

The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2008-2009
CONFERENCE 12
17 December 2008

Conference Moderator:

Tony Alves, DVM, Diplomate ACVP

CASE I – 08-22155 (AFIP 3107719)

Signalment: 3-7-year-old Texas Longhorn Bull (*Bos texanus*)

History: This bull is from an Amish dairy farm with approximately 15 Texas Longhorns. The bulls were bred in Ohio and moved to New York. Currently, they are kept on a separate but nearby pasture from where the dairy cattle are kept. The owner feeds "good quality" hay but not ad lib and it is unknown if grain is fed. The owner does not vaccinate the dairy cattle. The bull's vaccination history is unknown. The owner reported that five bulls had died in the previous month. In February of 2008, the referring veterinarian performed a field necropsy on the most recently deceased animals and submitted tissues to Cornell University for histopathological evaluation. Because of ambient conditions, the tissues were partially frozen at the time of necropsy and fixation.

Gross Pathology: The referring veterinarian could not perform a complete field necropsy since the carcasses were partially frozen. Both bulls were in poor body condition with a lack of perirenal and cardiac adipose tissue. Ruminal content consisted of a normal quantity of fiber and some stones. The feces were solid, and a small to moderate number of non-specified parasites were seen on fecal examination. Buccal and lingual ulcers were present in one animal, and the other animal had reddened Peyer's patches.

Laboratory Results: Virus FA results for Bovine Viral Diarrhea virus was negative

Histopathologic Description: Tongue: Skeletal muscle fibers are disrupted by multiple variably sized coalescing nodules composed of central aggregates of hyperesoinophilic, radiating, club-shaped hyalinized material (Splendore-Hoeppli material) that often contain moderate numbers of cocco-bacilli and a thin peripheral rim of densely basophilic granular material (mineral) (**Fig. 1-1**). These nodules are surrounded by concentric layers of degenerate neutrophils admixed with a moderate amount of pale eosinophilic fibrillar material (fibrin) and macrophages with occasional Langerhan's type multinucleated giant cells. Dissecting between the remaining myofibers and the inflammatory foci are dense bundles of collagen and regularly arranged fibroblasts admixed with moderate numbers of lymphocytes and plasma cells. The remaining myofibers are often hyperesoinophilic with floccular cytoplasm and lack cross striations (degeneration). There are multiple ulcers of the lingual epithelium and there are several dense intraepithelial aggregates of degenerate neutrophils, acantholytic keratinocytes, and nuclear debris (microabscesses).

Gram's stain of the tongue: There are moderate numbers of Gram negative cocco-bacilli within the Splendore-Hoeppli material.

Contributor's Morphologic Diagnosis: Tongue: Severe, multifocal to coalescing, chronic, pyogranulomatous glossitis with Splendore-Hoeppli material, mineralization, intralesional bacteria, fibrosis, ulceration and myonecrosis ('Wooden Tongue')

Contributor's Comment: Wooden tongue, or actinobacillosis, is caused by the bacteria *Actinobacillus lignieresii*. This bacterium has been isolated in a variety of species. The organism is ubiquitous and causes sporadic disease of primarily of cattle, sheep, and goats. Since the organism is a commensal of the oral

cavity in ruminants, development of disease is most frequently associated with damage to the oral mucosa. In cattle, the tongue is the most common site of infection and thought to be associated with their use of the tongue toprehend food. Comparatively, sheep, which use their lips to prehend food, most frequently have lesions associated with their lips and cheeks. The bacterium has also been isolated from the rumen.(6)

Clinically, affected animals present with weight loss and hypersalivation. This is due to the extensive destruction of the tongue and oral tissues. Gross lesions commonly manifest as variably sized, hard, circumscribed nodules that measure up to several millimeters in diameter. These nodules are most commonly found in the tissues and skin of the face, and can progress to form soft abscesses which can fistulate and discharge through the mouth or skin. The discharged purulent material is odorless and contains abundant granules.(6) This infection induces in the tongue a severe fibroblastic response causing it to become large and immobile making chewing and swallowing difficult. This hardening of the tongue is the genesis of the common name of the disease “wooden tongue”. The formation of granulation tissue within the fibrous connective tissue gives the nodular appearance to these lesions. Additionally, small yellow foci (sulfur granules) can often be seen within the dense granulation tissue. These represent the bacterial colonies within the lesion.

Oral infection with *A. lignieresii* will commonly spread via lymphatics to local lymph nodes. Infections are less commonly found in the forestomachs, lungs, skin and uterus.(4) Grossly, infected lymph nodes contain yellow-orange granulomatous nodules which frequently project above the normal nodal capsular contour and are surrounded by sclerosing inflammation.(2) Affected lymphatics are diffusely thickened with similar nodules.(2) The most commonly affected lymph nodes are the retropharyngeal and submaxillary nodes.

Histologically, these nodular lesions consist of multiple pyogranulomas surrounding aggregates of Gram negative cocco-bacilli embedded in homogenous eosinophilic material. The hypereosinophilic material along with the bacteria forms the club-shaped microcolonies which is characteristic of this disease.(6) These formations are thought to be associated with immune complex deposition.(2) Surrounding the central area of bacteria is a band of inflammatory cells composed predominantly of neutrophils with macrophages and occasional giant cells. The external layer of the pyogranuloma consists of a dense layer of fibrous connective tissue containing variable numbers of lymphocytes and plasma cells. These lesions are often concurrently infected with *Actinomyces pyogenes*, *Streptococcus* spp., and *Pseudomonas aeruginosa*.(4)

A. lignieresii has been isolated from laboratory rodents associated with middle ear infections and conjunctivitis (5) and from healthy and diseased horses.(3) There is one report of the bacteria being isolated from a horse with an enlarged tongue.(1) In swine it is seen as granulomas or abscesses of the teats associated with wounds from the sharp teeth of suckling piglets. In horses this bacteria infrequently causes lower airway disease and abscess.(4)

The most important differential diagnoses for actinobacillosis include *Actinomyces bovis*, *Staphylococcus aureus* (botryomycosis) and *Mycobacterium bovis*. *A. bovis* also contains sulfur granules within discharge. However, infections with this agent tend to be associated with infection of bone, in particular the mandible. (4) In addition, *A. bovis* is a Gram positive filamentous organism, and this infection does not consistently spread to local lymph nodes. Botryomycosis classically has chronic granulomas with centers of numerous Gram positive cocci embedded in a homogenous matrix and the purulent material associated with this infection has botryomycotic granules.(4)

AFIP Diagnosis: Tongue: Glossitis, pyogranulomatous, multifocal to coalescing, severe with Splendore-Hoeppli material, fibrosis, myocyte degeneration, necrosis, and loss

Conference Comment: The contributor provided an excellent review of this entity.

Splendore-Hoeppli material is intensely eosinophilic, club-shaped material that radiates around certain fungi, bacteria, parasites and biologically inert substances such as suture. The material is generally composed of antigen-antibody complexes, debris and fibrin.(7) The most common infections and conditions that result in the Splendore-Hoeppli phenomenon include botryomycosis, *Nocardia* sp., *Actinomyces* sp., and feline dermatophytic pseudomycetomas.(7) It has also been reported with *Pythium*

insidiosum, *Sporothrix schenckii*, *Candida albicans*, *Aspergillus* sp., and zygomycetes such as *Conidiobolus* sp. and *Basidiobolus* sp., *Coccidioides immitis*, nematodes, schistosomiasis, and hypereosinophilic syndrome.(7)

The presence of the Splendore-Hoeppli material led to a discussion of antigen-antibody complexes. Deposition of immune complexes causes tissue damage primarily by activation of the complement cascade and activation of neutrophils and macrophages through their Fc receptors.(8) Immune complexes also cause platelet aggregation and activation of Hageman factor which lead to microthrombi formation and kinin activation. Activated complement produces chemotactic factors such as C5a which recruits macrophages and neutrophils, and anaphylatoxins such as C3a and C5a which increase vascular permeability.(8) The leukocytes are activated by binding of their C3b and Fc receptors by the immune complexes. Activated leukocytes release prostaglandins, chemotactic substances, oxygen free radicals, and lysosomal enzymes that include proteases capable of digesting collagen.(8)

Contributing Institution: Cornell University, College of Veterinary Medicine, Department of Biomedical Sciences, S2-121 Schurman Hall, Ithaca, NY 14850, <http://www.vet.cornell.edu/>

References:

1. Baum KH, Shin SJ, Rebhun WC, Patten VH: Isolation of *Actinobacillus lignieresii* from a horse with an enlarged tongue. Journal of the American Veterinary Association, **185**:792-793, 1984
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 20-21. Elsevier Saunders, Philadelphia, PA, 2007
3. Christensen H, Bisgaard M, Angen, O, Olsen JE: Final classification of Bisgaard taxon 9 as *Actinobacillus arthritidis* sp. nov. and recognition of a novel genomospecies for equine strains of *Actinobacillus lignieresii*. International Journal of Systematic and Evolutionary Microbiology **52**:1239-1246, 2002
4. Henton MM, Van Der Lugt JJ: Actinobacillus Infections in Infectious Diseases of Livestock, eds. Coetzer JAW, Tustin, RC, 2nd ed., vol. 3, pp. 1648-1651 Oxford University Press, Cape Town South Africa, 2004
5. Lentsch RH, Wagner JE: Isolation of *Actinobacillus lignieresii* and *Actinobacillus equuli* from Laboratory Rodents. Journal of Clinical Microbiology **2**:351-354, 1980
6. Rycroft AN, Garside LH: Actinobacillus species and their role in disease. The Veterinary Journal **159**:18-36, 2000
7. Hussein MR: Mucocutaneous Splendore-Hoeppli phenomenon. Journal of Cutaneous Pathology **35**:979-988, 2008
8. Abbas AK: Diseases of immunity. In: Pathological Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 213-214. Elsevier Saunders, Philadelphia, PA, 2005

CASE II – NIAH-2 (AFIP 3106267)

Signalment: A 66-day-old, male, mixed, swine (*Sus scrofy*)

History: Seven of 41 piglets in a herd of 70 sows became affected at approximately 40 to 50 days of age. The affected piglets suddenly developed paralysis of the hind limbs and became recumbent. In spite of the severe flaccid paralysis of the hind limbs, they could move by using their forelimbs. In all cases, their body temperatures were within the normal range, their appetites remained normal, and none died spontaneously.

Gross Pathology: No gross abnormalities were seen in any of the piglets

Laboratory Results: Portions of the cerebrum, brainstem, cerebellum, spinal cord (cervical and lumber enlargement) and tonsils of 4 piglets were taken for virus isolation purposes. Each tissue was routinely homogenized in Earle's medium and inoculated onto porcine kidney cell line (CPK) cultures that were then observed microscopically for seven days. Three further passages were made of each sample. CPK cells were harvested when the cells displayed characteristic cytopathic effects. Viral RNA was extracted from the CPK cells with ISOGEN-LS (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. The RT-PCR was performed with a RNA PCR kit (Takara) according to the manufacturer's instructions. The amplified product was visualized by standard gel electrophoresis of 10µl of the final

reaction mixture on a 2% agarose gel. Amplified DNA fragments of specific sizes were located by ultraviolet fluorescence after staining with ethidium bromide. Fragment lengths were verified by comparison with a digested lambda standard on the same gel. PCR products were purified using a High Pure PCR Product Purification kit (Roche). The nucleotide sequences of the purified PCR products were determined by use of BigDye chemistry (Applied Biosystems, Foster City, CA) with the ABI 310 Sequencer (Applied Biosystems). The sequences of which were compared with each other and the PTV (Porcine Teschovirus) sequences available in the GenBank/EMBL/DDBJ using GENETYX-WIN version 4.0 (Software Development Co., Ltd, Tokyo, Japan). The cytopathogenic agents were recovered from the homogenate of cerebellum and tonsils of 3 piglets including this present piglet, and from the brainstem of this presented piglet. All cytopathogenic agents isolated from piglets were amplified in CPK cells, and the resulting extracted RNA was reacted with primers specific for PTV by RT-PCR. PCR products isolated from cerebellum and brainstem of this presented piglet were sequenced. These sequences were identical and the homology between this sequence and other PTV's were 91.2-95.6%. Based on this observation, the pathogenic agent isolated from this pig was identified as PTV. Virus was not isolated from the cerebrum and spinal cord.

Histopathologic Description: Histologically, lesions were limited to the central nervous system (CNS) and peripheral nerve fibers. All clinically affected piglets had similar histological changes. The changes observed were those of nonsuppurative encephalomyelitis, characterized by perivascular cuffing of the mononuclear cells, focal gliosis, neuronal necrosis and neuronophagia. The spinal cord was severely affected and the lesion was seen along the full length of the spinal cord. In the ventral horns, nerve cells were degenerated to varying degrees up to, and including, necrosis accompanied by neuronophagia, inflammatory or glial nodules, occasional hemorrhage, and a rather diffuse infiltration of mononuclear cells. In the white matter of the spinal cord, perivascular cuffing and infiltration of mononuclear cells and focal gliosis were also observed. In addition to the infiltrative changes, severe vacuolar changes and axonal swelling were observed in the white matter of the spinal cord. Infiltration of mononuclear cells was observed in the dorsal root ganglia, spinal nerves, and sciatic nerves. In some dorsal root ganglia, degenerated ganglion cells and neuronophagia were observed (**Fig. 2-1**). Swollen myelin sheaths and axonal spheroids were seen in the spinal roots, and in peripheral nerves, including the brachial plexus and sciatic nerves. The cerebellar nuclei and the gray matter of the brainstem were also severely affected. In the cerebral hemisphere, only slight perivascular cuffing was present.

Immunohistochemically, PTV antigens were detected in the cytoplasm of large nerve cells and glial cells in the cerebellar nuclei, the gray matter of the brain stem, and the ventral horn of the spinal cord of all examined pigs. In the spinal ganglia, PTV antigen was strongly detected in the cytoplasm of ganglion cells. In the nervous system, the distribution of PTV antigen was consistent with the lesion distribution. In the lesion, no antigens were seen in the central severe area. Antigens were mainly seen in the periphery of the severe lesions and, especially, in minimal to mild lesions around areas of perivascular cuffing.

Contributor's Morphologic Diagnosis: Spinal cord: nonsuppurative, necrotizing, myelitis, with vacuolar changes in the white matter, mixed pig, swine
Dorsal root ganglia: nonsuppurative, ganglionitis

Contributor's Comment: Enterovirus encephalomyelitis (previously known as Teschen/Talfan disease) caused by at least 9 serotypes of porcine teschovirus (PTV 1, and 2-6, 8, 12, 13 which are responsible for the milder form of the disease) of the picornaviridae family is considered to be of socioeconomic importance.(5) Infection appears to be selective for specific neuronal populations, resulting in a characteristic clinical syndrome of lower motor neuron paralysis. A diagnosis of enterovirus encephalomyelitis is made by virus isolation from the central nervous system of pigs showing neural signs (5) The disease was first described as Teschen disease.(5) Less virulent forms of the disease were later recognized in the United Kingdom (Talfan disease) and in Denmark (poliomyelitis suum).(5) These later diseases were described as resulting in lower morbidity and mortality, the clinical syndrome expressed as paresis and ataxia (which seldom progresses to complete paralysis).(1,2) The histological changes were those of nonsuppurative polioencephalomyelitis.(2,3,5) The lesions in the spinal cord in each syndrome were largely confined to the gray matter, particularly the ventral horns, but may selectively involve the dorsal horns in very young pigs.(3) Infections from PTV are most often asymptomatic, and PTV is still frequently isolated from the faces, tonsils and other non-neural organs of apparently unaffected pigs.(4)

In the present cases, the morbidity and mortality were low and the characteristic clinical signs were flaccid paralysis of the hind limbs. The nonsuppurative lesions were distributed mainly in the gray matter

of the brainstem and the spinal cord. These clinical and histological features of the present disease are similar to those of the disease produced by less virulent PTV strains, especially those of Talfan disease.(3,4) In previous reports of experimental Talfan disease, axonal degeneration was seen in the ventral root and sciatic nerves.(1) In the white matter of the spinal cord, slight degenerative changes were seen only in the dorsal funiculus.(1) In contrast, demyelination and axonal degeneration in the present cases, which resulted from a natural outbreak in Japan, appeared in the whole white matter, and in either the ventral or dorsal root.

Immunohistochemically, anti-PTV monoclonal antibody (no.9, IgM) (National Institute of Animal Health, Japan) 6 was used as the primary antibody. PTV antigens were detected in cytoplasm of nerve cells, glial cells and endothelial cells in the cerebellar nuclei, the gray matter of the midbrain, pons, and medulla oblongata and the ventral horn of the spinal cord and of ganglion cells in the spinal ganglion corresponding to those lesions characterized as nonsuppurative encephalomyelitis and ganglionitis in the pigs. The results suggest that nerve cells of the brainstem and spinal cord, and ganglion cells of the spinal ganglion permit PTV replication and represent the main target cell population of PTV.

The isolation of PTV from CNS is important for diagnosing enterovirus encephalomyelitis.(1) However, it has been reported that isolation of virus from CNS is quite difficult, and virus isolation is not always possible using routine techniques in the cases of enterovirus encephalomyelitis of pigs.(4) The optimum conditions for virus isolation from CNS, including the relationship of clinical signs to the presence of infectious virus and anatomic site where the virus is present in high density, have not been clarified in this disease. In the present cases, PTV was isolated from cerebellum and/or brainstem in the pigs slaughtered about three weeks after the onset of neural signs, but not from the cerebrum. These results suggest that sampling for virus isolation should be from the cerebellum or brainstem for the successful diagnosis of enterovirus encephalomyelitis.

AFIP Diagnosis: Spinal cord: Poliomyelitis and ganglioneuritis, nonsuppurative, multifocal, marked with neuronal degeneration and necrosis, neuronophagia, gliosis, astrocytosis, satellitosis, and spheroids

Conference Comment: The contributor provided an excellent review of porcine teschovirus (PTV) which targets selective neuronal populations in the ventral horn of the spinal cord, brain stem, and ganglion cells of the spinal ganglion resulting in neuronal necrosis, nonsuppurative polioencephalomyelitis and lower motor neuron paralysis. In naturally infected cases, the PTV antigens (but no lesions) were also present in bronchial epithelium, tonsillar epithelium, hepatocytes and the myenteric nerve plexus, but not in the cerebral hemispheres.(9) In addition to the typical neurological disease, some strains of the virus have been associated with female reproductive disorders, enteric disease, pneumonia, pericarditis and myocarditis. Lesions have been described in the kidney, liver, spleen, adrenal gland and thyroid gland.(9) PTV is frequently isolated from the feces, tonsils and non-neural organs of clinically normal pigs.(9) The proposed pathogenesis includes virus replication within the gut, mucosal lymphoid tissue and local lymph nodes; followed by viremia; and subsequent central nervous system invasion.(9)

Conference participants discussed other viruses affecting the nervous system of pigs. Pseudorabies (suid herpesvirus 1) causes nonsuppurative encephalitis primarily affecting the gray matter, neuronal necrosis and ganglioneuritis in the paravertebral ganglia.(7) Eosinophilic intranuclear inclusion bodies are present in the neurons and astroglia. Lesions are most severe in the cerebral cortex (differentiating it from porcine teschovirus), brain stem, spinal ganglia and basal ganglia.(7) Very young and aborted pigs typically have small areas of necrosis with eosinophilic intranuclear inclusions in the liver, tonsils, lung, spleen, placenta and adrenal glands.(7) Porcine hemagglutinating encephalitis virus (HEV) is a coronavirus that causes two clinical syndromes in young pigs: neurological signs occur in 4-7-day-old piglets and "vomiting and wasting disease" occurs in 4-14-day-old piglets.(7) Neurological lesions include nonsuppurative encephalomyelitis affecting the gray matter of the medulla and brain stem, and inflammation within the trigeminal, paravertebral and autonomic ganglia, and the gastric myenteric plexus.(7) Classical swine fever (porcine pestivirus) causes vascular lesions that result in hemorrhage, infarction, necrosis and disseminated intravascular coagulation.(6) Common lesions include hemorrhages in various organs (especially the lymph nodes), renal petechiae and splenic infarction.(6) Neural lesions occur in the gray and white matter, and primarily affect the medulla oblongata, pons, colliculi and thalamus.(10) There is endothelial swelling, proliferation and necrosis; perivascular lymphocytic cuffing; hemorrhage and thrombosis; gliosis; and neuronal degeneration.(10) In utero infections result in cerebellar hypoplasia and spinal cord hypomyelinoogenesis.(7) Two paramyxoviral diseases of pigs include porcine rubulavirus encephalomyelitis (Blue eye disease) and Nipah virus. Porcine rubulavirus causes encephalomyelitis,

reproductive failure and corneal opacity primarily in Mexico. There is nonsuppurative polioencephalomyelitis affecting the thalamus, midbrain and cortex. Additional lesions include anterior uveitis, corneal edema, epididymitis, orchitis and interstitial pneumonia.(7) Nipah encephalitis is an emerging disease causing severe and rapidly progressive encephalitis and pneumonia in pigs, other animals and humans.(7) Fruit bats are the natural reservoir. There is necrotizing vasculitis and fibrinoid necrosis of arterioles, venules and capillaries with endothelial syncytial cells resulting in large areas of hemorrhage and infarction. Eosinophilic intracytoplasmic and intranuclear inclusions are occasionally found in neurons and endothelial syncytia. Blood vessels in the lung, brain, glomeruli and lymphoid organs are most commonly affected.(7) Additional lesions include bronchointerstitial pneumonia with necrotizing bronchiolitis, lymphocytic and neutrophilic meningitis, nonsuppurative encephalitis and gliosis.(7)

Contributing Institution: National Institute of Animal Health (<http://niah.naro.affrc.go.jp/index.html>), 3-1-5 Kannondai, Tsukuba, Ibaraki, 305-0856 Japan.

References:

1. Edington N, Christofinis GJ, Betts AO: Pathogenicity of Talfan and Konratice strains of Teschen virus in gnotobiotic pigs. *J Comp Pathol* **82**:393-399, 1972
2. Harding JDJ, Done JT, Kershaw GF: A transmissible polio-encephalomyelitis of pigs (Talfan disease). *Vet Rec* **69**:824-832, 1957
3. Jubb KVF, Huxtable CR: The nervous system. *In: Pathology of Domestic Animals*, eds. Jubb KVF, Kennedy PC and Palmer N, 4th ed., vol. 1, pp. 267-439. Academic Press, San Diego, CA, 1993
4. La Rosa G, Muscillo M, Di Grazia A, Fontana S, Iaconelli M, Tollis M: Validation of RT-PCR assays for molecular characterization of porcine teschoviruses and enteroviruses. *J Vet Med B* **53**:257-265, 2007
5. Mádr V: Enterovirus encephalomyelitis (previously Teschen/Talfan disease). *In: Manual of standards for diagnostic tests and vaccines*, 4th ed., pp. 630-637. Office International des Epizooties, Paris, 2000
6. Maxie MG, Robinson WF: Cardiovascular system. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 3, pp. 78-82. Saunders Elsevier, London, UK, 2007
7. Maxie MG, Youssef S: Nervous system. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 1, pp. 321-433. Saunders Elsevier, London, UK, 2007
8. Yamada M, Kaku Y, Nakamura K, Yoshii M, Yamamoto Y, Miyazaki A, Tsunemitsu H, Narita M: Immunohistochemical detection of porcine teschovirus antigen in the formalin-fixed paraffin-embedded specimens from pigs experimentally infected with porcine teschovirus. *J Vet Med A* **54**:571-574, 2007
9. Yamada M, et al. Al.: Immunohistochemical distribution of viral antigens in pigs naturally infected with porcine teschovirus. *J Vet Med Sci* **70**:305-308, 2008
10. Zachary JF: Nervous system. *In: Pathological Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., p. 967. Mosby Elsevier, St. Louis, MO, 2007

CASE III – 0806396 (AFIP 3105581)

Signalment: 6-month-old, spayed female, Siamese cross cat, *Felus catus or domesticus*

History: Chronic vomiting and weight loss. Exploratory laparotomy revealed an abnormal ileocecal region that was resected and submitted for histologic examination. The cat did poorly following the exploratory laparotomy and was euthanized. Although the owner declined a complete postmortem examination, they consented to collection and submission of some tissues for identifying the fungal agent.

Gross Pathology: A 5.1 x 5.0 cm section of cecum with attached short segments of ileum and colon was submitted.

Laboratory Results: Fresh liver, small intestine, and a swab from the colon were submitted for definitive fungal identification. *Candida albicans* was cultured from all three sites.

Histopathologic Description: At the diverticulum of the cecum there was a focally extensive area of severe necrosis with coalescing bands and large aggregates of inflammatory cells (neutrophils, histiocytes [includes epithelioid and vacuolated forms], small lymphocytes and plasma cells) admixed with irregular streams of spindle cells with a plump nucleus. Multiple aggregates of nonpigmented, branching, pseudohyphae and septate hyphae (4 to 8 um thick) were present within the inflammatory infiltrate and on

the mucosal surface (**Fig. 3-1**). Sections from the ileum and colon were unremarkable.

Contributor's Morphologic Diagnosis: Severe, focally extensive, necrotizing, pyogranulomatous typhlitis due to *Candida albicans*

Contributor's Comment: Members of the genus *Candida* are saprophytic, dimorphic fungi in the family *Cryptococcaceae*.(3) In the yeast phase, *Candida* species normally inhabit the alimentary, upper respiratory and genital mucosae of mammals.(3,1) *Candida* species are first acquired by neonates as they pass through the birth canal, colonize the oral, gastrointestinal, upper respiratory and genital mucosae for the life of the animals.(3) Their presence normally evokes no reaction. Under certain circumstances, *Candida* species can invade deeper host tissues and proliferate as blastoconidia, pseudohyphae and branched, septate hyphae.(3) In other instances, they can disseminate via the bloodstream to many tissues.(3)

Pathogenic factors of *Candida* species are important in determining their relative virulence in the host.(3) *Candida albicans* can invade the columnar epithelium of the intestines.(3) The yeast form of *C. albicans* colonizes epithelia, while hyphae are the more invasive form and are found within deeper tissue invasion.(3)

Local proliferation of *Candida* species on mucosal surfaces is the first step in the spread of infection. Overgrowth of *Candida* species within the gastrointestinal tract is inhibited by mucosal microflora.(1) Factors that upset the balance of normal endogenous microflora may cause *Candida* organisms to proliferate. Intestinal candidiasis may be sequelae to parvoviral infections or alterations in microflora caused by systemic antibiotic therapy.(3,1,5) Mycotic ileitis and colitis in cats have been reported as a secondary complication of infection with feline panleukopenia virus.(5,7) Localized candidiasis has been reported to occur in chronically immunosuppressed cats and in those with nonhealing ulcers of the gastrointestinal tract.(3) However, in one study retroviral status (feline leukemia virus, FeLV and feline immunodeficiency virus, FIV) did not influence the ability to isolate *C. albicans* from feline cutaneous and mucosal samples.(6) The FeLV and FIV status of this patient was not established and routine bloodwork, that may have demonstrated panleukopenia, was not performed.

Histologic examination of lesions reveals multifocal abscesses or areas of necrosis that contain abundant blastoconidia, pseudohyphae and true hyphae surrounded by mixed inflammatory cells.(3) Infiltrates tend to be minimal in profoundly immunosuppressed or leukopenic animals.(3) The proprial infiltrate in the cecum from this cat would suggest it was not immunosuppressed or leukopenic.

Candida species grow well on blood agar and are often isolated from specimens submitted for bacterial culture. Organisms may sometimes be cultured from many tissues at surgery or necropsy, but such results should be interpreted cautiously.(3) Culture of *Candida* species from cutaneous, mucosal or exudates alone are not an indicator of infection.(3) Histologic confirmation of invasion and host reaction are essential.(3) Mucosal and cutaneous biopsies should be submitted for histologic and culture examination simultaneously. A definitive histologic diagnosis can sometimes be made in the presence of negative culture results.

AFIP Diagnosis: 1. Cecum (per contributor): Typhlitis, necrotizing and pyogranulomatous, diffuse, severe, with vasculitis, fibrin, hemorrhage, edema, intralesional hyphae and pseudohyphae, and rare eosinophilic intranuclear inclusion bodies.

2. Lymph node: Lymphoid depletion, diffuse, severe, with draining hemorrhage, edema, and rare eosinophilic intranuclear inclusion bodies.

Conference Comment: The contributor provides an excellent review of *Candida* sp. In addition, several eosinophilic intranuclear inclusion bodies were observed within lymphocytes in the lymph node in the intestinal epithelium. Immunosuppression is suspected to have predisposed this 6-month-old cat to concurrent viral and *Candida* infections. The inclusion bodies, along with the necrotizing lesion and lymphoid depletion, are suggestive of feline parvovirus (PV) infection. Inclusion bodies are generally found only early in infection.(2) Intestinal lesions of PV are similar to those seen with feline leukemia virus associated enteritis (FAE), feline enteric coronavirus (CoV), and enteritis of unknown etiology (EUE). Crypt necrosis is the primary lesion observed with PV, FAE and EUE, while necrosis of the villar tip is the primary lesion with CoV. The inflammation is marked in FAE, EUE and CoV, unlike in PV. Many macrophages are present in EUE. The inflammation is composed primarily of T lymphocytes in PV and FAE. There is lymphoid depletion with PV and EUE. Lymphoid tissue is normal to hyperplastic with FAE.

There is decreased bone marrow activity in PV. Bone marrow activity is slightly increased with FAE, CoV and EUE.(4) Intrauterine infection results in congenital cerebellar hypoplasia in kittens.(2)

	PV	FAE	CoV	EUE
Necrosis	crypts	crypts (less severe)	villar	crypts
Inflammation	↑ (T cells)	↑ ↑ (T cells)	↑ ↑ (lymphocytes)	↑ ↑ (macrophages)
Lymphoid tissue	depleted	Normal to hyperplastic		depleted
Bone marrow	↓	Slight ↑	Slight ↑	Slight ↑

Contributing Institution: Prairie Diagnostic Services, 52 Campus Drive, Saskatoon, Saskatchewan, Canada, S7N 5B4, www.usask.ca/pds

References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. *In: Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 2, p. 230. Saunders Elsevier, London, UK, 2007
2. Gelberg HB: Alimentary system. *In: Pathological Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 378-380. Mosby, St. Louis, MO, 2007
3. Greene CE and Chandler FW. Candidiasis and Rhodotorulosis. *In: Infectious Diseases of the Dog and Cat*, ed. Greene CE, 3rd ed., pp. 627- 632. Saunders Elsevier, London, UK, 2006
4. Kipar A, Kremendahl J, Jackson ML, Reinacher M: Comparative examination of cats with feline leukemia virus-associated enteritis and other relevant forms of enteritis. *Vet Pathol* **38**:359-371, 2001
5. Lorenzini R and De Bernardis F: Antemortem diagnosis of an apparent case of feline candidiasis. *Mycopathologia* **93**:13-14, 1986
6. Ochiai K, Valentine B and Altschul M: Intestinal candidiasis in a dog. *Vet Rec* **146**:228-229, 2000
7. Sierra P, Guillot J, Jacob H, Bussieras S, Chermette R: Fungal flora on cutaneous and mucosa surfaces of cats infected with feline immunodeficiency virus or feline leukemia virus. *American Journal of Veterinary Research* **61**:158-161, 2000

CASE IV – V08-03243 (AFIP 3106654)

Signalment: Two-year-old female bovine (*Bos taurus*), breed unknown

History: This producer had lost 10 head of young cattle (6-18 months of age) over a period of approximately 10 days. Most cattle were simply found dead; this one was seen “staggering, depressed”, and died before attending veterinarian could get to the premises.

Gross Pathology: The animal was in good nutritional and post-mortem condition. Age was estimated at approximately 1 year. There were multifocal variably sized and irregular shaped purplish-black areas of discoloration of skeletal muscle occurring with random distribution in the caudal neck, brisket, and quadriceps muscles; these were bilateral distribution on both sides of the animal. Lesions were present both on the surfaces of the muscles, and deeper into musculature. These lesions were quite “dry” and there was crepitus on palpation.

Laboratory Results: Bacteriology – *Clostridium chauvoei* was detected via both fluorescent antibody and anaerobic culture methods.

Histopathologic Description: Skeletal muscle fibers were in varying stages of necrosis, varying from early coagulation necrosis to complete fragmentation and collapse. There were large zones of hemorrhage with some aggregates of neutrophils and macrophages were found between muscle fibers, as well as edema fluid. Multifocal pockets of emphysema were also seen, primarily in fascial planes. Occasional rectangular bacterial organisms were seen, again primarily in fascial planes.

Contributor's Morphologic Diagnosis: Myositis, necrotizing, due to *Clostridium chauvoei* (blackleg)

Contributor's Comment: The lesions are virtually pathognomonic grossly, and bacterial FA techniques can verify a diagnosis quite rapidly. Differential diagnoses would include other clostridial disease (i.e., *C. septicum* or pseudo-blackleg) (3); however, *C. chauvoei* is the only disease that consistently produces gas pockets in the muscles in freshly dead animals, coupled with the prominent areas of discoloration (due to hemolyzed erythrocytes). Microscopic lesions are typical, but relatively moderate in severity. Death is caused by the production and absorption of a potent exotoxin by the organism, thus causing a toxemia with subsequent muscular hemorrhages and edema.(2,3) The progress of the disease is extremely rapid, thus in some cases it may produce only moderate histologic lesions associated with the toxemia, or they may be widespread and severe, as with this one.

This case was submitted because this condition is not commonly seen anymore due to better herd management practices and good vaccines being available and in use in most cattle operations.

However, in the Southwestern United States, we do see blackleg occasionally due to some management issues that are unique to our geography and climate. In a desert environment, large acreages are required to range (i.e., pasture) cattle; yearling cattle such as these would require a minimum of 40-60 acres per animal, depending upon availability of forage, drought, etc. Requirements for cow/calf pairs commonly range from 60-100 acres per animal; hence, a 200 cow herd would require 12,000-20,000 acres (20-30 sections). Many operations leave the bulls out with the cows all year, thus having year round calving and calves of many different sizes and weights. Thus, these large ranges (often in very rugged and inaccessible country, except by horseback) make doing a "gather" for any reason difficult, very labor intensive, time consuming, and requires experienced and savvy cowboys. As a result, cattle are often only gathered up once a year, usually to select and market heavier calves. Obviously, with these types of management problems, installation of a comprehensive herd health (including vaccinations) program is often a hit-and-miss proposition, as was the case with these animals. This particular animal had not been vaccinated (nor had any of the others).

AFIP Diagnosis: Skeletal muscle: Myocyte degeneration and necrosis, multifocal, moderate, with hemorrhage, emphysema, and few intralesional bacteria (**Fig. 4-1**)

Conference Comment: The pathogenesis of blackleg involves ingestion and passage of spores across the intestinal mucosa, and distribution to tissues where they are stored for long periods in the phagocytic cells. The latent spores germinate when there is muscle damage or low oxygen tension.(3) The large muscles of the pelvic and pectoral girdles are most often affected, but any striated muscle is susceptible including the tongue, heart and diaphragm.(3) Grossly, the peripheral tissue is dark red and edematous, while the center is red-black, dry, friable and emphysematous. Additional findings include a rancid-butter odor, rapid autolysis of tissues, and fibrinohemorrhagic pleuritis (without corresponding severe pneumonia). Histologically, leukocytes are sparse since they are destroyed by the exotoxins.(3)

Differentiating blackleg from pseudo-blackleg, gas gangrene and malignant edema has important management implications. Pseudo-blackleg is caused by the activation of latent spores of *C. septicum* in the muscle. *C. septicum* proliferates rapidly after death, unlike *C. chauvoei*, so the carcass must be examined immediately after death. Pseudo-blackleg lesions are often multiple and are much less emphysematous. *C. septicum* also causes hemorrhagic abomasitis (Braxy) in ruminants.(3) Blackleg results from activation of latent spores in muscle, whereas gas gangrene and malignant edema results from contamination of an open wound.(3) Gas gangrene and malignant edema can be caused by a single or mixed infection of *C. chauvoei*, *C. septicum*, *C. perfringens* and *C. novyi*. The most common cause of malignant edema is *C. septicum*. Ruminants, horses and swine are most susceptible.(3) Malignant edema causes primarily a cellulitis, rather than a myositis as in gas gangrene. Also, the gas characteristic of gas gangrene is not a feature of malignant edema.(3) *C. novyi* also causes "swelled head" in rams, resulting from the infected head wounds received during fighting.(3) *C. novyi* is also the cause of necrotic hepatitis (Black disease) in sheep, and *C. haemolyticum* causes bacillary hemoglobinuria in cattle and sheep. In both diseases, clostridial spores are ingested, cross the intestinal mucosam, and remain latent within Kupffer cells. Migrating larvae of the common liver fluke, *Fasciola hepatica*, cause necrosis which leads to activation of the latent clostridial spores. The spores release beta toxin, a necrotizing and hemolytic

lecithinase (phospholipase C), that causes necrosis of the surrounding tissue and hemolysis in bacillary hemoglobinuria. Grossly, there are large pale areas of necrosis surrounded by broad zones of hyperemia. The necrotic area in bacillary hemoglobinuria is usually focal and larger than in Black disease. Bacillary hemoglobinuria causes intravascular hemolysis with anemia and hemoglobinuria.(2)

Contributing Institution: NMDA/Veterinary Diagnostic Services, 700 Camino de Salud NE
Albuquerque, NM 87106, <http://nmdaweb.nmsu.edu/animal-and-plant-protection/veterinary-diagnostic-services>

References:

1. Jones TC, Hunt RD: Veterinary Pathology, 5th ed., pp. 579-580. Lea Febiger, Philadelphia, PA, 1983
2. Stalker MJ, Hayes MA: Liver and biliary system. *In: Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 2, pp. 355-356. Saunders Elsevier, London, UK, 2007
3. Van Vleet JF, Valentine BA: Muscle and tendon. *In: Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 1, pp. 259-264. Saunders Elsevier, London, UK, 2007