Signalment: Ten-month-old, male, Labrador retriever, (*Canis familiaris*).

History: A 10-month-old, intact male, Labrador retriever presented to the referring veterinarian with a one-month history of pain when rising after sleeping. Physical exam revealed pain (2/10) on palpation of the lumbar spine. Radiographs showed collapse disk space at L1-L2 and end plate lysis of L1-L2 and L4-L5. Blood culture was positive for *Brucella canis.* The dog was referred to the Veterinary Teaching Hospital at Colorado State University for castration where the owner was counseled about the human health risk of *B. canis* and that the dog could continue to shed the bacteria even after castration and antibiotic treatment. The owner elected euthanasia because of the zoonotic risk. The body was submitted for necropsy.

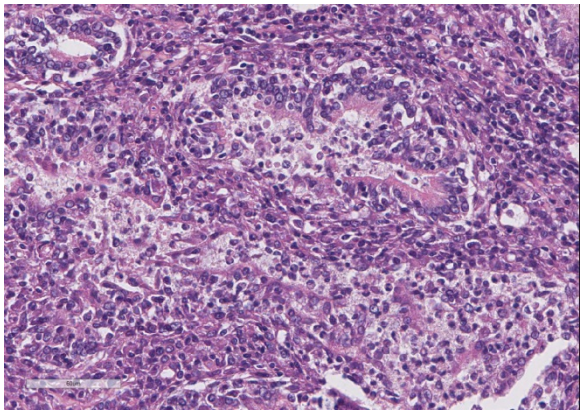
Gross Pathology: None. The vertebrae were not evaluated for intervertebral disk disease or vertebral disease due to the public health concern.

Laboratory results: Blood culture: Positive for *Brucella canis*.

Histopathologic Description: Prostate gland: Multifocal and coalescing infiltrates of lymphocytes, plasma cells, and macrophages obliterate normal architecture, expand the interstitium, and spill into remaining glandular lumina. Low numbers of neutrophils are present. There are multifocal sites of coagulative necrosis. Redundant fibrous connective tissue expands the interstitium, in some fields, up to 2-3x normal. Gram staining is negative



Testis, dog. There is diffuse glandular hypertrophy of the prostate. Aggregates of lymphocytes expand the interstitium, and multifocal areas of glandular tissue are effaced by an inflammatory infiltrate (arrow). (HE, 37X)



Testis, dog. Within inflamed areas, there is destruction of glands, necrosis of glandular epithelium, and infiltration by moderate numbers of neutrophils and macrophages. (HE, 360X)

for organisms.

Testis and epididymis: There is multifocal infiltration of the epididymal connective tissue stroma by small clusters of lympho-cytes, plasma cells, and few macro-phages with a perivascular to random distribution. The ducts contain a moderate amount of sperm. In the testis, there is a moderate to severe decrease in complete spermiogenesis, with many tubules lacking luminal spermatozoa. Gram staining is negative for organisms.

Contributor's Morphologic Diagnosis: 1. Prostate gland: Prostatitis, lympho-plasmacytic, multifocal to coalescing, chronic, severe, with parenchymal loss and fibrosis, Labrador retriever, *Canis familiaris.*
2. Epididymis: Epididymitis, lympho-plasmacytic, multifocal, chronic, moderate.

Contributor's Comment: *Brucella canis* is a gram-negative, rough or mucoid, facultative intracellular coccobacillus. Canids can be infected with four of the *Brucella* species (*B. canis*, *B. abortus*, *B. melitensis*, and *B. suis*) and serve as the reservoir species for *B. canis.* The bacteria was first isolated in 1966 in a colony of beagles and is now reported worldwide (the Americas, Europe, Asia, and South Africa).[12] The disease is especially prevalent in the southeastern United States.[5,6]

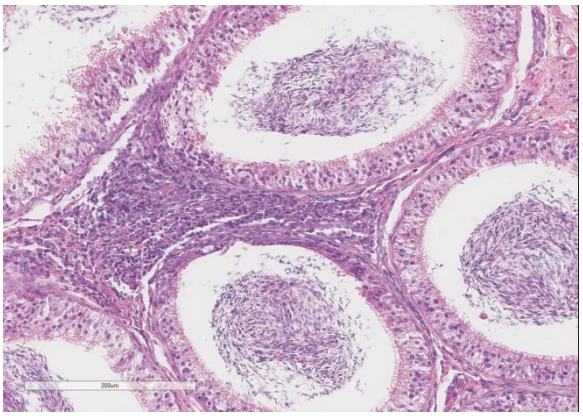
Infection occurs via bacterial penetration of mucus membranes, especially oral, conjunctival, and genital. Semen and vaginal fluids of infected canines have very high bacterial loads and exposure to these fluids is the most common route of mucosal transmission, with exposure to infected aborted fetuses, placental tissues, lochia, and urine (especially from male dogs) also serving as sources of infection.[4,12] In addition, *B. canis* has also been isolated from saliva, nasal and ocular secretions, milk, and feces, but the importance of these as sources of infection is unknown.[12]

Upon penetration of mucus membranes, the bacteria are taken up by phagocytes and trafficked to lymphatic organs and the genital tract. *Brucella canis* can persist intracellularly by evading phagosome-lysosome fusion and replicate within an endoplasmic reticulum-derived vacuole.[2,11] Bacteremia develops 1 to 4 weeks post infection and can persist, intermittently, for several years.[4,11]

Infected canids often show vague or non-specific clinical signs, including poor hair coat, lethargy, weight loss, back pain, lymphadenomegaly, and reproductive failure.[5] In bitches, there may be infertility, early embryonic death, fetal resorption, and late term abortion (45 to 60 days).[4,6] Aborted fetuses are born dead and partially autolyzed with subcutaneous congestion and edema.[12] Pups can be born alive but usually die shortly after birth. Brown to green vaginal discharge can occur for one to six weeks post abortion. In males, *Brucella* sp. can cause infertility, epididymitis, prostatitis, scrotal dermatitis (secondary to licking

trauma), testicular swelling or atrophy, and loss of libido.[4] Sterility can occur secondary to testicular damage that may result in autoimmune anti-sperm antibodies.[4,12] Less commonly, in both sexes infection can result in uveitis, diskospondylosis of the thoracic and lumbar spine, glomerulonephritis, and very rarely meningoencephalitis.

Gross lesions of brucellosis may be lacking or vague, often limited to splenomegaly, lymphadenomegaly, and in males, an enlarged epididymis. In the uterus, *Brucella* species cause chronic to subacute endometritis with glandular hyperplasia and reticular cell nodules.[4] In males, classic histologic findings are similar to what is presented in this case: lymphoplasmacytic interstitial epididymitis and prostatitis. Other findings can include focal hepatic necrosis, myocarditis, meningoencephalitis, hyaline thickening of the basement membrane of glomeruli, and granulomatous uveitis with retinitis.[4]



Epididymis, dog: The intertubular interstitium is expanded by moderate numbers of lymphocytes and fewer plasma cells. (HE, 196X)

The diagnosis of brucellosis can be made based on a history of reproductive failure and supportive serology with positive blood culture.[6] There is no effective treatment for *B. canis*. Treatment can eliminate active bacteremia but cannot eliminate bacteria residing in tissues; thus, infected tissues serve as sources for recurrent bacteremia.[12] Spaying and neutering infected dogs can help decrease shedding of the bacteria but does not eliminate infection.

Brucella canis is a zoonotic agent, but its significance as a human pathogen is poorly understood. Confirmed transmission to humans is not common, although it is likely that the disease is under-diagnosed.[4,6] Veterinarians, kennel workers, and people living with a *B. canis*-positive dog are at greatest risk of infection. In humans, *Brucella* causes vague clinical signs of fever, weakness, headache, joint pain, and enlarged lymph nodes;[12] it has also been associated with ocular lesions and endocarditis.[4,6] It is of greatest risk to immunosuppressed individuals, children, and pregnant women. In all cases, counseling about the potential health risks of living with a *B. canis*-positive dog should be provided.

JPC Diagnosis: 1. Prostate gland: Prostatitis, lymphocytic, multifocal to coalescing, chronic, severe.
2. Prostate gland: Hyperplasia, cystic, glandular, diffuse, moderate.
3. Epididymis: Epididymitis, lymphocytic, multifocal, mild.

Conference Comment: The contributor offers an excellent example of the typical lesions associated with *Brucella canis* in a male dog and a thorough summary of the epidemiology, clinical signs, pathogenesis, diagnosis, and public health implications associated with this pathogen.

Bacteria of the genus *Brucella* are most often associated with reproductive failure and abortion in a variety of mammals. *Brucella* consists of six classically recognized species based on the pre-

dominant host species and characteristic of the bacteria.[9] These include: *B. abortus* primarily affecting cattle; *B. melitensis* affecting sheep and goats; *B. suis* affecting pigs; *B. ovis* affecting sheep; *B. canis* affecting dogs; and *B. neotomae* affecting rodent species.[7] Two novel species of *Brucella*, *B. ceti* and *B. pinnipedialis*, have been isolated from marine mammals. *Brucella ceti* has been associated with abortion in cetaceans, while *B. pinnipedialis*, isolated from Northeastern Atlantic hooded seals, has a reduced pathogenicity due to its decreased ability to survive and multiply within macrophages. *Brucella microti* has recently been isolated from common voles and red foxes.[1,6,8,9]

Brucella species consist of both smooth and rough strains, with rough strains lacking the expression of the virulence factor O-side chain on the lipopolysaccharide (LPS) present on smooth strains.[8] The smooth strain LPS is non-endotoxic and allows entry into host cells via cholesterol-rich lipid rafts in the plasma membrane. In addition, the O-side chain prevents complement-mediated bacterial lysis and inhibits apoptosis of the infected cell. Both smooth and rough strains are adept at survival within macrophages due to expression of the VirB operon encoded type IV secretion system, which is induced by acidification of the phagosome during the respiratory burst. The VirB system neutralizes the pH of the phagosome and allows *Brucella* species to undergo intracellular replication and survival. Virulent strains also employ other methods to detoxify free radicals within the phagosome, including expression of superoxide dismutates. Intracellular survival and replication is the key to its virulence, and once infection is established, it tends to persist.[2,3,9,13]

Rough strain *Brucella* species are phagocytosed following recognition by toll-like receptor-4 (TLR-4) and are less virulent due to their lack of LPS O-side chain; however, as mentioned above, both have the ability to survive within phagocytes via the VirB operon.[3,9] *Brucella ovis* and *B. canis* are rough strain groups, while *B. abortus*, *B. melitensis*, and *B. suis* are more virulent and categorized under smooth strains.[3,7,9]

Conference participants discussed the emergence of zoonotic *B. canis* in underserved communities worldwide due to human interaction with populations of free-roaming canines. While *B. canis* is classified as a rough strain, and thus has a lower virulence, there have been sporadic reports of human infection. The incidence is likely under-reported due to its non-specific symptoms of low grade fever, joint pain, headache, and fatigue.[1,7]

Contributing Institution:

Colorado State University
College of Veterinary Medicine and Biomedical Sciences
Department of Microbiology Immunology and Pathology
http://csucvmbs.colostate.edu/academics/mip/

References:

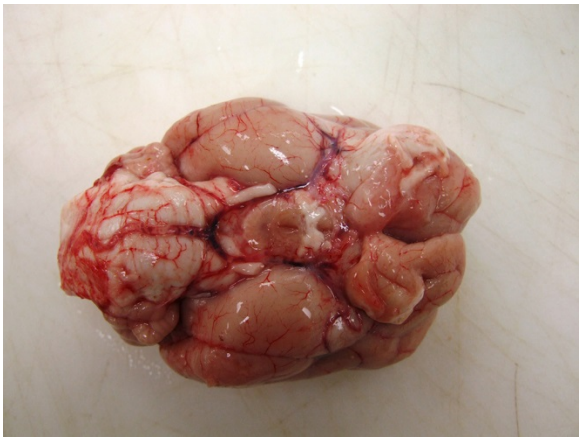
1. Centers for Disease Control and Prevention [CDC]. Brucellosis (*Brucella melitensis, abortus, suis*, and *canis*). CDC; 2012 Nov. Available at: https://www.cdc.gov/brucellosis/index.html. Accessed 6 January 2017.
2. De Figueiredo P, Ficht TA, Rice-Ficht A, Rossetti CA, Adams LG: Pathogenesis and immunobiology of brucellosis: review of *Brucella*-host interactions. *Am J Pathol*. 2015; 185(6)**:**1505-17.

3. De la Cuesta-Zuluaga JJ, et al. Identification of the virB operon genese encoding the type IV secretion system, in Columbian *Brucella canis* isolates. *Vet Microbiol*. 2013. 163:196-199.
4. Greene CE, Carmichael LE: Canine Brucellosis. In: Green CE, ed. *Infectious diseases of the dog and cat*. 4th ed. St. Louis, Mo.: Elsevier/Saunders; 2012:398-411.
5. Hollett RB: Brucellosis. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine : diseases of the dog and the cat*. 7th ed. St. Louis, Mo.: Elsevier Saunders; 2010: 882-6.
6. Hollett RB: Canine brucellosis: outbreaks and compliance. *Theriogenology*. 2006;66(3)**:**575-87.
7. Kazmierczak J. Public health implication of Brucella cais infection in humans. Summary findings of Brucella canis Workgroup. March 2012. Available at: http://www.nasphv.org/Documents/BrucellaCanisInHumans.pdf. Accessed 6 January 2017.
8. Larsen AK, Nymo IH, Boysen P. et al. Entry and elimination of marine mammal *Brucella* spp. by hooded seal (*Cystophora cristata*) alveolar macrophages in vitro. *PLoS One*. 2013; 8: e70186. doi: 10.1371.
9. Olsen SC, Palmer MV. Advancement of knowledge of Brucella over the past 50 years. *Vet Pathol*. 2014; 51(6):1076-1089.
10. Schlafer DH, Foster RA. Female genital system. In: Maxie MG ed. In: *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 3. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:402-406.
11. Von Bargen K, Gorvel JP, Salcedo SP: Internal affairs: investigating the Brucella intracellular lifestyle. *FEMS Microbiol Rev*. 2012;36(3)**:**533-62.
12. Wanke MM: Canine brucellosis. *Anim Reprod Sci*. 2004;82-83**:**195-207.
13. Zachary JF. Mechanisms of microbial infection. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:189-90, 197-8.

CASE II: D15-008849 (JPC 4066261).

Signalment: Six-year-old, castrated male, boxer, (*Canis familiaris*).

History: The dog presented with a 20-day history of seizure-like activity (lateral recumbency, drooling, and paddling) with increasing frequency. Phenobarbital administration controlled the symptoms for several weeks, but the dog gradually became lethargic and inappetant with head twitching behavior. The owners elected euthanasia due



Cerebrum, dog . Note the asymmetric enlargement of the right rostral cerebrum. (Photo courtesy of: Department of Diagnostic Medicine and Pathobiology, Kansas State Veterinary College of Veterinary Medicine, 1800 Denison Avenue, Manhattan, KS 66506, http://www.vet.k-state.edu/depts/dmp/index.htm)

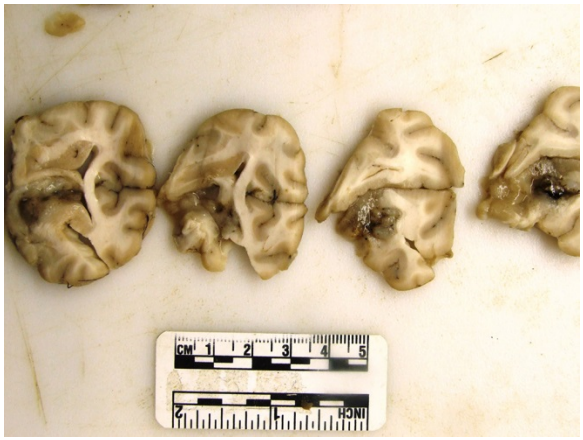
to concern about the dog's quality of life.

Gross Pathology: The rostral aspect of the right frontal lobe of the brain was enlarged, with shallow sulci and flattened gyri, and was deviated left of the midline. The brain was fixed in formalin and sectioned for examination. The cranioventral portion of the right cerebral hemisphere contained a gray, translucent, gelatinous, 2cm x 2cm x 4cm mass, extending from the frontal lobe of the cerebrum into the mesencephalon.

Laboratory results: Bloodwork was unremarkable.

Histopathologic Description: Within the white matter of the cerebral cortex, there is a poorly demarcated, unencapsulated, infiltrative neoplastic mass. The neoplasm is composed of round to polygonal cells arranged as a loose meshwork or occasionally as closely packed sheets with a honeycomb appearance and scant fibrovascular stroma. Neoplastic cells are 10-14 microns in diameter and have distinct cell borders; scant to moderate amounts of eosinophilic, fibrillar cytoplasm; a prominent perinuclear clear zone (perinuclear halo); and small, irregularly round, hyperchromatic nuclei with indistinct nucleoli. The mitotic rate is less than one per ten 400x high power fields. Capillary blood vessels within the mass are prominent with marked endothelial hypertrophy. Other variable features that are present in some slides: scattered foci of hemorrhage, glomeruloid microvascular proliferation, and pseudocystic cavities containing homogeneous, lightly basophilic (mucinous) material.

Neoplastic cells were negative for GFAP on immunohistochemical staining.

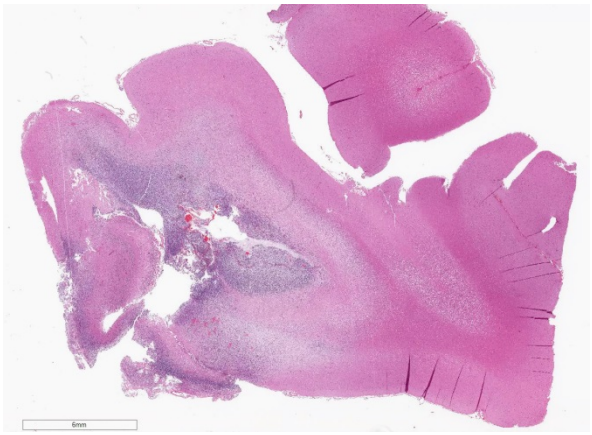


Cerebrum, dog. In the cranioventral portion of the right cerebral hemisphere, there is a gray, translucent, gelatinous mass causing deviation of midline. (Photo courtesy of: Department of Diagnostic Medicine and Pathobiology, Kansas State Veterinary College of Veterinary Medicine, 1800 Denison Avenue, Manhattan, KS 66506, http://www.vet.k-state.edu/depts/dmp/index.htm)

Contributor's Morphologic Diagnosis: Brain (cerebrum): Oligodendroglioma.

Contributor's Comment: This case had a classic signalment and presentation for canine oligodendrogliomas. Oligodendrogliomas occur most commonly in dogs, particularly in older animals and brachycephalic breeds (boxers, Boston terriers, and bulldogs), and rarely in other species including humans, cats, cattle, and horses.[1,2,9] In a study involving 173 dogs, it was found that dogs with oligodendrogliomas are 3.6 times more likely to have seizures compared with dogs with other types of primary brain tumors.[9] Other common presenting clinical complaints include mentation change, vision loss, neck pain, and vestibular syndrome.[9] Bloodwork is usually unremarkable, as it was in this case.

The most frequent location for oligodendrogliomas is the white or gray matter of the cerebral hemispheres, particularly the olfactory area and rostral lobes, but can



Cerebrum, dog. An infiltrative neoplastic mass is present within the white matter of the cerebral cortex, arranged as a loose meshwork or occasionally as closely packed sheets with prominent capillary blood vessels. This section shows a central pseudocystic cavity. (HE, 4X)

occur caudally or in the spinal cord.[5,9] Grossly, they appear as large, soft, gelatinous or mucoid, well-demarcated masses with grayish-blue matrix and gray to pink stroma on cut section.[1,2,5] Histologically, oligodendrogliomas are moderately to highly cellular, and characterized by dense sheets of uniform cells. [1,5] Delayed fixation causes an artifactual "honeycomb" cell pattern with a perinuclear halo effect. [1,2,5] Prominent microvascular proliferation is common, often with formation of a delicate "chicken-wire" pattern or vascular loops with glomerular-like tufts, as seen in this case. [1,2,5] Foci of hemorrhage, mineralization, or microcystic areas containing blue-staining mucinous material may also be present.[1,2,5] Tumors can extend along or through the leptomeninges or ependymal surfaces; intraventricular growth may be associated with widespread intraventricular metastases.[5]

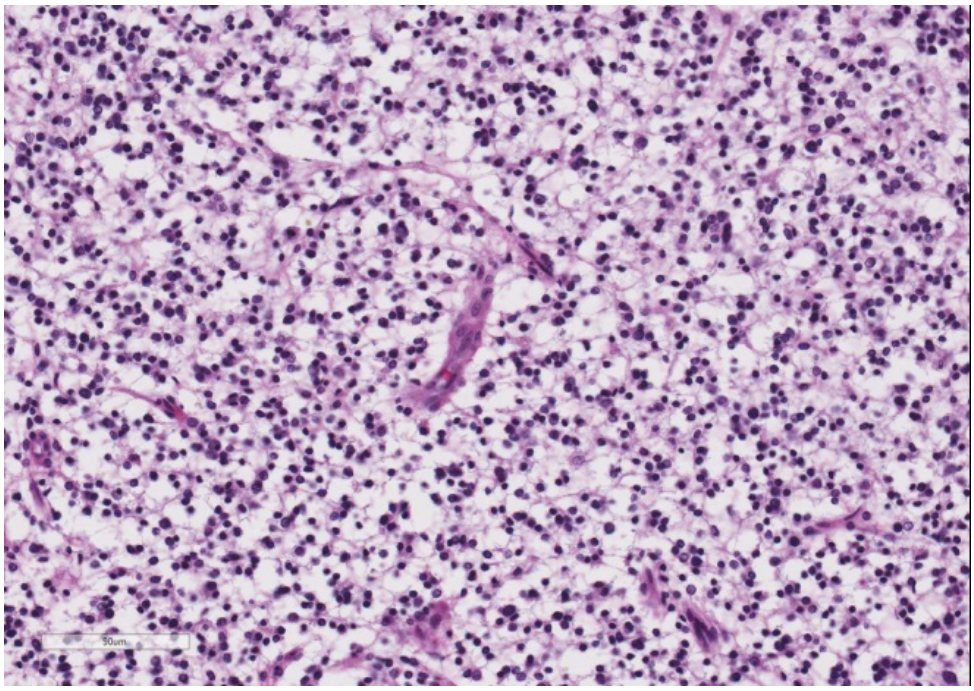
Anaplastic oligodendrogliomas are characterized by focal or diffuse anaplasia with prominent proliferation of glomeruloid vessels at the tumor margins, nuclear polymorphism, and increased mitotic index (1-2 per HPF), necrosis, and/or meningeal infiltration.[1] Intermingled astrocytes are common, and in some tumors polymorphic multinucleated giant cells may be numerous.[1] Often, areas of necrosis with peripheral glial cell palisading can be found.[5] Due to the lack of malignant features, in this case, the tumor was diagnosed as a benign oligodendroglioma.

Oligoastrocytomas are rare mixed glial tumors composed of neoplastic astrocytes and oligodendroglia, in which the two cell populations may be diffusely intermingled or in geographically distinct clusters. [1,2,5] Canine oligoastrocytomas are predominantly composed of oligodendroglial cells with at least 25-30% neoplastic astrocytes; lower percentages of astrocytic elements are interpreted as reactive proliferating cells within oligodendrogliomas.[1,5] Anaplastic (malignant) oligoastrocytomas are characterized by increased cellularity, nuclear atypia, high mitotic activity, vascular proliferation, and necrosis, and may be difficult to differentiate from high-grade astrocytomas.[1]

Ultrastructurally, oligodendrogliomas have no obvious distinguishing features, appearing as cells with sparse microtubules and few organelles in the cytoplasm and frequent desmosomal junctions between cells.[5]

Historically, diagnosis of oligodendrogliomas has relied on gross and microscopic tumor morphology and negative GFAP staining of cells, due to lack of specific markers.[2,4,5] In recent years, several markers have been investigated, including doublecortin, olig2, and CNPase.[3,4] Doublecortin is a cytoplasmic neuronal precursor marker which is frequently expressed in oligodendrogliomas and

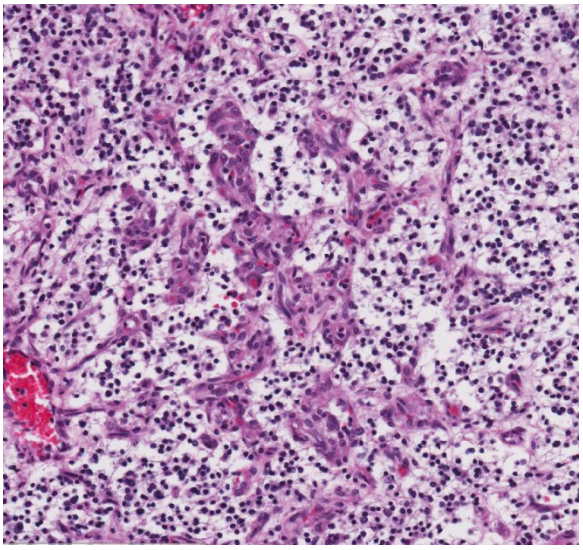
embryonal neoplasms such as neuroblastomas and PNETs, but infrequently expressed in astrocytomas.[3] Olig2 is a nuclear transcription factor that is required for oligodendrocyte differentiation but not astrocyte development, and has been shown to stain all oligodendrogliomas in one study.[4] Non-neoplastic astrocytes do not stain with Olig2; however, astrocytomas can have positive nuclear staining.[4] CNPase is a cytoplasmic phosphodiesterase that is expressed early in myelination.[4] It stains normal and neoplastic oligodendrocytes, but weak cytoplasmic staining of canine astrocytomas can also occur.[4] A combination of negative GFAP staining and positive Olig2 staining may be most helpful in identification of oligodendrogliomas. Positive staining for factor VIII-like antigen and smooth muscle actin highlights the neoplasm's characteristic microvascular proliferations.[5] In our case, the neoplasm stained negatively for GFAP. No staining for the other potential oligodendroglioma markers was attempted.



Cerebrum, dog. In many areas, the neoplastic cells are arranged as a loose meshwork with a characteristic "honeycomb" appearance. (HE, 224X)

JPC Diagnosis: Brain, cerebrum: Oligodendroglioma, boxer, *Canis familiaris*.

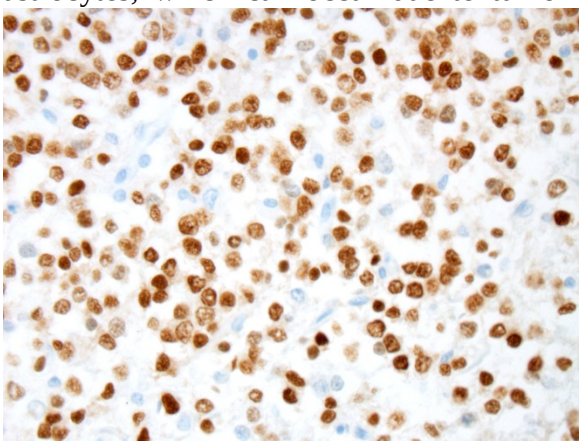
Conference Comment: Despite some moderate slide variability, the contributor provides a great example and com-prehensive review of oligodendrogliomas in the canine central nervous system (CNS). Oligodendrogliomas are the third most common primary neoplasm in the dog brain, after meningiomas and astrocytomas.[6] These soft gelatinous neoplasms typically arise in the telencephalic or diencephalic cerebral white matter, but can uncommonly occur in the brainstem, spinal cord, or within the ventricular system as a single tumor or as multiple concurrent oligodendrogliomas as a result of metastasis through the cerebrospinal fluid.[2,6,8]



Cerebrum, dog. Multifocally, prominent capillary blood vessels have endothelial hypertrophy and a proliferative "glomeruloid" pattern. (HE, 168X)

Oligodendrogliomas originate from oligodendrocytes, which normally function to provide support and myelination to axons within the white matter tracts of the CNS.[7] Each oligodendrocyte can envelop up to 50 axons based on the thickness of the myelin sheath needed. Oligodendrocytes also serve a role in the production of neurotropic factors important for the remyelination of demyelinated axons in the CNS.[7] Schwann cells provide an equivalent function in the peripheral nervous system (PNS); however, unlike oligodendrocytes, Schwann cells can only envelope one part of a single axon. Schwann cells are also extremely important in axonal regeneration in the PNS.[10]

As mentioned by the contributor, positive immunohistochemical staining for oligodendrocyte transcription factor-2 (Olig2) in combination with negative glial fibrillary acidic protein (GFAP) has been shown to be an extremely useful tool in the diagnosis of oligodendrogliomas. The proportion of Olig2-positive cells has been shown to be significantly higher in oligodendrogliomas when compared with other glial tumors such as astrocytoma and oligoastrocytoma, mentioned above.[4,6] Prior to the conference, an Olig2 and GFAP stains were performed by the Joint Pathology center. In this case, neoplastic cells showed strong and diffuse intranuclear immunoreactivity to Olig2. Expression of intranuclear Olig2 immunoreactivity is restricted to the neoplasm and resident oligodendrocytes within the unaffected section of cerebrum. Additionally, neoplastic cells are diffusely immunonegative for GFAP. The GFAP-positive cells noted by conference participants at the periphery and within the neoplasm are interpreted as reactive astrocytes, which can occur due to tumor-induced activation of the residential astroglial network. This staining pattern is consistent with the contributor's diagnosis of an oligodendroglioma, in this case.[2,4,6,8]



Cerebrum, dog. Neoplastic cells exhibit diffuse, strong intranuclear staining for olig-2. (anti-olig-2, 400X)

Contributing Institution:

Department of Diagnostic Medicine and Pathobiology
Kansas State Veterinary College of Veterinary Medicine
1800 Denison Avenue
Manhattan, KS 66506
http://www.vet.k-state.edu/depts/dmp/index.htm

References:

1. Burger PC, Scheithauer BW: Tumors of neuroglia and choroid plexus. In: *AFIP Atlas of Tumor Pathology, Series 4, Tumors of the Central Nervous System*. ed. Silverberg SG. Washington DC: ARP Press; 2007:225-233.
2. Cantile C, Youssef S. Nervous system. Maxie MG ed. In: *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:400.
3. Ide, Uchida K, Kikuta F, Suzuki K, Nakayama H. Immunohistochemical characterization of canine neuro-epithelial tumors. *Vet Pathol*. 2010; 47(4)741-750.
4. Johnson GC, Coates JR, Wininger F. Diagnostic immunohistochemistry of canine and feline intracalvarial tumors in the age of brain biopsies. *Vet Pathol*. 2014; 51(1)146-160.
5. Koestner A, Higgins RJ. Tumors of the nervous system. In: *Tumors in Domestic Animals*. ed. Meuten DJ. 4th ed. Ames, IA: Iowa State Press; 2002:703-706.
6. Kovi RC, Wünschmann A, Armién AG, Hall K, Carlson T, Shivers J, Oglesbee MJ. Spinal meningeal oligo-dendrogliomatosis in two boxer dogs. *Vet Pathol*. 2013; 50(5):761-764.
7. Kremer D, Gottle P. et al. Pushing forward: Remylination as the new frontier in CNS disease. *Trends Neurosci*. 2016; 39(4):246-263.
8. Rissi DR, Levine JM, et al. Cerebral oligodendroglioma mimicking intra-ventricular neoplasia in three dogs. *J Vet Diagn Invest*. 2015; 27(3):396-400.
9. Snyder JM, Shofer FS, Van Winkle TJ, Massicotte C: Canine intracranial primary neoplasia: 173 cases (1986-2003). *J Vet Intern Med*. 2006; 20:669-675.
10. Toy D, Namgung U. Role of glial cells in axonal regeneration. *Exp neurobiol*. 2013; 22:68-76.

CASE III: 05-7078-B (JPC 4085316).

Signalment: Three-month-old, Pinzgauer-cross, steer, (*Bos taurus*).

History: Since birth, the calf failed to grow hair and maintained a thickened crusty skin. Shortly before death, the calf became anorexic and lethargic. The calf had developed disseminated pocks, sub-cutaneous nodules, and interdigital ulcers. This calf was the first on this farm with this presentation.

Gross Pathology: This three-month-old Pinzgauer-cross steer calf presented with severe generalized hypotrichosis with the exception of the preputial skin. Large areas of the skin, especially the trunk, were

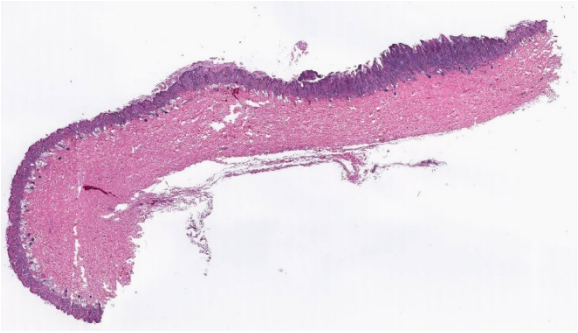
Haired skin, calf. Large areas of the skin, especially the trunk, were covered with thick layers of keratin resulting in a fish scale or elephant skin appearance. (Photo courtesy of Oregon State University Diagnostic Laboratory http://vetmed.oregonstate.edu/diagnostic)

covered with thick layers of keratin resulting in a fish scale or elephant skin appearance. The thickened keratin layer had criss-crossed, deep fissures that extended into the dermis. There were scattered dermal, up to 7mm in diameter nodules over the trunk, thigh, and shoulder. Over the carpal and tarsal joints, the skin was smooth. The interdigital skin had multiple small (up to 5 mm) areas of ulceration. All subcutaneous and internal lymph nodes were moderately to severely enlarged (e.g., right prescapular lymph node measured 7 x 3 x 2). The oral mucosa had extensive erosions and the tongue had a focal area of hyperkeratosis over the fossa linguae. There were a few small areas of epithelial hyperplasia and hyperkeratosis in the esophagus and forestomachs. No significant gross lesions were present in other organs and tissues including bone marrow, eye, and liver.

Laboratory results: Electron microscopic examination of the skin identified poxviral particles in areas of ballooning degeneration. Immunohistochemistry on a section the skin did not identify bovine viral diarrhea virus.

Histopathologic Description: The section contains skin lesions of increasing severity along the length of tissue with one end much

Forestomachs, calf. There were a few small areas of epithelial hyperplasia and hyperkeratosis in the esophagus and forestomachs. (Photo courtesy of Oregon State University Diagnostic Laboratory http://vetmed.oregonstate.edu/diagnostic)

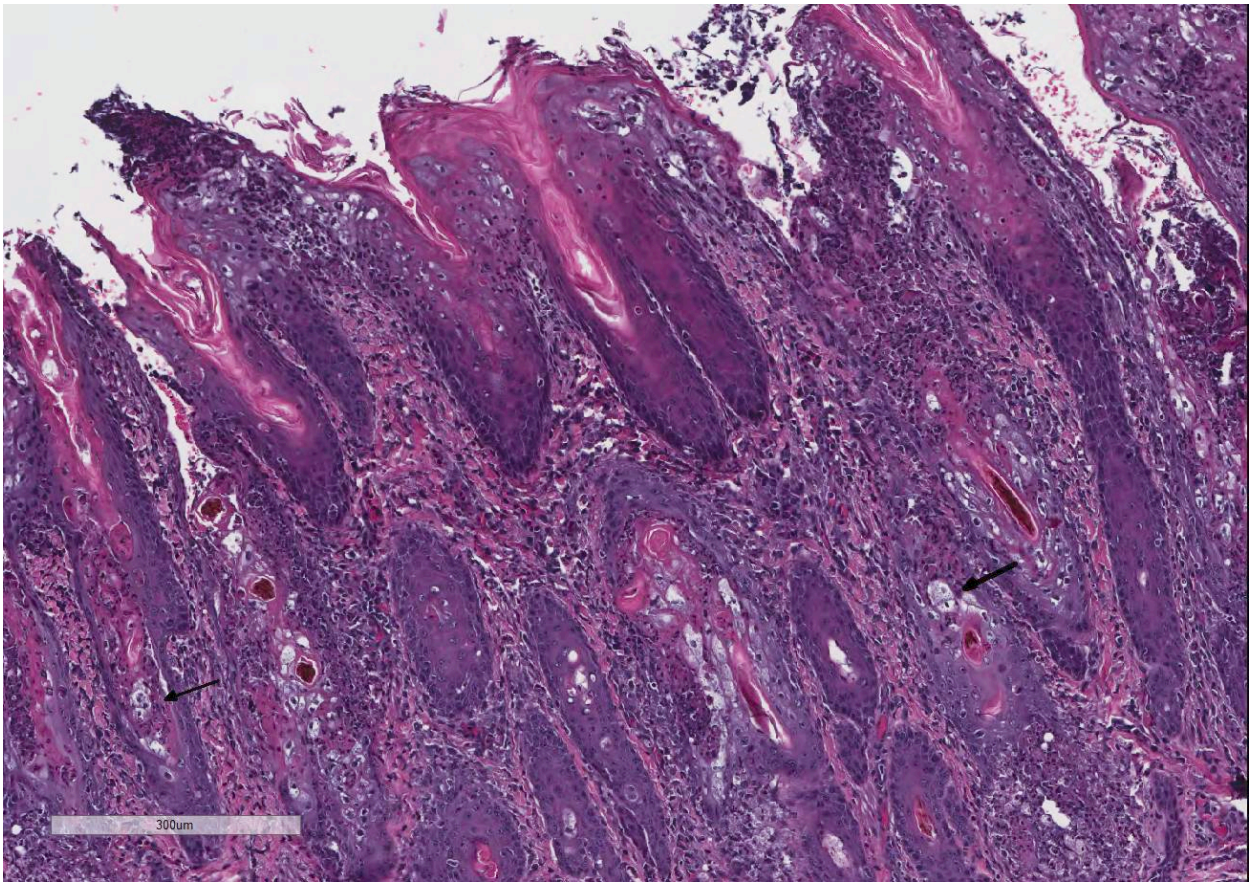


Haired skin. A section of haired skin demonstrates a diffuse hypercellularity of the epidermis and papillary dermis. 50% of the epidermis (at right), displays an increased level of hyperplasia. (HE, 4X).

more affected and distorted than the other. In the section of skin, there is mild to moderate epidermal hyperplasia with mild to severe compact orthokeratotic hyperkeratosis and segmental parakeratosis. In severely affected areas, hair follicles are rare and small for the follicles that are present. Some affected follicles contain clumps of keratin with no discrete hair shafts whereas other contains hair shafts that are angled, thin, and fragmented. Multifocally, the epidermis has severe ballooning degeneration with cytoplasmic eosinophilic inclusions in degenerate keratinocytes. This is associated with suppurative folliculitis and pustular epidermitis. Areas of hyperkeratosis are colonized by small numbers of *Malassezia* sp.

Contributor's Morphologic Diagnosis:
Severe epidermal compact hyperkeratosis
Severe follicular dysplasia and hyper-keratosis
Severe chronic ulcerative and pustular dermatitis with epithelial ballooning degeneration and intracytoplasmic viral inclusion bodies

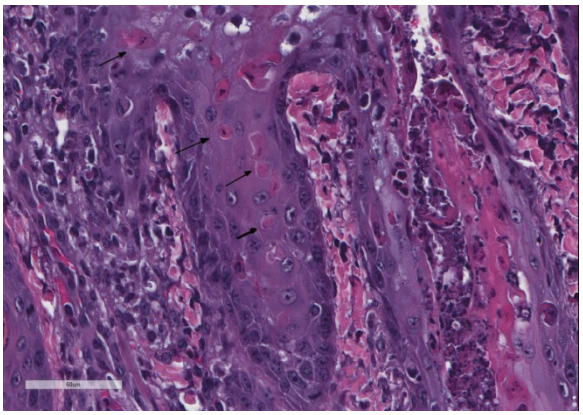
Contributor's Comment: This Pinzgauer-cross calf suffered from a combination of ichthyosis and follicular dysplasia, which was complicated by surface *Malassezia* sp.



Haired skin, calf. A thick plaque of keratin scale spans several hair follicles, extending deeply within them. There is marked hyperplasia of the stratum basale and spongiosum, and small groups of cells which exhibit hydropic degeneration (arrows). There is multifocal pustule formation in the epidermis, as well as suppurative folliculitis. (HE, 288X).

infection, suppurative folliculitis, and changes compatible with a poxviral dermatitis. This constellation of maladies has not, to the best of the authors' knowledge, yet been described. Autosomal recessive ichthyosis with hypotrichosis syndrome has been described in humans, but not well established in the veterinary literature. The skin of this calf tested negative for bovine viral diarrhea virus by immunohistochemistry and an underlying virus induced immune deficiency was excluded in this case.

There are at least 36 types of ichthyosis described in humans.[14] Ichthyosis in animals has not been well correlated with human forms. However, there are five human ichthyosis correlates described in animals, which include ichthyosis vulgaris, x-linked ichthyosis, epidermolytic hyperkeratosis, lamellar ichthyosis and harlequin ichthyosis.[9]

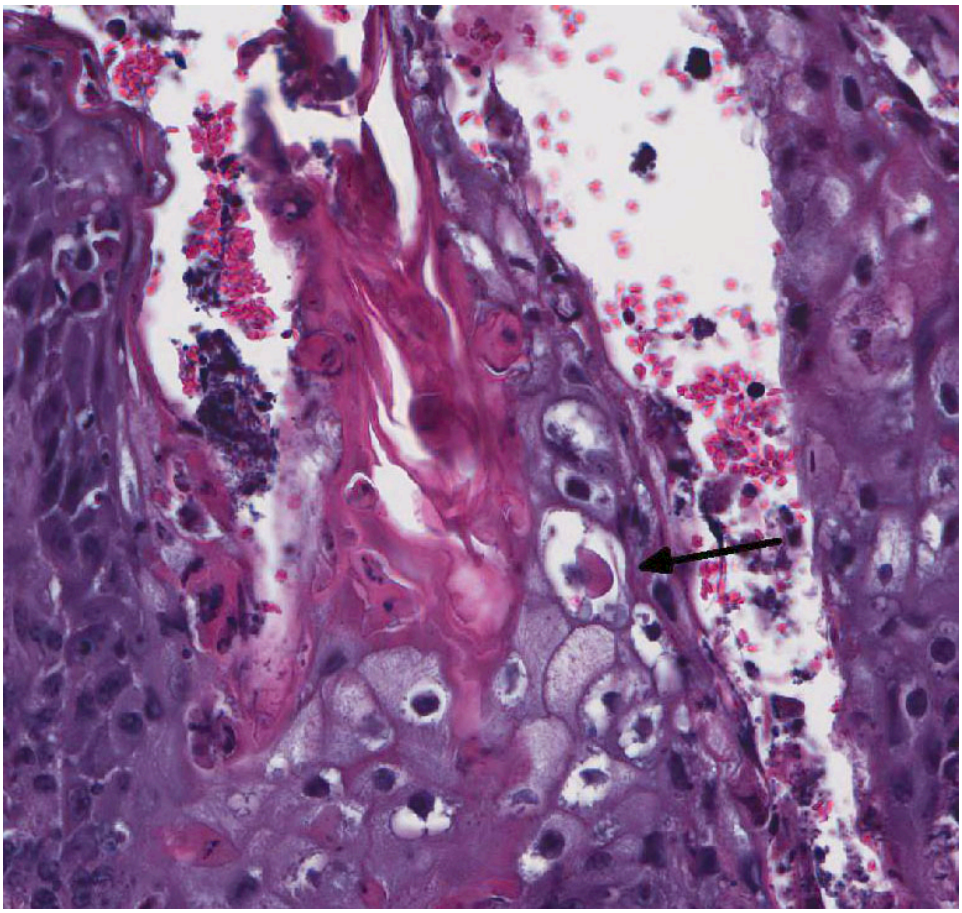


Haired skin, calf. There are numerous apoptotic cells scattered throughout the epidermis and follicular epithelium (arrows)

Ichthyosis is a rare skin condition that has been shown to affect cattle, dogs, pigs, chickens, mice and llamas[9], among others. Two inherited forms of ichthyosis, both through autosomal recessive genes, have been reported in cattle: ichthyosis fetalis and ichthyosis congenita. Ichthyosis fetalis is generally fatal and affected animals usually survive for only a few days after birth. This disease has been described in Norwegian red poll, Friesian, and brown Swiss calves, and this entity most closely resembles human harlequin ichthyosis.[2] Hairless skin is associated with ichthyosis fetalis and is characterized by large, horny, plates separated by deep clefts. Microtia, cataracts, thyroid hypoplasia, and eversion of mucocutaneous junctions are common features of this type of ichthyosis. These manifestations were not apparent in this case.

Ichthyosis congenita, a milder variant of ichthyosis, has been reported in Jerseys, Pinzgauer, Chianina, and Holstein-Friesian breeds.[9] The lesions of ichthyosis congenita are similar to those of ichthyosis fetalis, but less severe and often localized to the skin over the abdomen, inguinal region, joints and the muzzle. Ichthyosis congenita most closely resembles human lamellar ichthyosis. Due to the older age of this calf and clinical presentation, ichthyosis congenita is suspected in this case.

Eight genes have been linked to congenital ichthyosis: TGM1 (in people and Jack Russell terriers, but shown to not be the case in cattle[3]), ABCA12 [1], ABHD5(CG158)[7], 2 lipoxygenases (ALOXE3 and ALOX12B[5]), NIPAL4 (ICTHYIN, Golden retrievers[10]), LIPN[4], CYP4F22[13], and PNPLA-1(American bulldogs[16]). The listed mutations result in disruption of the normal protective barrier that the skin provides while ichthyosis represents the local response to restore that barrier[4]. For example, TGM deficiency results in the abnormal cross-linking of the cornified envelope. Lipoxygenase deficiency in ALOXE3 and ALOX12B mutations are directly associated with abnormal lipid metabolism, impairing the structural integrity of the lipid bilayers. GJB2 mutations affect gap-junction integrity. Accumulation of large amounts of keratin and impairment of the permeability barrier promote the colonization and subsequent infection of skin. The surface *Malassezia* sp. infection, suppurative folliculitis, and poxviral dermatosis likely represent secondary lesions to the ichthyosis.



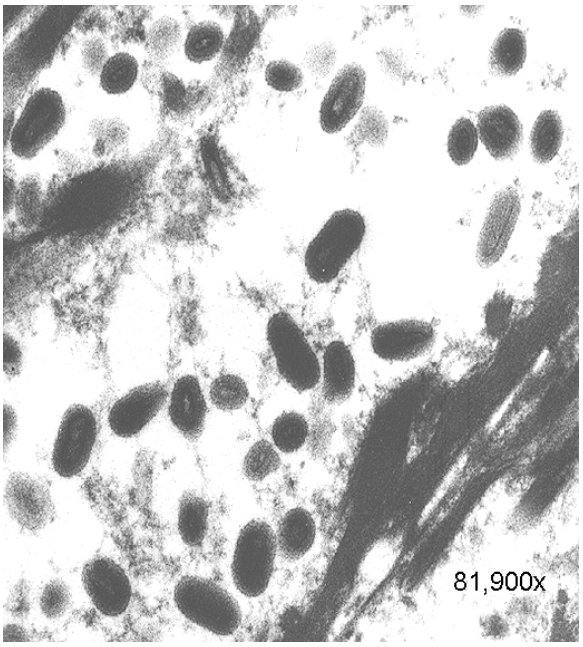
Haired skin, calf. Scattered throughout the proliferative epidermis, there are nests of cells which exhibit ballooning degeneration with large eosinophilic viral inclusions (arrow). (HE, 400X)

Follicular dysplasia or hypotrichosis is not an initial feature of ichthyosis congenita. However, hairlessness was present at birth in this calf, which suggests a primary rather than secondary process. Histologically, the follicular morphology is most consistent with follicular dysplasia. Based on genetic studies, an autosomal recessive component for follicular dysplasia has been described in cattle, such as for Herefords, Polled Herefords, Ayrshires, Guernseys, Jerseys, Holsteins and Black Angus.[8,12,15]

JPC Diagnosis: 1. Haired skin: Epidermal dysplasia, diffuse, severe, with focally extensive epidermal and follicular compact othokeratotic hyperkeratosis, suppurative epidermitis and folliculitis, and marked follicular dysplasia, Pinzgauer-cross, steer, *Bos taurus*. 2. Haired skin: Dermatitis, pustular, proliferative and ulcerative, focally extensive, severe with ballooning degeneration and intracytoplasmic viral inclusion bodies.

Conference Comment: Conference participants unanimously agreed that this is a fascinating case and both a diagnostic and descriptive challenge. The superimposition of the multiple processes in this case made it difficult to identify the etiology of the inflammatory process. In fact, given the degree of inflammation within the proliferative area, most participants did not initially identify poxviral intracytoplasmic inclusion bodies within areas of epidermal ballooning degeneration, confirmed by the contributor's provided transmission electron microscopy image. Readers are encouraged to review 2013 Wednesday Slide Conference 25 Case 3 for a review of poxviruses in various veterinary species.

Participants were struck by the diffusely malformed hair follicles numerous containing shrunken, twisted, coiled, fragmented and densely packed hair shafts consistent with follicular dysplasia.



Haired skin, calf. Numerous poxviral particles were identified in the cytoplasm of cells exhibiting ballooning degeneration.

Follicular dysplasia is the congenital abnormality of the morphogenesis of hair follicles leading to changes in the quality and/or quantity of the hair shaft and resulting in hypotrichosis.[8,9,11] Congenital hypotrichosis has been widely reported in all domestic veterinary species, but is most common in calves. Various reported cases of congenital hypotrichosis in cattle have either been associated with an X-chromosome linked ectodermal dysplasia, autosomal dominant, or autosomal recessive hereditary conditions.[9,11] Cattle affected by X-linked ectodermal dysplasia also have concurrent dental disease characterized by incomplete dentition and lack of secondary tooth development and is known as hypotrichosis and anodontia (HAD). In Holsteins, this has been associated with an inherited defect in the ectodysplasin-1 (ED1) gene.[9,11]

Both lethal and viable variants of autosomal recessive and dominant forms of congenital hypotrichosis have been reported with generalized alopecia associated with excessive scaling, infundibular hyper-keratosis, easily broken and misshapen hair shafts, keratinocyte degeneration, and dilated apocrine glands. Other reported findings include subcapsular hepatic fibrosis, anemia, and neurologic deficits.[8,11] Non-genetic causes of hypotrichosis in calves include iodine deficiency, adeno-hypophyseal hypoplasia, maternal ingestion of the toxic plant *Veratrum album*, and intrauterine infection with bovine pestivirus.[9]

Contributing Institution:

Veterinary Diagnostic Laboratory
Oregon State University
134 Magruder Hall
Corvallis, OR 97331
http://vetmed.oregonstate.edu/diagnostic

References:

1. Charlier C, Coppieters W, Rollin F, et al. Highly effective SNP-based association mapping and management of recessive defects in livestock. *Nat Genet*. 2008;40(4):449-454.
2. Chittick EJ, Olivry T, Dalldorf F, Wright J, Dale B a, Wolfe B a. Harlequin ichthyosis in two greater kudu (Tragelaphus strepsiceros). *Vet Pathol*. 2002;39:751-756.
3. Dardano S, Gandolfi B, Parma P, et al. Characterization of bovine TGM1 and exclusion as candidate gene for ichthyosis in Chianina. *J Hered*. 2008;99(1):81-83.
4. Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. *J Lipid Res*. 2008;49(4):697-714.
5. Jobard F, Lefèvre C. Lipoxygenase-3 (ALOXE3) and 12 (R)-lipoxygenase (ALOX12B) are mutated in non-

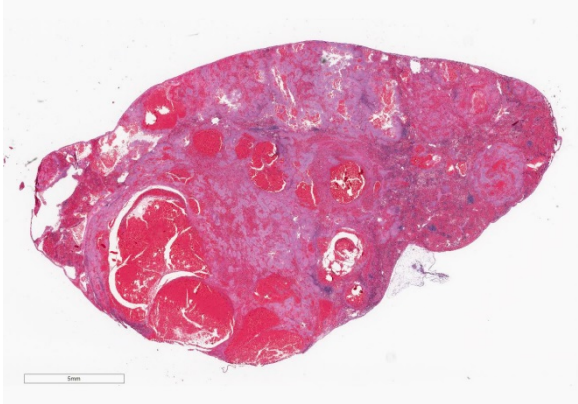
bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome. *Hum Mol Genet*. 2002;11(1):107-113.
6. Jubb, Kennedy & Palmer's Pathology of Domestic Animals 5th edition
7. Lefèvre C, Jobard F, Caux F, et al. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. *Am J Hum Genet*. 2001;69(5):1002-1012.
8. Mansell JL. Follicular dysplasia in two cows. *Vet Dermatol*. 1999;10(2):143-147.
9. Mauldin EA, Peters-Kennedy J. Integumentary system. In: Maxie MG ed. In: *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:530-532, 538-540.
10. Mauldin EA, Wang P, Evans E, et al. Autosomal Recessive Congenital Ichthyosis in American Bulldogs Is Associated With NIPAL4 (ICHTHYIN) Deficiency. *Vet Pathol*. 2015;52(4):654-662.
11. Mecklenburg L. An overview on congenital alopecia in domestic animals. *Vet Dermatol*. 2006;17(6):393-410.
12. Miller WH, Scott DW. Black-hair follicular dysplasia in a Holstein cow. *Cornell Vet*. 1990;80(3):273-277.
13. Ohno Y, Nakamichi S, Ohkuni A, et al. Essential role of the cytochrome P450 CYP4F22 in the production of acylceramide, the key lipid for skin permeability barrier formation. *Proc Natl Acad Sci U S A*. 2015;112(25):7707-7712.
14. Oji V, Tadini G, Akiyama M, et al. Revised nomenclature and classification of inherited ichthyoses: Results of the First Ichthyosis Consensus Conference in Sorèze 2009. *J Am Acad Dermatol*. 2010;63(4):607-641.
15. Scott DW. *Color Atlas of Farm Animal Dermatology*. 1st ed.; 2008.
16. Tamamoto-Mochizuki C, Banovic F, Bizikova P, Laprais A, Linder KE, Olivry T. Autosomal recessive congenital ichthyosis due to PNPLA1 mutation in a golden retriever-poodle cross-bred dog and the effect of topical therapy. *Vet Dermatol*. May 2016.

CASE IV: N9882548 (JPC 4084539).

Signalment: Three-year-old castrated male domestic shorthair cat (*Felis catus*).

History: History of inflammatory bowel disease treated with prednisolone. Presented with lethargy, anorexia, and a change in behavior (less social).

Gross Pathology: Hemoabdomen with multiple splenic masses on abdominal ultrasound and exploratory laparotomy with subsequent splenectomy.



Spleen, cat: The normal splenic architecture is effaced by coalescing nodules of inflammation which alter splenic hemodynamics, often resulted in markedly dilated sinusoids and vessels. (HE, 5X)

On gross evaluation of the spleen, the spleen was enlarged with diffuse nodularity and rounded edges. Expanding the right ventral aspect, there was an approximately 7 x 3.5 x 2.5 cm mass-like swelling. On cut section, the parenchyma was dark red-purple with foci of hemorrhage.

Laboratory results: Moderate regenerative anemia (Hct 18.7%, reticulocyte count 171,000/uL), WBC within reference (8,900/uL), and moderate thrombocytopenia (90,000/uL).

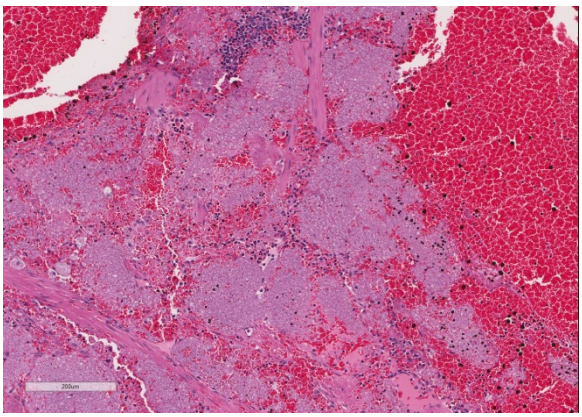
Histopathologic Description: Multifocally throughout the splenic parenchyma, there are large areas of congestion, hemorrhage, necrosis, fibrin deposition and extensive accumulation of extracellular pale basophilic to amphophilic foamy to stippled material composed of 2-4 um diameter round structures each with a thin wall and a central basophilic granular core, consistent with cyst and trophozoite structures. There is a mild to moderate infiltrate composed of macrophages with fewer neutrophils and occasional macrophages contain similar round structures in the cytoplasm. In the intervening congested red pulp, there are numerous hematopoietic precursors including predominantly erythroid and megakaryocytic cells.

Special stain: The cell wall of the round structures is multifocally faintly positive for GMS (Gomori methenamine silver) stain and acid-fast negative.

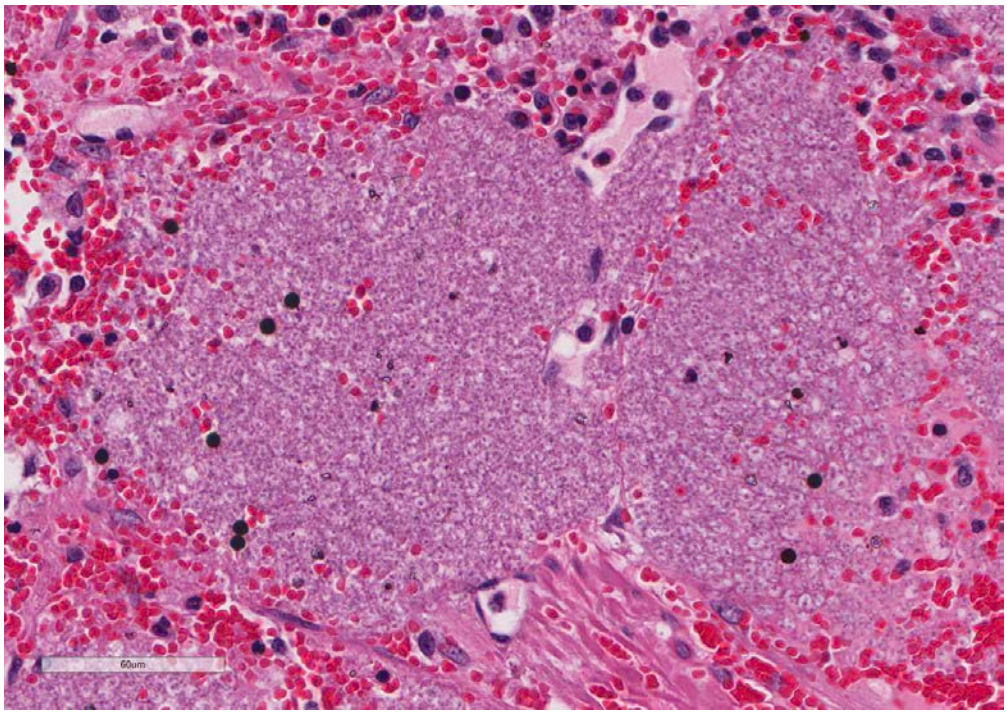
Contributor's Morphologic Diagnosis: Spleen: Severe multifocal to coalescing congestion, hemorrhage, and necrosis with myriad extracellular and intrahistiocytic cysts and trophozoites, consistent with *Pneumocystis* spp.
Pneumocystis spp. confirmed with immuno-histochemistry, electron microscopy, and PCR testing. Sequencing suggests a novel species/strain.

Contributor's Comment: Given the lack of clinical signs referable to the respiratory tract and the extensive splenic involvement by the infection, the findings are consistent with extrapulmonary pneumocystosis in this case. A small splenic choristoma in the pancreas was also affected and a minor intravascular population of these organisms was identified in the liver at the time of biopsy.

Pneumocystis is a saprophytic organism that has somewhat uncertain taxonomy.



Spleen, cat. The splenic red pulp is markedly expanded by vague nodules of foamy exudate. (HE, 48X)

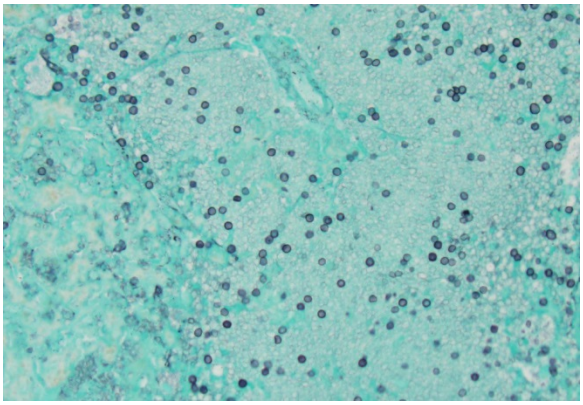


Spleen, cat. The foamy exudate is composed of numerous trophozoites with a central nucleus, consistent Pneumocystis carinii. (HE, 360)

Pneumocystis has been classified as a unicellular protozoan of the phylum *Sarcomastigophora*, subphylum Sarcodina. The reproductive behavior is similar to the ascospore formation of yeast cells and the staining properties resemble that of pathogenic fungi. Based on 16S-like rRNA, *Pneumocystis* is phylogenetically most closely related to the fungi of the class *Ascomycetes*. Multiple species have been designated, although there is some controversy in this area and several strains likely exist. The most widely referenced species are *Pneumocystis carinii* in dogs and *P. jiroveci* in humans, with several species indicated in the laboratory animal literature. The life cycle of *Pneumocystis* involves a tropho-zoite and a cyst form, both of which occur in the infected tissue.[5]

Pneumocystis is best known as an opportunistic organism that most commonly causes pneumonia, in particular in immuno-compromised people and animals. Clinical pneumonia occurs in many cases, but subclinical or latent infections are also common in many animal species, including cats. Clinical disease may occur via new infection or reactivation of a latent infection under conditions of stress, immuno-suppressive therapy, or with other underlying infection, such as canine distemper virus in dogs.[5] In dogs, most reported cases of pneumocystic pneumonia occur in animals with underlying immuno-deficiency, such as miniature Dachshund, Pomeranian and Cavalier King Charles spaniel dogs with combined immunodeficiency syndrome.[2,4] In cats, subclinical or latent infections are most common, and although *Pneumocystis* has been identified in the lungs in the cat, natural cases of clinical disease have not been reported.[5] Under experimental conditions, immunosuppression with glucocorticoid administration has led to pneumocystic pneumonia in cats.[5]

Extrapulmonary infection with *Pneumo-cystis* has been reported in humans, particularly with HIV/AIDS, and involves the spleen in some cases.[1,3] A case of extrapulmonary pneumocystosis has also been reported in a dog.[5] In humans with extrapulmonary infections, some cases have concurrent pneumocystic pneumonia, while



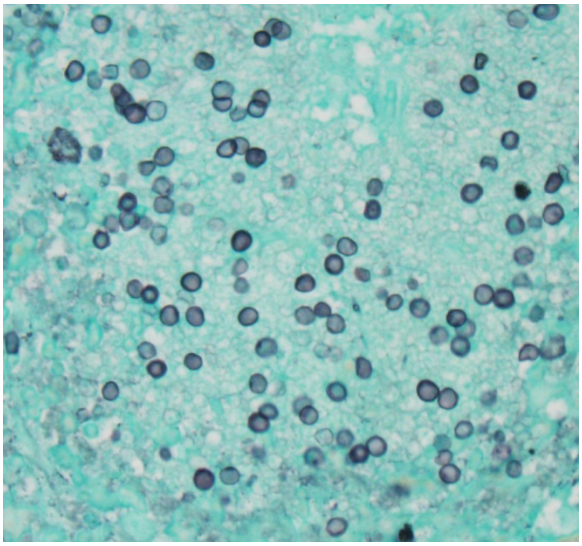
Spleen, cat. The exudate contains numerous argyrophilic viable P. carinii trophozoites. (GMS, 200X)

others do not, and those that do not are thought to either acquire a primary extra-pulmonary infection or have reactivation of a latent infection at extrapulmonary sites.[1] Other than spleen, reported sites for extrapulmonary involvement in humans include lymph node, liver, bone marrow, adrenal gland, gastro-intestinal tract, kidney, thyroid gland, heart, pancreas, central nervous system, bone, eyes or ears.[1,3] In a minority of cases, intravascular organisms are identified in the tissues,[1] which was present in the liver biopsy from this patient.

In pneumocystic pneumonia, the classic histologic appearance is of intra-alveolar aggregates of foamy eosinophilic material with only a minor infiltrate of macrophages and without significant neutrophilic inflammation. GMS staining highlights the 4-7um diameter cyst wall and more easily demonstrates the ovoid, irregular or crescent shape of the cysts. At extrapulmonary sites, *Pneumocystis* has similar tissue destruction with necrosis and a similar appearance of foamy eosinophilic material.[1,3] Methods of confirmation of *Pneumocystis* include cytologic or histologic morphologic appearance and GMS staining characteristics, PCR evaluation, immuno-histochemistry or immunofluorescence.[3,5]

JPC Diagnosis: Spleen: Splenitis, necrotizing and hemorrhagic, diffuse, severe with numerous extracellular and intra-histiocytic trophozoites, domestic shorthair, *Felis catus*.

Conference Comment: We thank the contributor for providing a thorough review of the epidemiology, pathogenesis, and comparative pathology of *Pneumocystis* spp. in humans and veterinary species. This outstanding case provided conference participants the opportunity to describe and identify a relatively common opportunistic pathogen in an extremely rare extra-pulmonary location and an uncommonly affected species. Despite the lack of case reports of feline splenic pneumocystosis in the veterinary literature, many conference participants included *Pneumocystis* sp. on their list of differential diagnoses due to the classic histomorphology, characterized by the presence of abundant extracelluar and intrahistiocytic foamy lightly eosinophilic material and absence of overwhelming inflammation. Although definitive vasculitis and thrombosis are not seen, conference participants also readily identified the



Spleen, cat. Higher magnification of Pneumocystis trophozoites. (GMS, 400X)

numerous brilliant hemodynamic changes present in the tissue section, indicated by severe congestion, marked dilation of sinusoids, and hemorrhage. Multifocal areas of extramedullary hematopoiesis are also identified, although its association with the infectious etiology is unclear.

As mentioned above, extrapulmonary pneumocystosis is rare but has been reported in a dog and immunocompromised people, typically affected with HIV/AIDS.[3,5,6] Postmortem analysis from previously reported human cases indicates that *Pneumocystis* sp. can disseminate throughout the body via hematogenous and/or lymphatic routes. It is thought that the vast majority of cases of extrapulmonary pneumocystosis result from hematogenous or lymphatic spread from the lung. However, in cases where the lungs are unaffected, such as in this cat, there may be reactivation of latent infection in extra-pulmonary organs due to severe immunosuppression.[6]

Contributing Institution:
Animal Medical Center
New York, NY
http://www.amcny.org/

References:

1. Cohen OJ and Stoeckle MY. Extrapulmonary *Pneumocystis carinii* infections in the acquired immunodeficiency syndrome. *Arch Intern Med*. 1991; 151:1205-1214.
2. Kanemoto H, Morikawa R, Chambers JK, et al. Common variable immune deficiency in a Pomeranian with *Pneumocystis carinii* pneumonia. *J Vet Med Sci*. 2015; 77(6):715-719.
3. Karam MB and Mosadegh L. Extra-pulmonary *Pneumocystis jiroveci* infection: A case report. *Braz J Infect Dis*. 2014; 18(6):681-685.
4. Lobetti R. Common variable immunodeficiency in miniature dachshunds affected with *Pneumocystis carinii* pneumonia. *J Vet Diagn Invest*. 2000; 12:39-45.
5. Lobetti R. Pneumocystosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*. 4th ed. St. Louis, MO: Elsevier Inc; 2012:689-695.
6. O'Neal CB, Ball SC. Splenic pneumocystosis: An atypical presentation of extrapulmonary *Pneumocystis* infection. *AIDS Reader*. 2008; 8:503-508.