Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 12

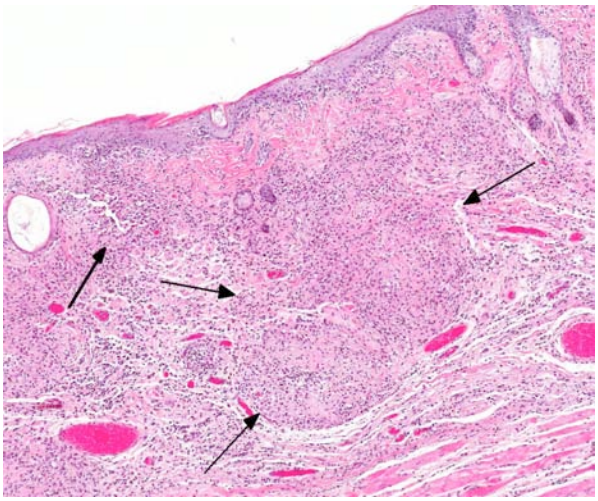
15 January 2014

CASE I: 12-1353 (JPC4033123).

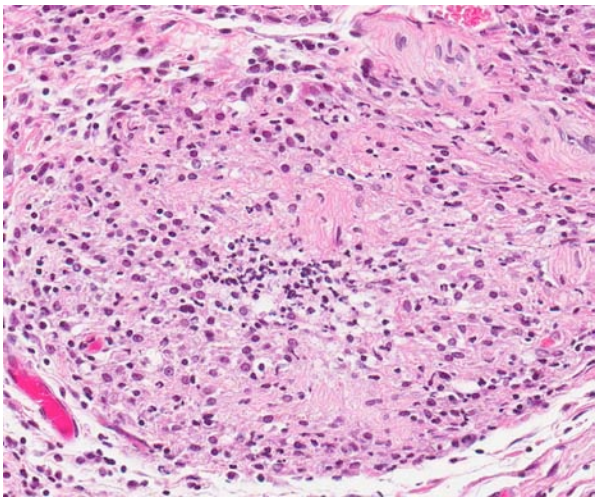
Signalment: Adult male Djungarian hamster (*Phodopus sungorus*).

History: Four adult dwarf hamsters with no significant prior medical history were on display at a museum petting exhibit. The animals were in the process of being retired from the exhibit with the intention of adoption when skin lesions were noticed on two hamsters. A male hamster described to have one necrotic pinna was culled and both pinnae were submitted for biopsy. Within one week of receiving biopsy results, a second male adult hamster with an abdominal skin lesion was culled and submitted for autopsy. The submitted tissue section is from the second hamster.

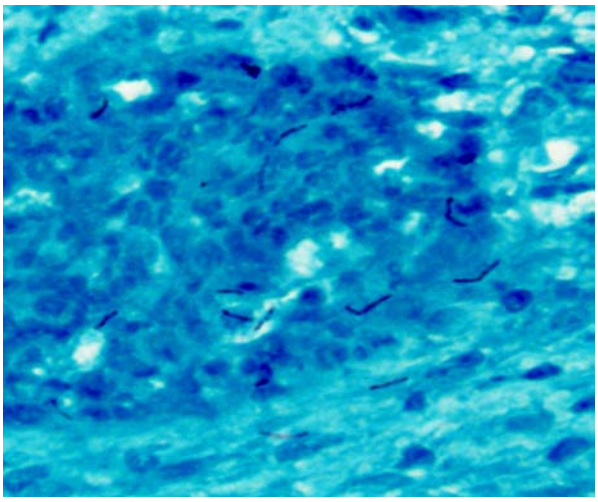
Gross Pathology: A 50g adult male hamster is presented for postmortem examination. The carcass is in good postmortem condition with a



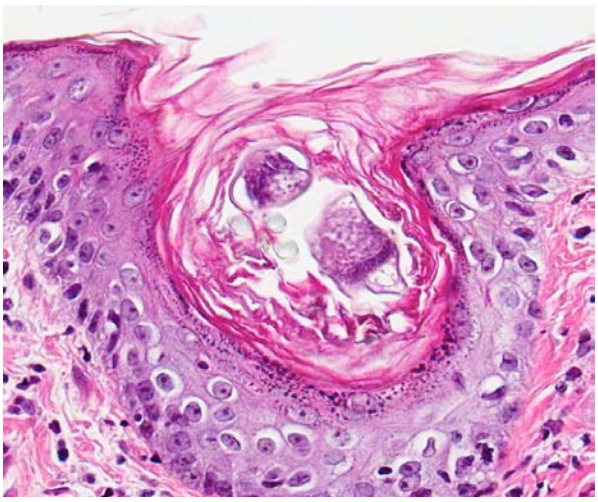
1-1. Haired skin, hamster: The superficial dermis is expanded by multifocal to coalescing aggregates of foamy macrophages. (HE 88X)



1-2. Haired skin, hamster: Some of the granulomas have a central core of viable neutrophils. (HE 120X)



1-3. Haired skin, hamster: Scattered within granulomas are low numbers of filamentous, beaded, acid-fast bacilli. (Fite-Furaco 400X)



1-4. Haired skin, hamster: Epidermal pits and hair follicles contain cross-section of mites. (HE 400X)

euthanasia-to-necropsy interval of 13 hours. Throughout the body, adequate adipose stores are present. A focally extensive area of caudal abdominal skin, extending from the umbilicus to the perineal region and encompassing both the left and right inguinal areas, has an asymmetric, poorly haired, moist, red patch with a 0.1 cm in diameter paramedian ulcer and several small irregular brown crusts. No internal gross abnormalities are noted. Caudal abdominal skin is submitted.

Laboratory Results: Formalin-fixed, paraffin-embedded tissue was submitted for mycobacterial PCR, and the resulting sequence most closely matched that of *Mycobacterium marinum* and *M. ulcerans* (greater than 99% sequence identity with GenBank acc#AB026701 and CP000325).

Histopathologic Description: Abdominal skin: The dermis and panniculus are expanded by multifocal to coalescing nodular infiltrates of abundant histiocytes and neutrophils that form multifocal pyogranulomas with abundant central degenerative neutrophils and necrotic cellular debris surrounded by a thick rim of epithelioid macrophages. Low numbers of gram-positive, acid-fast bacilli are present in scattered histiocytes. The overlying epithelium is multifocally, irregularly hyperplastic with multifocal intracellular edema, frequent neutrophil and lymphocyte transmigration which frequently disrupts the basal layer, few intraepidermal neutrophil aggregates, and mild orthokeratotic hyperkeratosis. (The original pinna biopsy submission from the male hamster has similar histological findings.)

Contributor's Morphologic Diagnosis: Haired skin: Marked, multifocal to coalescing pyogranulomatous dermatitis and panniculitis with intralesional acid -fast bacilli (mycobacteriosis).

Contributor's Comment: Mycobacteriosis in mammals typically occurs via contamination of skin wounds or traumatic inoculation and rarely via inhalation.[6] Direct contact with environmental inhabitants generally results in local disease, but can pose a greater risk to immunocompromised individuals. *M. marinum* is ubiquitous in fresh, brackish, and salt water, and causes disease in fish and humans ("fish tank granulomas" or "swimming pool granulomas"). Human infection typically results from direct inoculation into skin abrasions while handling fish or tank water, leading to slow growing nodules at the site of inoculation and local lymphadenopathy.[3] *M. ulcerans* is the causative agent of Buruli ulcer, the third most common mycobacterial disease in humans. Epidemiologic factors are not completely understood, as the disease is most common in dry periods in wetlands of tropical or subtropical regions.[6] The toxin mycolactone is believed to cause the characteristic ulcerative and necrotic cutaneous and subcutaneous lesions.[2] *M. ulcerans* is a rare cause of atypical mycobacteriosis in cats, causing ulcerative and nodular skin lesions.[7] Koalas and possums in Australia are naturally infected with

M. ulcerans.[5] Recently it has been proposed that small mammals may serve as a reservoir for *M. ulcerans*.[1]

The source of mycobacterial infection in the museum hamsters was not identified, although there are numerous aquatic displays at the museum. Despite the zoonotic potential, no human cases of infection were reported.

JPC Diagnosis: 1. Haired skin and subcutis: Dermatitis, pyogranulomatous, multifocal to coalescing, moderate, with edema and rare intrahistiocytic acid-fast bacilli.
2. Haired skin, epidermis: Hyperplasia, multifocal, mild to moderate with mild orthokeratotic hyperkeratosis, intracorneal pustules and occasional acarid parasites.

Conference Comment: *Mycobacterium ulcerans* and *M. marinum* are closely related, slow growing, opportunistic pathogens most notable for causing skin disease in humans[8] (see WSC 2013-14 conference 2, case 2 for a summary of the mycobacterial classification system). Aquatic *Acanthamoeba* sp. has been implicated as a natural host of *M. marinum* and *M. ulcerans*, and may play an important role in disease transmission;[9] however, in this case there is no indication that the affected hamsters were housed near the museum's aquatic displays.

In addition to the striking dermal lesions in this hamster, attributed to infection with *M. marinum/ ulcerans*, there are rare intrafollicular and intracorneal segments of arthropods, up to 40 µm in diameter and 200 µm in length, with a thin, eosinophilic, chitinous exoskeleton, short jointed appendages, a hemocele, striated muscle, and digestive and reproductive tracts. These arthropods are associated with epidermal hyperplasia, orthokeratosis, and occasional intracorneal pustules. The differential diagnosis includes *Demodex* sp. and *Notoedres* sp. *Demodex criceti* and *D. aurati* infestation is common in many hamster colonies; however, these mites exhibit low pathogenicity and rarely cause clinical signs. *Notoedres notoedres*, a mite that burrows into the stratum corneum, is less common, although it can be enzootic in some hamster colonies. Males typically have a higher parasite load than females, and factors such as advanced age or stress from handling may predispose skin lesions in animals with no previous clinical signs.[4]

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http://www.cvm.ncsu.edu/dphp/path/anatomicpath.htmlhttp://www.cvm.ncsu.edu/dphp/path/anatomicpath_eduinfo.html#resprogram

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CASE II: AFIP1 Pfizer (JPC 4001270).

Signalment: 4.5- month-old male New Zealand white rabbit (*Oryctolagus cuniculus*).

History: Several rabbits presented with anorexia and loose feces.

Gross Pathology: Necropsy of one of the rabbits revealed that the stomach was distended with food and the remainder of the gastrointestinal tract contained loose digesta. Based on the necropsy findings and clinical observations, enteropathy was diagnosed and routine histology sections were processed.

Electron microscopy: Intestine: The image consists of portions of four enterocytes, identified by the presence of numerous normal and swollen microvilli. Some of the microvillar border is replaced by randomly arranged, oval to elongate, approximately 0.8 to 1.0 µm diameter, encapsulated bacilli. The bacilli are located outside the cell membrane on the apical surface of enterocyte where they create an indentation (cup and/or pedestal) where they abut the cell surface. At the attachment site, the apical portion of affected enterocytes is thickened by a homogeneous band of lightly electron dense material that disrupts the terminal web. In the swollen microvilli, the actin cystoskeleton is not recognizable. Both nucleated cells present in the image exhibit mild hydropic changes in the apical cytoplasm, indicative of degeneration.

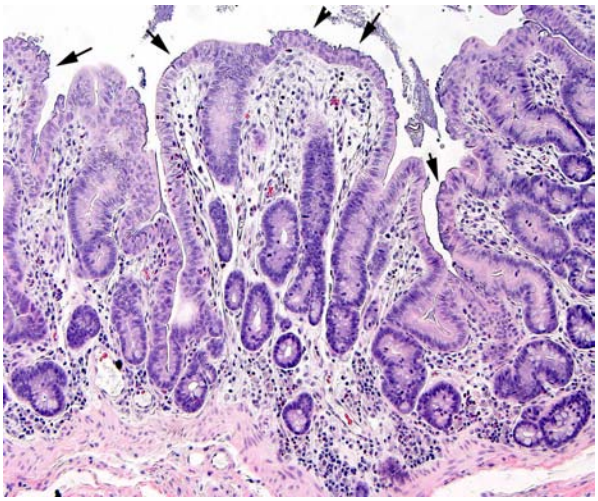
Histopathologic Description: Multiple sections of large intestine are examined and all contain similar lesions. Along the apical surface of enterocytes, there are myriad, often adherent, short (approximately 1 x 2 µm), plump bacilli. The epithelium is often disorganized and crowded containing irregularly shaped enterocytes with slightly basophilic cytoplasm and prominent nuclei (regenerative epithelia) admixed with scattered necrotic epithelial cells. Occasionally, crypts are dilated with heterophils and necrotic cellular debris (crypt abscesses) and lined by flattened, frequently hyperplastic cells. The mucosa is variably edematous and contains moderate numbers of heterophils infiltrating the lamina propria.

Bacteria attached to the apical surface of enterocytes are gram-negative bacilli.

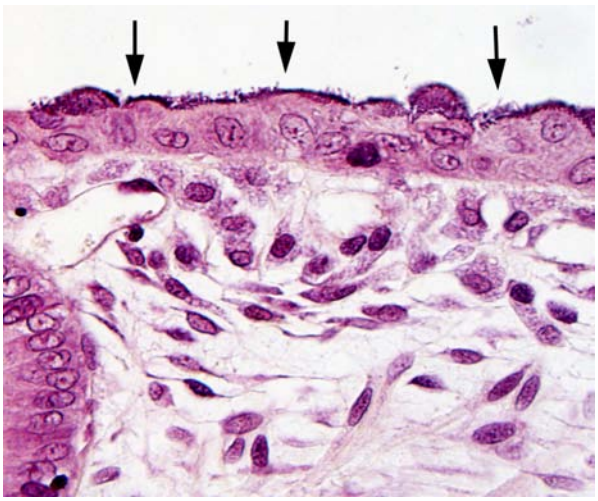
Other findings include renal tubular necrosis and regeneration, hepatic and myocardial necrosis with mineralization, focal heterophilic subpleural pneumonia, and tracheitis.

Contributor's Morphologic Diagnosis: Cecum, Colon: Typhlocolitis, erosive and heterophilic, mild, diffuse, subacute, with myriad attaching gram-negative bacilli.

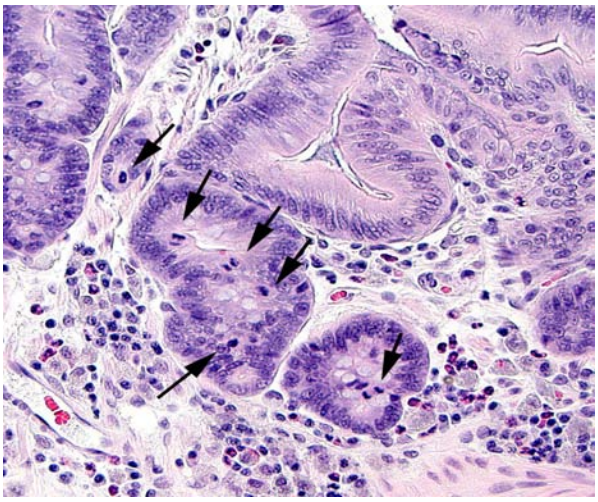
Contributor's Comment: Attaching and effacing *Escherichia coli* (AEEC) is a prominent enteric pathogen in rabbits, and is a major cause of disease in commercial farms and occasionally



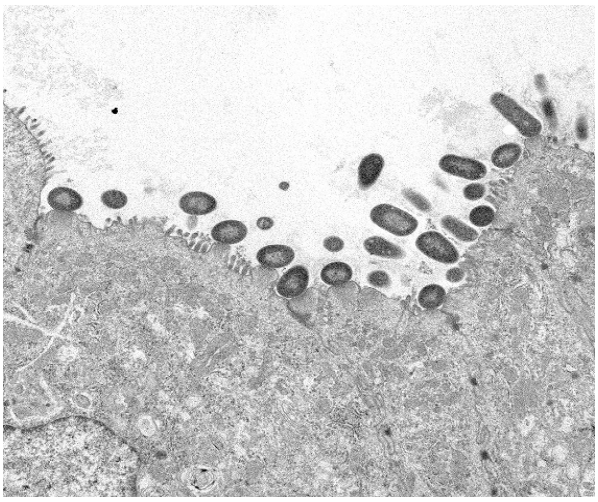
2-1. Colon, rabbit: Edema markedly expands the colonic lamina propria, separating glands. Even at low magnification, the apical brush border of colonic epithelium is prominently basophilic. (HE 80X)



2-2. Colon, rabbit: The microvillar border of the colonic epithelium, both on the luminal surface and within glands is segmentally lined by large numbers of robust gram-negative rods, consistent with attaching and effacing E. coli infection. (Brown-Hopps 400X)



2-3. Colon, rabbit: Mitotic figures are numerous with glands (arrows). (HE 400X)



2-4. Colon, rabbit: Ultrastructurally, E coli attach to the microvillar border with formation of characteristic "cups and pedestals". (Photo courtesy of: Pfizer Inc., Global Research and Development, Groton/New London Laboratories, Eastern Point Road MS 8274-1330, Groton, CT., www.pfizer.com)

in research facilities.[9] Age, history, clinical signs, gross and microscopic findings are useful criteria in the diagnosis. Characterization of the isolate is recommended to determine whether the strain is likely to be a primary pathogen.[9] Mortality varies from very low to very high, and when dealing with low pathogenic strains, the infection can be controlled with hygiene and antibiotic treatments. In the case of highly pathogenic strains, most antibiotics fail and the whole rabbit stock must be killed and replaced.[7] The definitive method for the determination of AEEC lesions is the observation of the characteristic ultrastructural appearance of the lesion, which consists of bacteria attached to the apical surface of enterocytes and goblet cells, with swelling and effacement of microvilli and disruption of the actin cytoskeleton, followed by epithelial desquamation, villous atrophy and malabsorption.[5-7,11] Isolates of enteropathogenic *Escherichia coli* (EPEC) can be subdivided into strains affecting suckling rabbits and those affecting weanlings. In suckling rabbits, the infection is associated with yellowish watery diarrhea in animals ranging from 7 to 12 days old. Lesions are usually found throughout the small and large intestine, accompanied by mucosal ulcerations and hemorrhages. The serotype O109:H2 appears to be restricted to suckling rabbits and causes minimal disease in weaned animals.[8,9]

In weaned rabbits, bacterial attachment occurs in the ileum, cecum and colon. Weanling rabbits are typically affected at 4-6 weeks of age; diarrhea occurs approximately six days after infection and the gross findings include watery intestinal contents, serosal ecchymosis, edema of the intestinal walls and mesenteric lymphadenomegaly. Microscopically, bacteria adhere to enterocytes and have a typical bacillary appearance. Colonized cells appear degenerate and many are hyperchromatic, rounded-up and/or pyknotic, with frequent mucosal erosions and detachment. At low magnification, this confers a "cobblestone" appearance to the mucosal surface. The inflammatory component of the lesion is variable, ranging from mild edema to focal polymorphonuclear infiltrates infiltrating the lamina propria.[9,11] Serotypes frequently found in weaned rabbits are O15:H-, O26:H11, O103:H2 and O109:H2.[8,9]

Initial attachment of the bacteria is non-intimate and restricted to the follicle-associated epithelium of Peyer's patches in the ileum, hence the differences of strain causing disease in suckling and weanlings rabbits. It has been proposed that the resistance of suckling rabbits to weanling-associated strains is due to the fact that Peyer's patches do not develop before two weeks of age.[3]

Primary non-intimate adhesion is critical for the virulence of rabbit strains of EPEC, and is accomplished by a variety of adhesins, often targeted at the follicle associated epithelium of the ileal Peyer's patches, similar to the process

observed in humans.[10] Rabbit AEEC rarely produce enterotoxin or Verotoxin and in contrast to other host species, the presence of *eae* gene in the rabbit is closely associated with diarrheal disease.[2]

The attaching-effacing lesion is due to an interesting mechanism, which is well studied *in vitro* using enteropathogenic *E. coli* (EPEC) O127:H6. This mechanism consists of four stages, responsible for the pathologic and ultrastructural findings (reviewed by Wales et al, 2005).[11] Briefly, the first stage is initial non-intimate attachment. EPEC secreted proteins (Esp) Esp-A, -B and -D are exported via a type III secretion system into the host eukaryotic cell. Esp-B and -D, in addition, may create a pore forming structure in the cell membrane, and these elements form a channel for the introduction of bacterial macromolecules in the host cytoplasm. During this stage, there is translocation of the translocated-intimin-receptor protein (Tir) into the host cell. During the second stage, there is signal transduction leading to cytoskeletal reorganization and microvillus effacement. At the site of intimate attachment, there is considerable cytoskeletal reorganization, with depolarization of actin, formation of F-actin and accumulation of α-actinin, myosin light chain, talin and ezrin; all of these changes are associated with effacement of microvilli and disruption of the intestinal barrier function of enterocytes. During this second stage, Tir is inserted in host cell membrane. The third stage is known as intimate attachment. In the third stage, Tir focuses filamentous actin, forming a "pedestal." Intimin, encoded by the enterocyte attaching and effacing gene (*eae*), is a surface-exposed outer membrane protein. Intimin binds Tir, and this binding is indispensable for the development of AE lesions. The fourth stage is invasion. Intracytoplasmic bacteria have been observed in vacuoles and free in the cytoplasm of the host cell, and rarely in the lamina propria. In the rabbit AEEC is not considered to be enteroinvasive.[9]

In conclusion, even in absence of bacteriologic culture, the diagnosis of this case was possible based on the histopathological and electron microscopy findings. Culture and serotyping of the bacteria, plus histopathology, are the preferred methods of diagnosis. No other cases were identified in the colony after this event.

JPC Diagnosis: Colon: Colitis, heterophilic and proliferative, diffuse, mild, with edema and numerous enterocyte surface-associated Gram negative bacilli.

Conference Comment: Histochemical staining with Brown & Hopps stain reveals numerous gram-negative bacilli adhered to the large intestinal mucosa. This, in combination with the characteristic electron microscopy findings previously described, supports the contributor's diagnosis of enteropathogenic *Escherichia coli*.

Escherichia coli is a gram-negative, non-spore-forming bacillus. Non-virulent strains are often part of the normal intestinal flora, while virulent strains are capable of causing multiple disease syndromes in humans and animals via assorted combinations of virulence factors.[1,4] Specific serotypes of *E. coli* (e.g., O157:H7) are named based on the presence of the following antigens:[4]

- O-antigen (somatic): located on the lipopolysaccharide molecule
- K-antigen (capsular): outermost structural component composed of carbohydrates
- H-antigen (flagellar): composed of flagellin protein
- F-antigen (fimbrae or pili): adhesins which project from the bacterial cell wall

Enteric disease is a common manifestation of colibacillosis. The pathology of enteropathogenic, or attaching and effacing (AEEC) *E. coli* is described in detail by the contributor. In addition to rabbits, this form of colibacillosis can cause diarrhea in pigs, dogs and humans. Some strains of enterohemorrhagic *E. coli* (EHEC), also known as Shiga toxin-producing or verotoxin-producing *E. coli*, are attaching and effacing as well. This subset produces cytotoxins that are structurally similar to the Shiga toxins generated by *Shigella dysenteriae*, which bind the host cell glycolipid receptor globotriaosylceramide (Gb3), causing inhibition of protein synthesis with subsequent necrosis. Shiga toxins primarily affect intestinal epithelium and vascular endothelium, due to the presence of Gb3 receptors in these tissues. Enterohemorrhagic strains of *E. coli*, such asserotype O157:H7, are most notorious as a cause of human foodborne illness; however, they

are also associated with erosive fibrinohemorrhagic enterocolitis in calves under four weeks old. In dogs, EHEC has occasionally been linked with dysentery and, in greyhounds, a hemolytic uremic syndrome with cutaneous edema and ulceration known as cutaneous and renal glomerular vasculopathy. Furthermore, edema disease in weaned pigs is caused by enteric colonization with Shiga toxin-producing *E. coli*, mediated by F18ab fimbriae. This syndrome is characterized by central nervous system signs or sudden death due to vasculitis and edema. It is often associated with outbreaks of post-weaning *E. coli* enteritis (EHEC) and only occasionally produces diarrhea. Typically, edema disease results in a classical enterotoxemia without significant gross or microscopic lesions.[1]

Enterotoxigenic *E. coli* (ETEC) is a common cause of neonatal diarrhea in calves and piglets less than four days old. Among its most important virulence factors is its ability to colonize the intestine and produce enterotoxin. Fimbriae (also known as pili), composed of pilin, project from the bacterial cell wall, and attach to enterocyte surface receptors. Fimbrial adhesins are antigenically distinct and somewhat species-specific; immunohistochemical stains can thus be helpful in reaching a definitive diagnosis. Enterotoxins produced by ETEC include heat-labile toxin and heat-stable toxin. Heat-labile toxin is composed of two subunits, known as LTI and LTII. LTI resembles cholera toxin, while LTII utilizes an adenylate cyclase pathway (i.e., cAMP) to initiate irreversible intestinal secretion of electrolytes and water, leading to secretory diarrhea. The heat-stable toxin STa causes inhibition of Na/Cl co-transport and water absorption via an increase in cyclic guanosine monophosphate (cGMP), while STb (primarily associated with ETEC in pigs) promotes secretion by stimulating production of prostaglandin E_2 and 5-hydroxytrypamine. Although ETEC results in severe osmotic diarrhea, dehydration, and potentially death, this syndrome serves as another example of a classical enterotoxemia with minimal gross and microscopic lesions.[1]

Some strains of *E. coli* are able to invade intestinal enterocytes, eventually disseminating throughout the body and resulting in septicemic colibacillosis. These enteroinvasive strains are best described in humans; however, invasive strains of *E. coli* also affect fowl, resulting in myriad clinical syndromes including septicemia and enteric disease.[4] Other strains of *E. coli* induce systemic infection by avoiding host defense mechanisms, especially in young, compromised animals such as neonates subjected to failure of passive transfer of immunity, or those with concurrent, debilitating disease. Several important virulence factors of potentially septicemic *E. coli* strains, such as the capsule, outer membrane proteins, toxins, or fimbriae, are encoded on bacterial colicin V plasmids. When these bacteria die they often release endotoxin from lipopolysaccharide on the outer membrane, leading to the clinical signs and gross and microscopic lesions associated with endotoxic shock.[1]

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CASE III: 13-V212 (JPC 4032443).

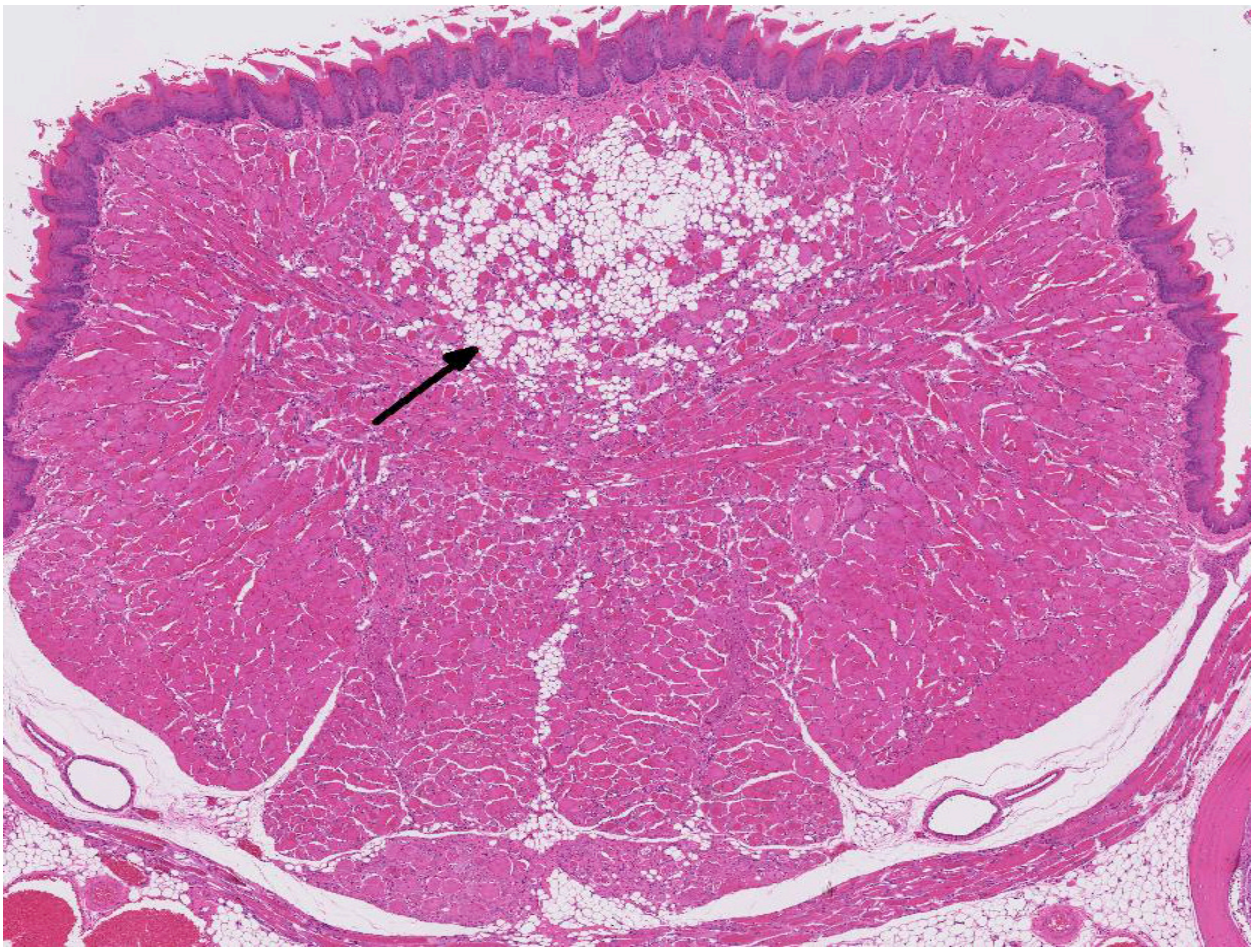
Signalment: 14-month-old female mouse αβ crystallin/HSPB2 knockout, C57Bl6 background (*Mus musculus*).

History: Genetically engineered mouse involved in ocular research relating to lens proteins. Three of three cagemates present with similar clinical signs, including hunched posture, rough haircoat, and poor body condition (body condition score 2/5). Marked kyphosis noted in all three mice. No improvement with supportive care over two days.

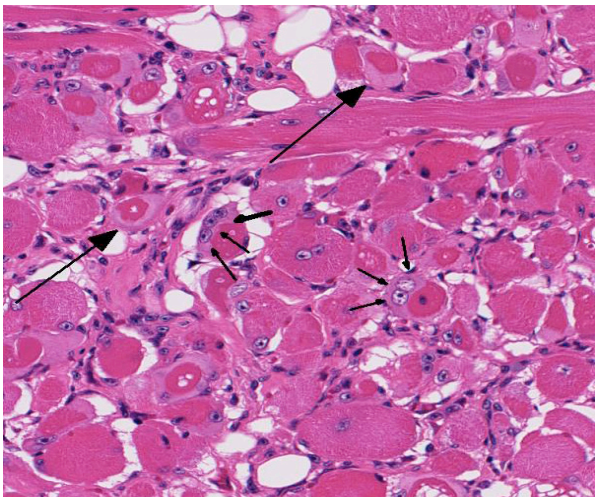
Gross Pathology: Reduced subcutaneous, peritoneal, and reproductive fat deposits. Marked kyphosis with prominent dorsal deviation of the mid-thoracic spine. No other gross abnormalities detected.

Laboratory Results: Splenic culture: No aerobic or anaerobic growth.

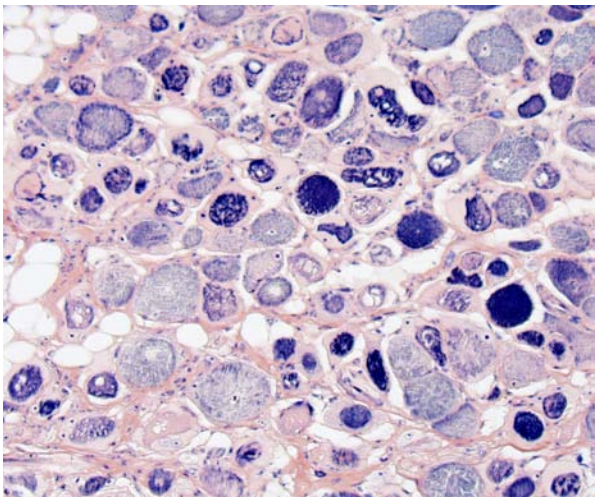
Histopathologic Description: On decalcified cross sectional views of the head, there is a focal, marked loss of the normal muscle architecture of the posterior tongue characterized by a large central region of myofiber loss and replacement by white fat. Diffusely throughout the remainder of the tongue, there is moderate myofiber size variation, myofiber pallor and cytoplasmic vacuolation, and myofiber loss and replacement by perimyseal white fat and endomysial fibrous connective tissue. Moderate multifocal myofibers have uneven cellular staining with central strongly eosinophilic homogenous material (hyaline degeneration of the sarcoplasm). There is mild, multifocal lymphocytic and histiocytic inflammation. Other muscles of the head show less pronounced changes, although mild hyaline degeneration of the sarcoplasm, central nuclei,



3-1. Tongue, αβ crystallin/HSPB2 knockout c57Bl6 mouse: Skeletal muscle of the tongue exhibits uneven staining characteristics, and is focally replaced by adipose tissue. (HE 40X)



3-2. Tongue, αB crystallin/HSPB2 knockout c57Bl6 mouse: Myofibers exhibit vacuolation and central hyaline myodegeneration (arrows). Hypertrophic satellite nuclei (arrowheads) as well as nuclei located within myofibers indicate attempts at regeneration. (HE 270X)



3-3. Tongue, α B crystallin/HSPB2 knockout c57Bl6 mouse: Phosphotungstic acid hematoxylin (PTAH) stain demonstrates the extent of myofibrillar degeneration in affected myocytes. (PTAH 360X)

myofiber size variation, and perimyseal fibrosis are present in the muscles ventral to the bulla, the lateral muscles of the head, and the muscles of the hyoid apparatus.

There are moderate artifacts of processing and decalcification in the sections. Bilaterally, the lens is rotated, shrunken, fragmented, and there is artifactual retinal separation. Multifocal moderate speckles in the lens are due to contraction. One lens has mild focal ballooning and swelling of the peripheral lens fibers consistent with early cataractous change. The other lens has mild multifocal granular debris (liquefaction) and scattered Morgagnian globules containing eosinophilic material (aggregates of lens crystallins).

Contributor's Morphologic Diagnosis: Tongue: Moderate, multifocal to coalescing chronic myopathy with myodegeneration, fibrous and fatty connective tissue replacement, and hyaline degeneration of the sarcoplasm.

Contributor's Comment: The αβ-crystallin knockout mouse is a genetically engineered model with a targeted deletion of the αβ-crystallin gene (and the nearby HSPB2 gene), created initially on a 129Sv background.[1] It has been proposed that αβ -crystallin functions as a molecular chaperone for intermediate filament proteins.[3] Alpha β-crystallin and HSPB2 are stress -inducible heat shock proteins, and αβ-crystallin is expressed predominantly in the lens but also in tissues such as cardiac and skeletal muscle.[1] These mice are viable and fertile and at approximately 40 weeks of age, they begin to develop skeletal muscle atrophy, kyphosis, osteoarthritis, and weight loss.[1] Myopathic changes in the tongue and axial musculature in αβ-crystallin knockout mice which appear similar to inherited myofibrillar myopathies seen in humans have been reported.[1,2] Many human patients with dominant negative αβ-crystallin mutations have an adult onset of the disease.[5,6] Axial muscles in the mice were examined and showed similar myopathic changes with marked fatty replacement and endomysial fibrosis and minimal associated inflammation. A predilection for tongue and truncal muscles has been previously reported in αB crystallinopathy; this predilection is thought to be secondary to increased expression of αβ-crystallin in type I muscle fibers which are more abundant in axial musculature.[2] Trichrome staining was performed and highlights the perimyseal collagen deposition in this case. Additionally, mild to moderate cardiomyopathy (characterized by cardiomyocyte hyaline and vacuolar degeneration, variable fiber size, and interstitial fibrosis with mononuclear inflammation) was identified in all three mice examined in this case, which has not been previously reported in αβ -crystallin knockout mice. Cardiomyopathy in human patients with αβ-crystallin mutations is rarely reported. The presence of cardiomyopathy in the mice we

examined may be related to genotype, background strain (increasing influence of C57BL6), or may represent an unrelated typical aging change in these mice. There are mild cataractous lens changes; however, significant artifact is present as these tissues were formalin fixed. Complete ocular phenotyping would require proper fixation and sectioning of the eyes.

JPC Diagnosis: 1. Skeletal muscle, tongue: Degeneration, necrosis and regeneration, focally extensive, marked, with fibrosis and fat infiltration.
2. Middle ear: Otitis media, suppurative, minimal.
3. Nasal cavity: Rhinitis, suppurative, minimal.

Conference Comment: As stated by the contributor, α β-crystallin is expressed primarily within the lens, as well as skeletal and cardiac muscle, where it is important for normal structure and function. Humans with αβ-crystallin mutations frequently present with cataracts, in addition to abnormal myofiber structure with subsequent myopathy.[1,5] The αB-crystallin knockout mouse was developed to study this rare human condition. In this case, lesions are confined to the tongue, while adjacent skeletal muscle appears relatively unaffected. Histochemical staining with Masson's Trichrome highlights moderate collagen deposition surrounding degenerate lingual myofibers, while PTAH outlines the well-defined, deep blue-purple striations of myofibers in the adjacent, normal skeletal muscle. This is a significant contrast to degenerate muscle fibers within the tongue, which often lack striations and exhibit patchy, irregular uptake of PTAH.

In addition to the key histological features discussed above, the moderator noted the presence of rare accumulations of a homogenous, eosinophilic substance within the interstitium of the anterioventral nasal septum. Although participants were unable to demonstrate this material during conference, deposition of amyloid-like material within the nasal septum is a documented background finding commonly reported in aging mice. Its composition remains unidentified, however PAS positivity and a lack of birefringence with polarized light after Congo red staining suggests that the substance is not amyloid.[4]

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http://depts.washington.edu/compmed/

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CASE IV: 12-0122-7 (JPC 4035410).

Signalment: 10-week-old female Swiss-Webster ICR/CD-1 (Institute of Cancer Research/ Caesarean-Derived from ICR stock) mouse (*Mus musculus*).

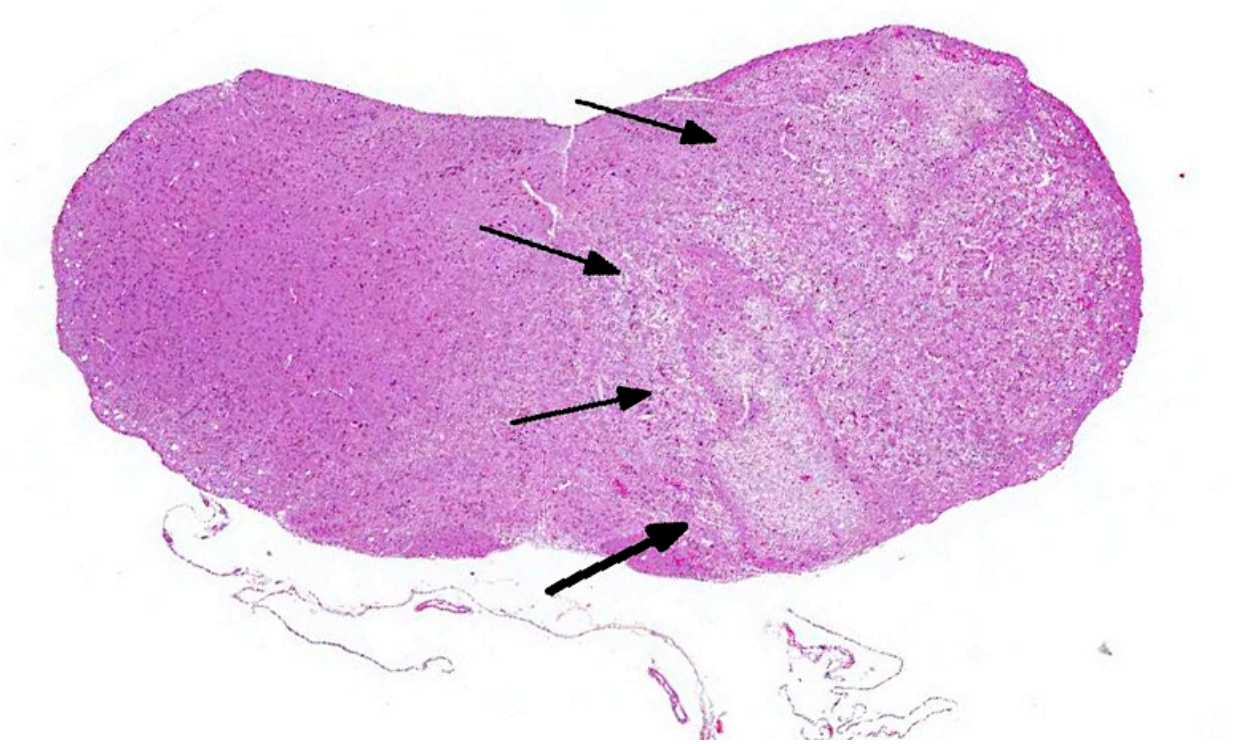
History: This mouse presented with an acute onset of intermittent rolling to the left separated by brief periods of lateral recumbency. The animal's body weight/body condition score and coat were within normal limits and the mouse appeared to otherwise be in good health. After a brief period of observation, during which a video of the rolling behavior was recorded, humane euthanasia was elected and a necropsy was performed. The gross necropsy findings were unremarkable and selected tissues were submitted for histopathology.

Gross Pathology: No significant gross lesions were seen.

Histopathologic Description: Extending from the caudal mesencephalon to the medulla oblongata there is unilateral, sharply-demarcated pallor and rarefaction of the neuropil with variable, mild to marked, multifocal vacuolization (malacia), neuronal cell loss and necrosis, capillary congestion, and mild, multifocal hemorrhage. This lesion is ventral and lateral in the caudal-most portions of the mesencephalon but unilateral and diffuse in the more caudal portions of the brainstem including the pons and medulla oblongata (involving trigeminal nerve sensory nucleus and motor fiber tracts, vestibulocochlear nuclei, and facial nerve motor nuclei and fiber tracts).

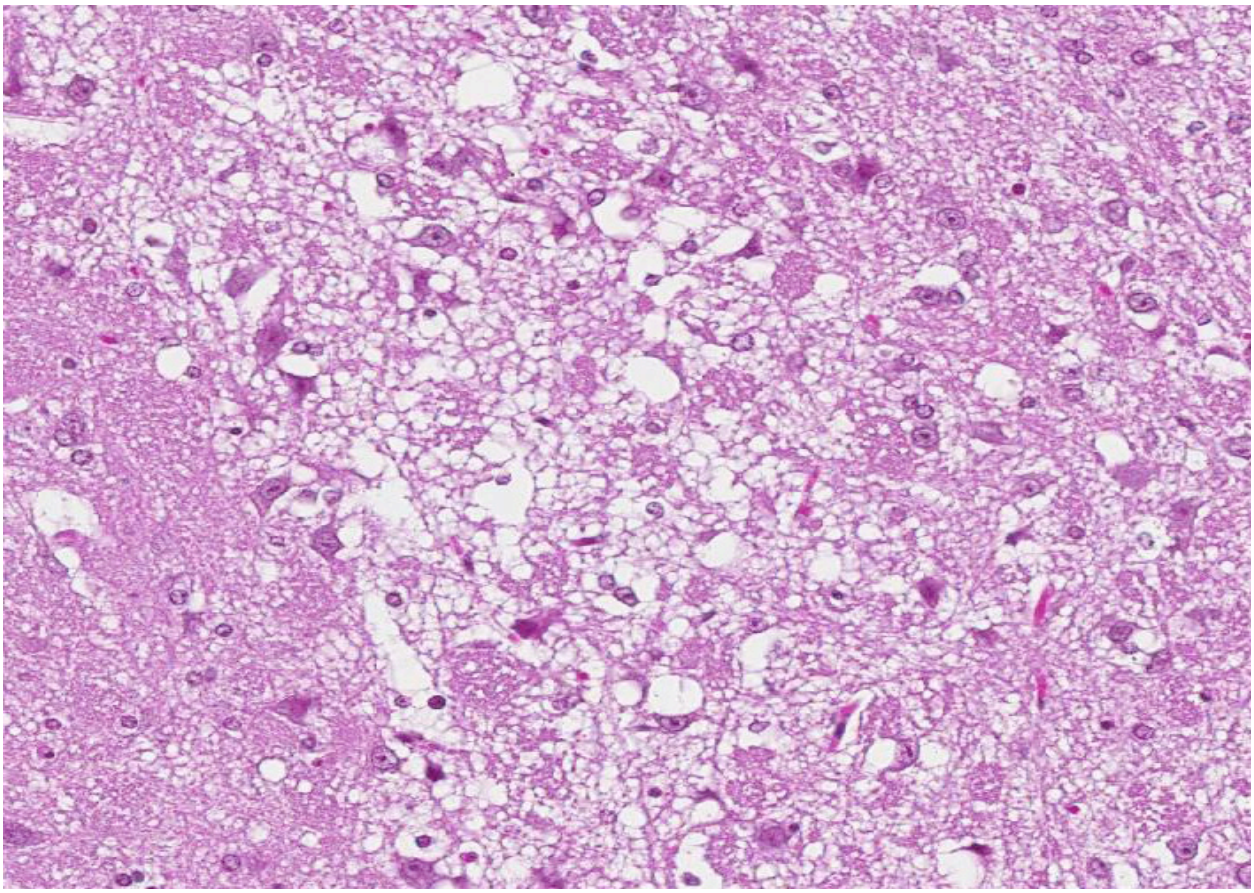
Contributor's Morphologic Diagnosis: Caudal mesencephalon to the medulla oblongata: Severe, subacute, unilateral encephalomalacia with neuronal cell necrosis and loss, and mild multifocal hemorrhage and edema.

Contributor's Comment: Our differential diagnoses in this mouse included otitis media and/ or interna (bacterial: *Mycoplasma pulmonis, Pasteurella pneumotropica, Pseudomonas aeruginosa, Streptococcus* sp. or viral: reovirus) or, despite the young age of this mouse, a CNS tumor involving the brainstem. Although no occlusion was identified in the intracranial vertebral artery or its branches in this mouse, the histopathologic lesions in the brainstem are consistent with subacute, unilateral brainstem



4-1. Brainstem, Swiss-Webster ICR/CD-1 mouse: There is a focally extensive and unilateral area of encephalomalacia extending from the pons to the caudal brainstem (arrows). (HE 0.63X)

infarction. The vestibular clinical signs (intermittent rolling to one side) are secondary to severe, unilateral ischemia affecting the pontine or vestibulocochlear nuclei, resulting in central vestibular disease. There were no lesions in the outer, middle, or inner ears in this mouse.



4-2. Brainstem, Swiss-Webster ICR/CD-1 mouse: The encephalomalacia is characterized by severe neuropil edema. (HE 100X)

This lesion has been previously reported by Southard and Brayton in Swiss ICR/CD-1 mice as "spontaneous vestibular syndrome" and "spontaneous unilateral brainstem infarction in Swiss mice."[11] Spontaneous vestibular syndrome in this outbred mouse stock is thought to be secondary to occlusion or dissection of the extracranial or intracranial vertebral artery and/or its branches.

The paired extracranial vertebral arteries arise from the subclavian artery, or rarely, directly from the aortic arch. The largest branch of the intracranial vertebral artery supplies the dorsal medulla and cerebellum and is termed the posterior inferior cerebellar artery (PICA). At the base of medulla oblongata the two intracranial vertebral arteries merge at the midline to form the basilar artery which then branches to form the posterior cerebral arteries and the posterior communicating arteries comprising the Circle of Willis.[1-3]

The clinical and histopathologic features of this syndrome in Swiss ICR/CD-1 mice have been compared by Southard and Brayton to a constellation of clinical signs seen secondary to occlusion or dissection of the intracranial vertebral artery or posterior inferior cerebellar artery in humans termed Wallenberg syndrome or lateral medullary syndrome.[9,10,12] However, these Swiss mice do not share the most common risk factors associated with stroke in humans, including hypertension, diabetes mellitus, smoking, and hyperlipemia leading to atherosclerosis[1,6,8] (in humans this constellation of clinical signs is sometimes also attributed to neoplasia involving the brainstem[12]). Currently, the underlying etiology in these Swiss mice, such as an underlying congenital intravertebral artery

stenosis, is not known and deserves further investigation.

Vertebral artery occlusions leading to medullary infarction in humans are associated with both gender and race predilections. Intracranial vertebral artery occlusions or dissections are more common in women and people of African and Asian descent, while extracranial vertebral artery occlusions or dissections are more common in white males.[1] Medullary infarctions are further classified as lateral medullary infarctions (LMI, Wallenberg, or Wallenberg's syndrome) and medial medullary infarctions (MMI or Dejerine syndrome).[4,5,7] The major symptoms associated with LMI include sensory disturbances affecting the face, dysarthria, vertigo, Horner's syndrome, cerebellar ataxia, and decreased pharyngeal reflexes. The major symptoms associated with MMI include motor weakness and sensory disturbances of the extremities, however, the clinical signs of LMI and MMI commonly overlap depending on the areas and extent of the brainstem affected.[4,5] It is not surprising that the vascular supply to the medial and lateral medulla differs (and sometimes varies). As mentioned above, the lateral medulla receives its arterial supply from the intracranial vertebral artery and PICA, while the upper and lower medial medulla receives arterial blood from the anteromedial medullary arteries. These arise from the intracranial vertebral artery in the upper medulla and from the anterior spinal artery in the lower medulla.[5] The reported prevalence of medullary infarctions in humans varies widely and is continually changing owing to the development and widespread use of magnetic resonance imaging as a diagnostic tool, which has improved anatomic localization of the lesions. The incidence of spontaneous medullary infarction in Swiss ICR/CD-1 mouse is not known.

JPC Diagnosis: Brainstem: Necrosis, unilateral, focally extensive, with edema.

Conference Comment: The contributor provides an excellent summary of unilateral brainstem infarction, a spontaneous condition rarely described in young Swiss mice. The histological lesions in this case are consistent with an early infarct, however, thrombi are not detected within examined sections, so the etiology cannot be confirmed. Examination of serial step sections of the head and neck may be helpful in definitively determining the underlying cause of these lesions. Many thrombi dissolve or break down and the affected area is reperfused, which causes additional damage, so the absence of thrombi does not necessarily indicate that they were not the inciting cause of the necrosis.

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