47th AAVLD Histopathology Slide Conference

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UKENTUCKY CASE 2

A UNIQUE PATTERN OF PLACENTITIS IN MARE REPRODUCTIVE LOSS SYNDROME

Manu Sebastion, Ralph Giles, Lenn R Harrison, James Donahue, Mary L Vickers, Thomas Tobin, and KB Poonacha
University of Kentucky, LDDC, 1429 Newtown Pike, Lexington KY – 40511

A placenta from a foal born dead during the period of mare reproductive loss syndrome was submitted to the University of Kentucky, Livestock Disease Diagnostic Center for macroscopic examination. The cervical star was intact and the allantochorion was torn at the juncture of the body and pregnant horn. The umbilical cord was diffusely yellow and edema in the amnionic segment which extended to the allantoic segment in a lesser degree was evident. Histopathological examination of the allantochorion revealed a multifocal, mild to moderate perivascular infiltrate of neutrophils and lymphocytes in the coelomic space/splanchenic mesoderm. The villi were free of inflammatory cell infiltrates. The amnion had diffuse edema. The attached superficial areas of umbilical cord were heavily infiltrated by neutrophils with minimal involvement of Wharton's jelly (funisitis).

In many of the placentas identified as being a Mare reproductive loss syndrome case, the inflammation of the allantochorion was limited to the coelomic space of the allantochorion which is a unique pattern of inflammation when compared to the prototypic ascending placentitis and placentitis due to systemic infections like Leptospira. Similar lesions were observed in the placentas of the abortions induced by dosing mares with eastern tent caterpillars. In all these cases funisitis (inflammation of the umbilical cord) was observed. Close examination of the sections from the allantochorion, demonstrated inflammation extended from the umbilical cord to the allantoic surface of the allantochorion. Since this pattern of inflammatory reaction has not been described previously, this lesion is apparently unique to the allantochorion of mare reproductive loss syndrome. The specific mechanism responsible for this lesion is not clearly identifiable, however the pattern indicates the inflammation extends from the amniotic cavity into the allantois.

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UNL-VDC 10197-05

MAMMOMONOGAMOUS LARYNGEUS IN A HOLSTEIN HEIFER

J.N. Henningson

A two year old Holstein heifer from Puerto Rico was positive at the University of Nebraska-Lincoln Diagnostic Lab for Bovine Viral Diarrhea (BVD) by IHC on skin. The heifer subsequently died due to mucosal disease. The veterinarian at necropsy found parasites attached to the laryngeal mucosa. The larynx with parasites attached was submitted along with the ear sample. Grossly, there were multifocal groups of parasites that were blood red to reddish brown, Y-shaped, and 9-20 mm in length attached to a mottled laryngeal mucosa. Histologically, there is a nematode with a thick-walled buccal capsule attached to a hyperplastic stratified squamous epithelium. The lumen of the buccal capsule and intestines contain epithelial debris and red blood cells. The female parasites contain numerous ova. There are multiple epidermal erosions with one focal area of ulceration that contains multiple colonies of cocci and serocellular debris with suppurative inflammation. The lamina propria and submucosa have a moderate, diffuse infiltration of neutrophils, plasma cells, lymphocytes, and macrophages. The ova are ovoid to ellipsoid, non-operculated and range from 78-95 micrometers by 42-54 micrometers in size.

The nematodes were identified as Mammomonogamous laryngeus, a nematode, which is a rare finding in North America. The parasite's geographic range primarily includes Brazil and the Caribbean Islands, but has been found in the Philippines, Thailand, and Jamaica. Mammomonogamous laryngeus of mammals is a member of the family Syngamidia, which includes genera Syngamus and Cyathostoma in birds. Mammomonogamous is a parasite of mainly domestic ruminants, especially cattle, and cats, with zoonotic potential. The worms of the family Syngamidia characteristics include: a large buccal capsule, male and female joined in permanent copulation, and infection of the upper respiratory tract.

The nematodes had a typical Y appearance due to the male and female worms being attached in permanent copulation. The male attaches by his bursa to the side of the female worm at the level of the vulva. Both males and females have a thick-walled buccal capsule, with short or long ribs, and eight small teeth at the base. At the oral opening there is a cuticular rim surrounded by lips. Male worms range from 3.0-6.3 mm long and 360-380 micrometers in diameter with or without spicules. The larger female worms range from 8.4-23.5 mm lengthwise and 550-570 micrometers in diameter.

The life cycle begins with adult worms in the laryngotracheal region of ruminants, in which ova are coughed up and swallowed. The ova are then passed in the feces or sputum and mature to infective larvae outside the host. Ruminants are infected by ingesting the infective larvae. The worms can also be ingested through ingestion of carrier hosts such as earthworms, snails, and arthropods.

There have been a handful of human cases in North America, but all have a history of visiting the Caribbean Islands or Jamaica. In humans, the most common clinical signs are a severe, chronic, nonproductive cough with a low grade fever and a possible persistent lump in the throat.

IP-2

BONE MARROW CHANGES IN POULTRY CONDEMNED FOR INFLAMMATORY PROCESS [IP]

Floyd Wilson [Presenter], Sue Ann Hubbard, Tim Cummings, Danny Magee & Lanny Pace
MS Veterinary Research & Diagnostic Laboratory & Poultry Laboratory,
College of Veterinary Medicine, Mississippi State University

History: A number of market age broilers submitted for processing were condemned for inflammatory disease [IP] during routine poultry inspection procedures.

Gross Pathology: All condemned birds were observed to have variable but generally severe focal to multifocal areas of skin thickening and yellow discoloration associated with sheet-like subcutaneous masses of yellow caseous exudates.

Gross Diagnosis: Skin/Multiple Sites: Marked Cellulitis

Disposition Category: Inflammatory Process [IP]

"Probable" Etiologic Diagnosis: E. coli Cellulitis

Histopathology: Bone Marrow was collected from the birds as part of an ongoing disposition survey of bone marrow changes in inflammatory diseases in poultry. The bone marrows were judged to be general markedly hypercellular, largely due to a dramatic increase in mature heterophils. A variable reduction in adipocytes was also appreciated.

Histologic Diagnosis: Bone Marrow: Marked Diffuse Heterophilic Hyperplasia

Morphometrics: Bone marrows were subjected to histomorphometric evaluations to determine the concentration of mature heterophils. 5 micron H&E stained sections were examined at 400-x magnification using a one mm square ocular grid. The number of mature heterophils present in each of ten grid fields was enumerated for a total of ten fields for each of the marrows. A marked elevation in mature heterophils within the bone marrow of IP birds was observed relative to historic control values.

Comments & Discussion: Current regulations from the USDA require that all birds manifesting with severe inflammatory disease be condemned if there is evidence also of systemic disease [1]. The birds presented in this case report were judged to be severely infected and to exhibit probable systemic involvement. While cultures of the skin lesion were not performed, the historic data indicates that the vast majority of cases of cellulitis in poultry are due to infections with E. coli [2]. The results for morphometric evaluations obtained from the current case were compared to historic data that has been accumulated for bone marrows from market age broilers from both normal control and birds manifesting with IP. The results demonstrate marked changes in the bone marrow as reflected in elevations in the numbers of heterophils providing evidence of systemic effects in severe cases of cellulites. Similar elevations in heterophils were observed for some birds with less severe localized IP that were presented for leg trim. The report also documents the value of bone marrow examinations for inflammatory diseases in poultry.

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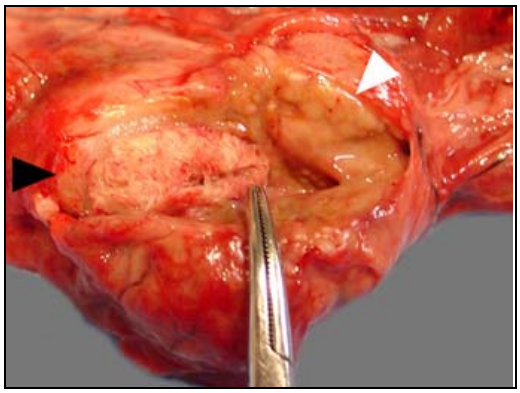
04C694

INTRA-ABDOMINAL GOSSYPIBOMA IN A DOG

D. O'Toole[1], T. Cornish[1], S. Kerr[2]
[1]Wyoming State Veterinary Laboratory, 1174 Snowy Range Road, Laramie, WY 82070.
[2] Bear Creek Veterinary Services, 6617 Hwy 85, Torrington, WY 82240

A 41.9-kg two and a half year old spayed St. Bernard bitch was presented to a Wyoming veterinarian with the complaint that she was lethargic and failed to gain weight since being spayed a year earlier in South Dakota. She was vomiting, and the frequency and severity of episodes was increasing. Masses were palpated intra-abdominally. Exploratory laparotomy was performed. Multiple abscesses with intralesional foreign bodies (gauze sponges) were found. There was a serosal abscess with adhesions and occlusion of distal small intestine. Masses were removed and the affected distal small intestine was resected. Tissues were submitted for histological examination, along with digital images of the appearance of the masses at surgery.



The laboratory received two pieces of tissue: a 13 x 8 x 2 cm segment of small intestine with associated abscess and a 32 x 15 x 8 cm intra-mesenteric mass. The mesenteric mass contained 6, 4 x 4 inch cotton swabs, all invested by fibrous connective tissue. Histologically, affected mesentery consisted multifocal granulomatous inflammation with fibrosis, and marked diffuse mesothelial hyperplasia with plasmacytic infiltration. Epithelioid macrophages contained linear, faintly basophilic, brightly birefringent cotton fragments. The intestine was segmentally occluded, tunica muscularis was replaced by granulation tissue, and mucosa was ulcerated. The dog made an uneventful recovery and regained weight.

Medical euphemisms for this condition are gossypiboma (*gossypium* (L): cotton), or the more revealing if vile term “textiloma.” A rich and alarming literature testifies to the numerous locations in which surgeons misplaced sponges. A search in Pub Med with gossypiboma as key word found 48 reports of the condition, some multiple cases. Among other locations, gauze sponges are left in cranial vaults, nasal passages and sinuses, pericardial sacs, intramuscular sites, abdominal cavity, bladders, and pleural sacs. In addition to gainful employment for our legal brethren, gauze sponges can evoke neoplasia. The most common complication is a large foreign body reaction that may be mistaken for neoplasia. Occasionally the reactions are sufficiently severe that patients die. The presence of myriad birefringent fibers in epithelioid macrophages should alert diagnosticians to the possibility of surgical sponge-induced granulomatous inflammation and fibrosis, even if the sponge has disintegrated or was overlooked

grossly. A recent review of the disease by surgeons at Duke University recommended the following steps: avoid small sponges during laparotomy; use sponges one-by-one on forceps; use only sponges containing radiopaque material; and take one final look at the engine before closing the hood.

The dog made an uneventful recovery. She will stay out of South Dakota.

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Kiupel

PAPILLOMAVIRUS IN AN EGYPTIAN FRUIT BAT

Kiupel M[1], McKnight CA[1], Wise A[1], Wise C[2], Maes R[1]

[1] Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI

[2] Crossroads Animal Hospital, 3232 N. Dettman Road, Jackson, MI 4901

A 5-year-old, intact female Egyptian Fruit Bat (Rousettus aegyptiacus) was acquired by The Organization of Bat Conservation, in Bloomfield Hills, MI, from a privately owned colony of 20-25 Egyptian Fruit Bats in February, 2003. Upon arrival, the bat had a small raised pigmented mass located at the lateral canthus of the left eye. Over the next eleven months, the mass progressively increased in size, and was surgically removed in January, 2004. At the time of surgery, the mass was 12mm in diameter, and extended 6mm into the underlying subcutaneous tissue. Further examination identified multiple (approximately 6) variably sized, raised, smooth to cauliflower-like skin masses randomly distributed throughout the left wing membranes. Over the next month, partial resolution of 2 of the wing membrane masses was noted. An additional three masses were removed in February, 2004. All excised tissues were submitted to the Diagnostic Center for Population and Animal Health at Michigan State University for microscopic examination.

All four masses appeared microscopically similar and were characterized by elongate to polygonal neoplastic cells with prominent intracellular bridging that were arranged in lobules and thick pegs extending from the overlying hyperplastic epithelium. Neoplastic cells had variable amounts of eosinophilic cytoplasm and large vesiculate nuclei with prominent, sometimes multiple nucleoli. Many lobules surrounded central areas of keratinization with numerous intermixed dyskeratotic cells. There were 1-3 mitoses per high power field (HPF). A diagnosis of multiple basosquamous carcinomas was made. Due to the multiple masses identified and the history of partial resolution of 2 of the masses, immunohistochemical staining for papillomavirus was done. Slides had positive intranuclear staining in all examined tissue.

DNA extracts from formalin fixed-paraffin embedded tumor tissue were tested by PCR, using degenerate primers designed to amplify a 450 bp segment of the L1 region of the human papilloma virus genome. A 450-bp product was obtained and directly sequenced. A BLAST analysis of the sequence data showed that there was 42.9% sequence identity with the L1 region of human papillomavirus.

This is the first report of a papillomavirus-associated carcinoma in a bat. Papillomaviruses have been associated with a number of hyperplastic and neoplastic lesions in a wide variety of vertebrate species, including humans.[1, 2, 3] Whereas papillomavirus in bovines most commonly results in benign lesions, such as fibropapillomas and papillomas, papillomavirus-induced lesions in humans may progress to squamous cell carcinomas.[1, 5, 7] A similar progression to carcinoma has been documented for other species-specific papillomaviruses, such as canine oral papillomavirus, cottontail rabbit cutaneous papillomavirus (Shope papillomavirus), and rodent (*Mastomys natalensis*) papillomavirus.[4, 6, 8] Interestingly, bovine papillomaviruses (BPV-1, BPV-2) have been associated with neoplastic lesions in other species, such as cutaneous sarcoids in horses.[1, 2, 3] In the case presented here, papillomavirus antigen was detected in all 4 examined carcinomas, strongly suggesting a role of this virus in tumorigenesis. It is uncertain whether the

papillomavirus identified in this bat represents a novel species–specific bat papillomavirus, or cross-infection of a known papillomavirus from another species. Further genomic sequencing is in progress.

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04-3001

LAWSONIA ENTERITIS IN A MINIATURE HORSE

Rob Bildfell and John Schlipf
Oregon State University VDL and VTH

These tissues are from a 4.5 month-old miniature horse presented to the Oregon State University VTH with a history of anorexia (10 days), and watery diarrhea (2 days). Findings on initial work-up included hyponatremia (120.4 mEq/l; norm 133 – 142), hypoproteinemia (3.8 g/dl), hypoalbuminemia (0.57 g/dl; norm 2.9 – 3.8) and a mild metabolic acidosis with respiratory compensation. Indicators of an inflammatory component included a neutrophilic leukocytosis and hyperfibrinogenemia. Treatment with a plasma transfusion, antibiotics (gentamicin, penicillin and metronidazole), anti-inflammatories and supportive fluids failed to resolve clinical signs. Fecal flotation for parasite ova, blood and fecal cultures for *Salmonella*, toxin assays for *Clostridium difficile* and *C. perfringens* enterotoxins were all negative. The filly was euthanized 4 days post-admission.

Gross necropsy findings in the gastrointestinal tract included multifocal ulceration of the gastric squamous mucosa. Small intestinal and cecal contents were watery while the ingesta in large colon and small colon was of normal consistency. The ileal mucosa was markedly thick due to corrugated mucosal folds and often had a granular surface consistent with erosion. Scattered along this mucosa were oval 5 – 15 mm long plaques interpreted as hyperplastic lymphoid tissue. No abnormal findings were noted in the duodenum, proximal jejunum or mesenteric lymph nodes. No significant bacterial isolates were recovered on aerobic cultures of ileum and liver.

Microscopic findings include a severe erosive to ulcerative ileitis with blunting of villi and crypt epithelial hyperplasia in less affected areas. Infiltrates of a variety of leukocytes are present throughout the lamina propria with extension into the edematous submucosa. Included in this infiltrate are multinucleate giant cells and plump macrophages. Examination of a mesenteric lymph node reveals a few foci of granulomatous inflammation in the paracortex (may not be visible on all slides). Acid-fast stains of these lesions were negative but Warthin-Starry stain reveals small numbers of bacterial rods in the cytoplasm of some of the cryptal enterocytes. Immunohistochemical stains performed at Iowa State University confirmed these bacteria as *Lawsonia*-like. Clumps of *Lawsonia* antigen were also identified in proprial multinucleate giant cells and in the histiocytic aggregates within the lymph node.

Lawsonia intracellularis is known to affect a variety of species, including horses[1]. Changes documented in equine cases include ileal thickening and hyperplasia of crypt epithelial cells. The granulomatous character of the inflammatory response in this case is unusual for *Lawsonia* infections but there is at least one report of similar changes in pigs[2]. Multinucleate giant cells in previous reports of equine proliferative ileitis are described as “rare” or “few”. Other proposed etiologies of equine granulomatous enteritis include various mycotic agents, *Mycobacterium avium* and *Campylobacter sp*.

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2784040

SYSTEMIC MYCOBACTERIOSIS IN A MARINE TOAD (*BUFO MARINUS*)

Scott D. Fitzgerald[1*], Thomas W.J. deMaar[2], Jennifer S. Thomas[1]
[1] Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI 48824, USA
[2] Binder Park Zoo, 7400 Division Drive, Battle Creek, MI 49014, USA

Non-tuberculosis mycobacteriosis is infrequently reported in frogs and toads. This case report describes a captive marine toad (*Bufo marinus*) that presented with spontaneous fracture of the left tibia. In spite of amputation and antibacterial therapy, this toad continued to decline, and so was euthanatized and necropsied. Microscopic evaluation of the fracture site, and additional organs including one front limb, lungs, heart, liver and kidneys exhibited granulomatous inflammation associated with numerous acid-fast bacilli. Mycobacterium isolation and identification revealed two different mycobacterium organisms, *M. marinum* and *M. terrae*. Mycobacterium infection can vary in reptiles from in apparent infection, to severe disseminated disease. In this case, it proved to be the cause of the limb fracture and widespread disseminated disease. An additional histologic finding associated with several sites of granulomatous inflammation were scattered 10-15 micron diameter pigmented yeast organisms, consistent with chromomycosis. *Mycobacterium marinum* has been previously shown to be a serious opportunistic infection in humans with occasional musculo-skeletal system involvement, and therefore is of zoonotic concern.

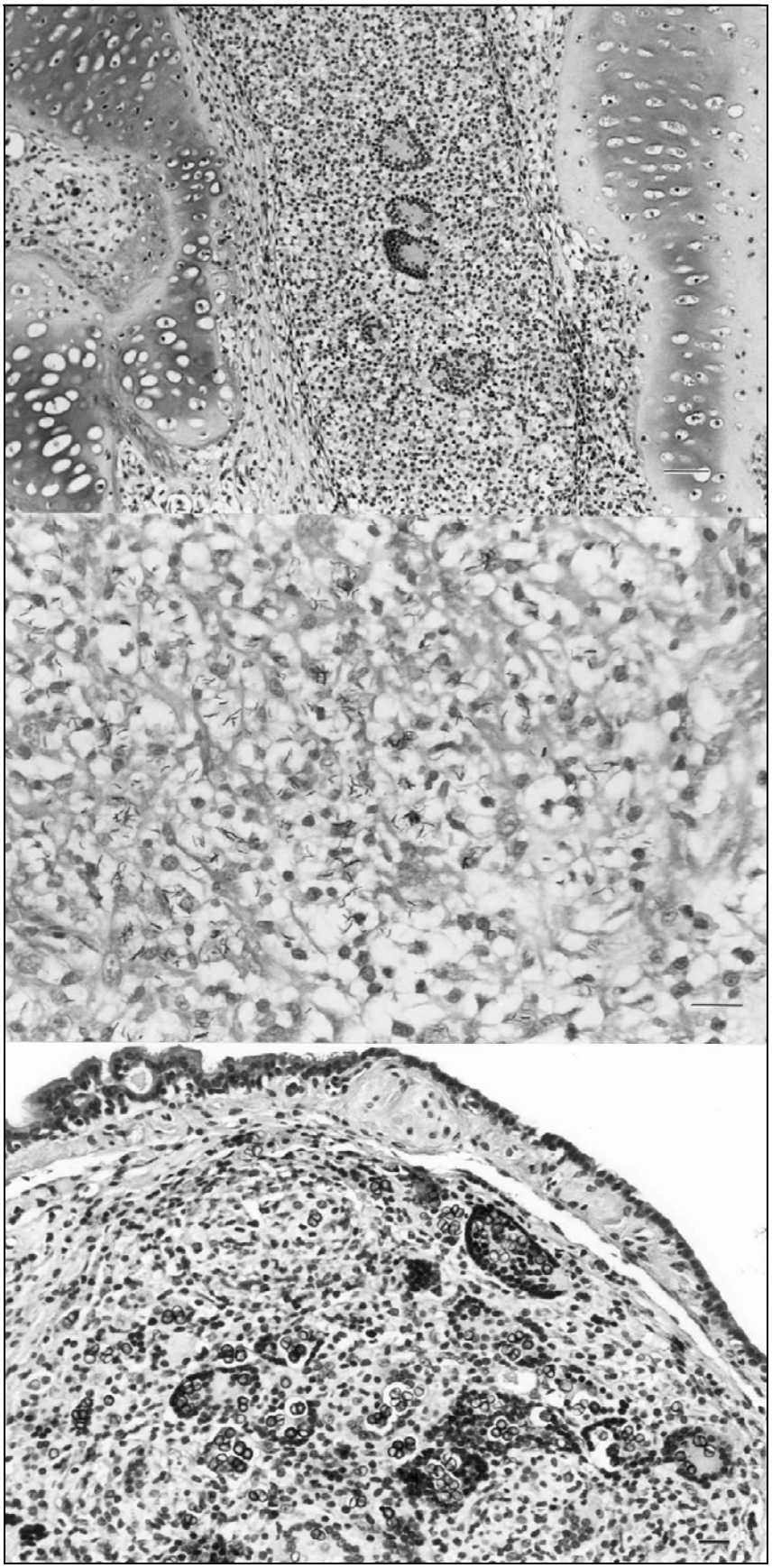

Fig. 1

Fig 2

Fig 4

S0403383 2a

SARCOCYSTIS FALCATULA PNEUMONIA IN A COCKATOO

Uzal F.A.[1], Shivaprasad H.L.[2]
[1]San Bernardino Branch, [2]Fresno Branch, California Animal Health and Food Safety Laboratory, University of California, Davis. 105 W Central Ave, San Bernardino, CA, USA

A 2-month-old male Umbrella cockatoo showed progressive dyspnea 2 days after it was imported into Southern California from Florida and it died 2 days after the beginning of clinical signs. The necropsy showed severe, diffuse congestion of both lungs, along with hepato and splenomegaly.

Histologically, the lungs showed diffuse interstitial pneumonia, characterized by increased number of macrophages and cuboidal cell hyperplasia along the atrial and air capillary epithelium. Inflammatory exudate was minimal and it consisted of diffuse infiltration of lymphocytes and plasma cells. A moderate amount of elongated merozoites, approximately 50-100 μm long by 15-25 μm wide, were observed within the blood capillary endothelium.

A diagnosis of interstitial pneumonia was established based on histopathological findings, while the etiology (*Sarcocystis falcatula*) was established by immunohistochemistry using antibodies against this parasite. *S. falcatula* is a protozoa described in North America, that has been associated with acute deaths in a variety of psittacine species.

A striking histological feature of this case was the proliferation of cuboidal cells in the alveolar walls of the lung with no much inflammatory exudate, a characteristic which in mammals is usually suggestive of some fume toxicosis. However, most fume toxicosis in birds are hyperacute with minimal or no histological changes in the lungs, except for severe edema.

9153168-7

NECROTIZING ENCEPHALITIS IN BHV-1 ABORTED FETUSES

Alexandra Brower[1,2] , Philip Bochsler[1,2] , Robert Porter[1,2], Karen Woods[1], Sue Ubl[1], Kelly M Homb[1], David Krueger[1] and Kathy Toohey-Kurth[1,2]

[1]Wisconsin Veterinary Diagnostic Laboratory (WVDL), University of Wisconsin, Madison 53706

[2]Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison 53706

From January through February 2004, the Wisconsin Veterinary Diagnostic Laboratory (WVDL) received 6 late term aborted fetuses from Angus cattle as part of a diagnostic investigation of an abortion storm on a farm in the southern part of the state. Diagnosis of herpes virus (BHV-1) abortion in all 6 fetuses was based on positive results from laboratory tests that included gross examination and histopathology, indirect fluorescent antibody staining, PCR, and virus isolation. Various combinations of these tests were conducted on multiple tissues from each fetus. The most severe gross and histopathologic lesions were in the liver in all of the cases, and were typical of BHV-1 abortions.

In all six cases there was also multifocal encephalitis. Brain tissue from two cases was tested with PCR and IFA. Both of the brains were PCR positive for BHV-1, and one of them was also IFA positive. Glial nodules were identified in all of the cases, and additional findings in some lesions included mononuclear inflammation with cavitation in the cerebral cortex, neuronal necrosis, and micro hemorrhage. Intranuclear inclusion bodies were not seen. Because the brain lesions were severe in some of the cases and thought to be atypical for BHV-1 abortions, brains from three additional WVDL IBR abortion cases, not associated with the described outbreak, were reviewed. In all three cases glial nodules and neuronal necrosis were also identified. BHV-1 immunohistochemistry using a monoclonal antibody revealed intralesional positive staining neuron cell bodies, processes, and cells interpreted as oligodendrocytes in all of the cases examined.

It was not clear if the brain lesions were an unrecognized or underappreciated manifestation of infection by BHV-1, an atypical manifestation of BHV-1, or the result of a different strain of BHV. (Because of the known neurotropism of BHV-5 in calves, this strain was considered a possible candidate.) Herpes virus from one of the aborted fetuses in the abortion storm was isolated and the sequence alignment of the thymidine kinase (tk) gene of BHV-1 (Accession Number D00438) was examined. The sequence of the isolate matched the predicted sequence of the BHV-1 target area and not that of BHV-5. Primers are being designed to sequence a larger region of approximately 500 to 600 base pairs in order to confirm this data. Many of the cases reviewed had negative *Neospora* sp. serology from the dam's sera and/or fetal thoracic fluid, and they all lacked the typical cardiac lesions associated with *Neospora* sp. infection, but *Neospora* sp. immunohistochemistry will be done on all of the cases to rule out the possibility of duel infections. These preliminary findings suggest that encephalitis with neuronal necrosis is a consistent lesion in IBR abortions.

03-3179

CANINE PYTHIOSIS SUSPECTS: EMERGING OOMYCOSIS VS MYCOSIS

Carol A. Lichtensteiger and April Paulman
Veterinary Diagnostic Laboratory and Department of Pathobiology,
University of Illinois, Urbana-Champaign, IL 61802

Pythium, known for decades as the cause of cutaneous granulomatous masses in horses (swamp cancer; kunkers, *P. insidiosis*), is one of two Oomycete infections emerging in dogs. Although *Pythium* infections in the United States were thought limited to the Gulf coast area, canine *Pythium* infections have been diagnosed in the lower Midwest. The other emerging Oomycete is *Lagenidium*, recently confirmed to cause natural infections in dogs affecting the skin, lymph nodes, and great vessels. The hyphae formed by Oomycetes species in tissue sections cannot be differentiated from each other or from fungal hyphae such as those of Zygomycetes. Irregular width, broad branching rarely septate hyphae in granulomatous masses in dogs are often labeled *Pythium* suspects.

The submitted tissue section (03-3179) is from a mesenteric mass in a young (1.75 year) spayed female Boxer crossbred dog. The dog had lost weight for two months. The mesenteric mass was found during a exploratory surgery and involved much of the jejunum. The dog was euthanized at surgery and biopsy sections were submitted for histopathology. The mass was a mesenteric lymph node with eosinophilic granulomatous lymphadenitis with marked perinodal fibrosing eosinophilic granulomatous peritonitis and intralesional silhouettes of hyphae. The hyphae were about 5 μm in width with slightly non parallel walls that stained well with the GMS stain and poorly with PAS stain. A top differential for the hyphae was *Pythium*; however, *Lagenidium* or saprophytic fungi such as zygomycetes could not be ruled out as additional tissue was not available. Infection prevalence and hyphae morphology is compatible with a *Pythium* diagnosis. *Pythium* hyphae tend to be smaller (2 to 8 μm) than *Lagenidium* (7 to 25 μm) and Zygomycetes (5 to 20 μm). In addition, *Pythium* tends to stain poorly with H&E where as *Lagenidium* stains well and Zygomycetes often form a silhouette in a dense eosinophilic sleeve. *Pythium* also contrasts with its poor PAS staining.

In the past eight years, the Diagnostic Laboratory has had eight biopsy submissions with granulomatous reactions and hyphae where *Pythium* was given as a differential: two with mesenteric masses (focal peritonitis; includes presented case), three with dermatitis, two with gastritis, and one with jejunitis. One of the dermatidities was confirmed *Pythium* by culture and serology (ELISA and Western blot analysis). Two other assays are available: a PCR assay using cultures or fresh or ethanol fixed tissues and immunohistochemistry. More extensive and accurate testing is needed in patients with hyphae associated with granulomatous diseases to identify the causative agents; prognosis and appropriate therapy varies with agent.

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6991061

AUDYCOPTID MANGE IN BEARS

Scott D. Fitzgerald[1*], Michael M. Garner[2], Thomas M. Cooley[3]
[1]Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI 48824, USA
[2]Northwest ZooPath, Monroe, WA 98272, USA
[3]Rose Lake Wildlife Disease Laboratory, Michigan Department of Natural Resources, East Lansing, MI 48823, USA

Skin biopsies were collected as part of a routine surveillance protocol from a captive Polar bear (*Ursus maritimus*). Rare intrafollicular arthropod parasites were present without any associated dermatopathology. These organisms were identified as *Ursicoptes americanus*, an audycoptid mite, which as been previously described associated with mange in both Polar and American black bears (*Ursus americanus*). Both sarcoptic and audycoptid mange are associated with alopecia, lichenification and crusting dermatitis in bears. Histologically, both types of mange are characterized by epidermal hyperplasia, marked hyperkeratosis, and the presence of intralesional organisms. Both parasites are in the sarcoptiform group of mites and share many anatomic features. The principal differentiating features of the two parasites are their location in the skin, with *Ursicoptes* being limited to hair follicles, and the elongated body shape of *Ursicoptes* mites compared to *Sarcoptes*.

A04-011163

HEPATOTOXICOSIS AND COPPER ACCUMULATION IN A LABRADOR RETRIEVER AFTER CARPROFEN THERAPY

Margaret A. Miller, José A. Ramos-Vara, Alok Sharma, and Joanne R. Smith
Animal Disease Diagnostic Laboratory and Veterinary Teaching Hospital, School of Veterinary Medicine, Purdue University, West Lafayette, IN

An obese (44.3 kg), 8-year-old, spayed female Labrador retriever dog suddenly developed anorexia, vomiting, and icterus after 14 days of oral carprofen therapy (2.3mg/kg every 12 hours). Carprofen therapy was discontinued and the dog was referred 5 days later to the Veterinary Teaching Hospital with marked elevation of the following serum enzyme activities (reference range in parentheses): alanine transaminase, 1996 IU/L (3-69 IU/L); alkaline phosphatase, 616 IU/L (20-157 IU/L). Enzyme activity declined over the following 5 days, but remained well above the reference range. Serum total bilirubin concentration increased during the same period from 13 to 32 mg/dl (0.1-0.8 mg/dl). Serum creatinine was 2.3 mg/dl (0.5-1.5 mg/dl). The liver was hypoechoic upon ultrasound evaluation. The dog developed progressive abdominal effusion and peripheral subcutaneous edema, and then thrombocytopenia and melena 4 days after admission.

Major histologic features in a percutaneous tru-cut hepatic biopsy 4 days after admission were apoptosis, periportal and centrilobular fibrosis, infiltration by macrophages and lymphoid cells, bile stasis, and accumulation of pigment in Kupffer cells and hepatocytes. The dog was euthanized 5 days after admission. At necropsy, the carcass was icteric with subcutaneous edema. The liver was slightly firm, yellow-brown with accentuated lobular pattern, and had scattered pinpoint subcapsular depressions. Bile distended the gall bladder without bile-duct obstruction. Gastrointestinal mucosa and serosa were petechiated; colonic content was bloody. Edema expanded the mucosa of the gastrointestinal tract and urinary bladder. The spleen was enlarged, meaty, and did not exude blood when incised.

Histologic changes in the liver resembled those noted in the biopsy; however, focal loss of hepatocytes, usually centrilobular or paracentral, was more obvious. There was at least a relative increase in fibrous stroma in these areas of lytic necrosis with patchy infiltration by macrophages, neutrophils, and lymphocytes. Portal and centrilobular lymphatic vessels were frequently dilated. Bile distended canaliculi. Golden brown pigment in hepatocytes and macrophages was interpreted as a combination of bile, hemosiderin (Perl's Prussian blue stain), and copper (Rubeanic acid stain).

Other important lesions included arteritis (in brain, heart, gastrointestinal tract, and urinary bladder) and tubular nephrosis with bilirubin casts. The cause for arteritis, which was not observed in the liver, was not determined; however, this lesion explained the observed hemorrhages and appeared more acute than hepatic lesions.

Carprofen is a nonsteroidal anti-inflammatory drug used for its analgesic and anti-inflammatory properties in dogs with musculoskeletal pain (usually due to osteoarthritis). Hepatocellular toxicosis with inappetence, vomiting, hyperbilirubinemia, and elevated serum activity of hepatic enzymes has been reported in 21 dogs, of which 13 were Labrador retrievers.[1] All Labrador retrievers reportedly developed clinical signs 14 or more days after the beginning of carprofen therapy regardless of the duration of therapy. Seventeen dogs (and all 13 Labrador retrievers) recovered; four died or were euthanized

3 to 5 days after presentation. Reported hepatocellular changes resembled those in this case, except that hepatocellular vacuolation was not appreciated in the current case. Copper accumulation, which was likely secondary to hepatocellular injury in the current case, was not reported in the series; however, histologic detection of copper requires special stains. Carprofen was considered a likely cause of hepatocellular toxicosis in this dog because of the apparent breed predilection, the interval between initiation of therapy and onset of clinical signs, and the histologic changes in the liver.

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F01-856 #27

PROVENTRICULAR DILATION DISEASE IN A PEREGRINE FALCON

H. L. Shivaprasad, CAHFS – Fresno, University of California Davis.

A five-year-old male Peregrine Falcon (Zado) was submitted to the laboratory with a history of odd behavior of one month duration and regurgitation of two days duration. The bird was severely dehydrated, emaciated, and weighed 520 Gms. The esophagus and crop were severely distended with ingesta and feathers. The duodenum and proximal jejunum were impacted with ingesta.

Microscopic examination of various organs revealed infiltration of lymphocytes mixed with a few plasma cells in the subserosal nerves and ganglia of the crop/esophagus, proventriculus, gizzard, intestine and heart. Other lesions noted were lymphoplasmacytic infiltration in the adrenal gland, brain, spinal cord, peripheral nerves (including optic nerve) and choroid.

Comment: Proventricular dilation disease (PDD) is one of the most common diseases of psittacines; the cause remains unknown. PDD has been reported in various species of birds including Canada geese, toucans, weaver finches, canaries, honey creepers and spoon bills, but not in raptors.

2873229

PYOGRANULOMATOUS AND NECROTIZING MENINGOENCEPHALOMYELITIS IN A 6-YEAR-OLD GREYHOUND ASSOCIATED WITH TOXOPLASMA GONDII

Rehagen D[1, 2], Kiupel M[1], Murphy A[1], Elsheikha H[1], Ducharme I[3], Bolin SR[1]
[1]Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI
[2]MPI Research Inc., Mattawan, MI
[3]Michigan Veterinary Specialists, 21600 West Eleven Road, Southfield, MI 48067

A 6-year-old, spayed female Greyhound had neck pain, which progressed to tetraparesis over a period of one week. A neurological examination indicated a cervical spinal cord white matter lesion. Cerebrospinal fluid analysis revealed mild pleocytosis with a white blood cell count of 200/µl. A complete blood count and serum chemistry were unremarkable. Myelography showed leakage of dye from the meninges at the level of C_6-C_7. Four hours following the myelogram, the dog died and was submitted to the Diagnostic Center for Population and Animal Health for a complete necropsy.

On gross examination, the only lesions were observed in the spinal cord. On cut section, there were multiple, elongate, randomly distributed, coalescing pink areas between C_2 and C_6. These areas were of similar consistency as the surrounding neural parenchyma. Microscopically there were multiple, randomly distributed areas of necrosis involving both the white and gray matter in the spinal cord. The areas of necrosis were characterized by fragmentation and edema of the neuropil, myelin degeneration, hypereosinophilic swollen axons, and variable degrees of hemorrhage with fibrin deposition. Inflammatory infiltrates varied from predominantly neutrophilic to pyogranulomatous with moderate numbers of neutrophils, epithelioid macrophages, lymphocytes and plasma cells. There was a comparable area of pyogranulomatous inflammation and necrosis within a cerebellar peduncle. Severe non-suppurative perivascular cuffing and meningitis extended from the cerebrum to the cervical spinal cord. A diagnosis of a necrotizing and pyogranulomatous meningoencephalomyelitis was made.

Immunohistochemical (IHC) staining of the spinal cord revealed rare intracellular, pyriform shaped structures, which stained positively with an antibody against *Toxoplasma gondii* and negatively with antibodies against *Neospora caninum* and *Sarcocystis spp*. No fungal organisms were observed with mucicarmine or PAS staining. No pathogenic bacteria were cultured from brain tissue. DNA extracts from frozen brain tissue tested positively by PCR using general Apicomplexan primers. The DNA extract also tested positively with primers specific to *T. gondii* and negatively with primers specific to *N. caninum*. Amplification and sequencing of the PCR product provided 393bp of the ITS-1 sequence. A BLAST analysis showed that the sequence was identical to ITS-1 sequences of *T. gondii*.

The case presented here most closely resembles the lesions described as pyogranulomatous meningoencephalomyelitis of Pointer dogs (PMEP). However, the lesions in the spinal cord of this dog were more neutrophilic and necrotizing than those described in Pointer dogs. A diagnosis of granulomatous meningoencephalomyelitis (GME) was considered as a differential. The etiology and pathogenesis of PMEP and GME are unknown and the diagnoses are based on their clinical presentations and microscopic appearances as well as the exclusion of other known etiologies.

Interestingly, in the case presented here, intralesional *T. gondii* antigen and nucleic acid were detected by IHC and PCR, respectively, suggesting a possible role of this organism in the pathogenesis of this lesion. According to the current literature, protozoal encephalomyelitis is uncommon in dogs and infections with *T. gondii* are usually associated with a non-suppurative inflammatory reaction. Affected dogs are often concurrently infected with canine distemper virus. Canine distemper virus was not identified in this case. Both the distribution and microscopic appearance of the lesion described here are uncommon for an infection with *T. gondii*. Further investigation of a possible role of *T. gondii* in the development of this particular lesion as well as pyogranulomatous meningoencephalomyelitis and GME in dogs is necessary.

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04-160/175

TOXOPLASMOSIS IN NICOBAR PIGEONS (*CALOENAS NICOBARICA*)

H. L. Shivaprasad[1] and Rick Last[2]
[1]CAHFS – Fresno, University of California Davis
[2]Vetdiagnostix, PO Box 13624, Cascades 3202, South Africa.

Formalin fixed lungs, liver, spleen, heart and kidney were submitted from three Nicobar pigeons. These pigeons were part of ten breeding pairs and 5 other birds. The three pigeons died in a span of one month without any apparent clinical signs. The only significant gross lesions were severe pulmonary congestion and edema.

Microscopic examination revealed severe pulmonary congestion, edema and mild lymphoplasmacytic inflammation, especially around vessels in the lungs. The livers had multifocal necrosis of hepatocytes and periportal inflammation. The spleens had severe fibrin exudation in the vascular sinuses and inflammation in and around small vessels. Scattered through out the lung, liver and spleen were large numbers of basophilic staining zoites either free or surrounded by a thin wall. These zoites were present in the macrophages, endothelial cells, hepatocytes and epithelial cells. Immunohistochemistry for toxoplasma was strongly positive for Toxoplasma gondii in these organs, as well as in heart and kidneys.

Comment: Toxoplasmosis is not common in pigeons. It has been reported in a variety of species of birds, most commonly in canaries.

UKENTUCKY CASE 1/2

MALIGNANT CATARRHAL FEVER IN A GOAT?

Manu Sebastian, Ralph C Giles, Mary L Vickers, Stephen F Sells, Robin Wharton
University of Kentucky, LDDC, 1429 Newtown Pike, Lexington, KY-40511.

A 6-month old female Boer goat was submitted for necropsy to the University of Kentucky, Livestock Disease Diagnostic Center. For the last one week the goat had reduced appetite and leathery skin, before it died. The animal was treated with LA-200 every other day for a total of two treatments. The goat's eyes were swollen and watery.

External examination revealed large areas of dermatitis over much of the body. The skin was markedly thickened, with hairless areas in the groin and axillae, and on the left side of the head, between the horn and base of the ear. Skin over the remainder of the body was dry and flaky. The abomasum was distended with fetid green fluid contents.

Microscopic examination of the organs revealed the following findings. Brain: Mononuclear cells infiltrated the meninges over the cerebrum and cerebellum. These cells were also present in the perivascular space around blood vessels in the deeper gray and white matter. These mononuclear cells consisted of a mixture of lymphocytes, plasma cells and macrophages. Adjacent neurons in the gray matter showed degenerative change. Skin: Epidermis was thickened and hyperkeratotic. Neutrophils densely infiltrated the epidermis. Mononuclear cells infiltrated the dermis. Squamous epithelial cells were vacuolated and some had undergone necrosis. There was necrosis of the epithelium of hair follicles. Syncytial cells were present in the areas of epidermal and follicular necrosis. Thyroid and adrenal: Mononuclear cells focally infiltrated interstitial regions of thyroid and medulla of adrenal. Larger number of mononuclear cells infiltrated portal areas of liver. Tracheobronchial lymph nodes had moderate depletion of lymphocytes. Intestine: Neutrophils focally infiltrated the crypts and lining epithelial cells were denuded. Abomasum: Mucosa was eroded and densely infiltrated by neutrophils. Blood vessels in submucosa were infiltrated by mononuclear cells and fibrin was deposited along subintima. Tissue from the goat were positive for Ovine Herpes Virus-2 by polymerase chain reaction.

Ovine Herpes Virus–2 is the virus responsible for the world wide sheep associated malignant catarrhal fever, (MCF); however, it has never been isolated from its natural host or clinically susceptible species. Classical malignant catarrhal fever is a serious and often lethal infection of many species of the families of Bovidae and Cervidae. Its hallmarks are a syndrome of widespread lymphoproliferation, vasculitis, and inflammatory lesions affecting many tissues.

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Ohio 5715-04

ULCERATIVE GLOSSITIS AND GLOSSAL MYOSITIS IN AN ONAGER (*EQUUS HEMIONUS*) ASSOCIATED WITH *ACTINOBACILLUS LIGNIERESII* AND *ACTINOBACILLUS EQUULI*

J. Hayes [1], R. Gandalf [2], S. Rajeev [1], M. Atkinson [2]
[1]Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH
[2]The Wilds, Cumberland, OH

A dead 17-year-old female Persian onager weighing 422 pounds was presented in February, 2004 with a history of mild depression for 2 days, which progressed over the next 2 days to severe depression and recumbency. The animal was sedated for evaluation and movement into a barn. Physical examination revealed ulcers on the distal and lateral aspects of the tongue, and a distended mass was detected by rectal palpation. The onager died as reversal drugs were being administered. A complete blood count revealed leukocytosis (35x 10^3/µL, reference range 5.5-12.5) with marked neutrophilia (30,450, reference range 2600-7500) and monocytosis (1050, reference range 0-1000). Erythrocytes and hematocrit were decreased (4.24 x 10^6 µ/L [reference range 7.0-12.0] and 28.5% [reference range 32.0-50.0], respectively.

Multiple epithelial ulcers were found at three different sites of the tongue. Two ulcerated areas (6x4 and 10x8 mm) were present on the dorsal aspect of the tip of the tongue. A 15x5 mm region along the left lateral aspect of the tongue near the base contained linear and coalescing ulcers that were covered by a reddish brown crust. Two 1cm linear brown fissures were present on the dorsal midline of the caudal aspect of the tongue.

Microscopic lesions were most severe at the tip of the tongue. Numerous colonies and mats of individual coccobacilli adhered to surface epithelial cells and colonized exposed lamina propria where full thickness ulcers were present. Large numbers of viable and degenerate neutrophils were present at ulcerated sites, extended into subjacent connective tissue, perimysium and endomysium, and could be found within the sarcolemmal membrane of individual myofibers. Multifocal myofiber degeneration was evidenced by loss of cross striations, hyalinization and fragmentation of sarcoplasm and fraying of myofibrils. Myofiber atrophy and foci of mineralization were noted, also. Neutrophils were noted within the tunica media of a mid-size artery in connective tissue of the lesion at the tip of the tongue (vasculitis). In sections of the dorsal and lateral aspects of the tongue, fissures within hyperplastic epithelium were colonized by similar coccobacilli, but ulcers and inflammation were not present. Gram staining identified numerous small gram negative coccobacilli and a few gram positive cocci. Bacterial culture of these lesions revealed moderate growth of *Actinobacillus lignieresii*, *Actinobacillus equuli* and *Micrococcus* sp. Antibiograms of the two *Actinobacillus* isolates were identical, with resistance to penicillin, intermediate susceptibility to clindamycin and spectinomycin, and full susceptibility to all other antibiotics tested.

Actinobacillus lignieresii is a commensal organism of the bovine and ovine oropharynx. It has been reported in association with unusual infections in horses, including the tongue and muzzle[1,2]. Isolation of both *Actinobacillus lignieresii* and *Actinobacillus equuli* from a wound of a man bitten by a horse has been reported[3], suggesting these organisms may be commensals of the equine alimentary tract. This is the first report of glossitis associated with these two organisms in an onager to our knowledge. The pathogenesis is not known; opportunistic infection of traumatic lesions cannot be excluded. Severe cirrhosis and

hemochromatosis of the liver and splenic fibrosarcoma were also found at necropsy; perhaps these conditions contributed to a compromised immune system.

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B04-18465

SEPTICEMIC LISTERIOSIS IN AN ADULT DAIRY COW

Peter G. Moisan*, David J. Waldrep
Rollins Animal Disease Diagnostic Laboratory, Western Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture. *Presenter

A 33-month-old, 3-months pregnant, dairy cow from a herd of about 1000 cows exhibited lethargy, ruminal atony, and diarrhea for 1 week before death. No other sick cows were reported in the herd. The gross necropsy revealed severe (10%) dehydration, fluid-distended rumen, scant small intestinal content, and watery colonic material. Mesenteric lymphadenopathy was seen, and many of the mesenteric lymph nodes in the region of the distal jejunum were 8cm in diameter. The nodes were tan and bulged from the cut surfaces. The mediastinal lymph nodes were similar in appearance, though smaller. The liver was firm and pale. Congestion of most viscera was noted. Ecchymoses were located over the epicardial surfaces.

Microscopic lesions included necrosuppurative hepatitis, splenitis, lymphadenitis, and enteritis. The hepatic lesions were random and coalescent and the lesions in the lymph node, spleen, and small intestine were multifocal. Small, individual, gram positive and silver stain positive bacilli were located in all affected foci and in blood vessels throughout most tissues. Cultures of lung, lymph node, small intestine, and liver yielded 1+ to 4+ *Listeria monocytogenes*.

The encephalitic form of listeriosis is the most common manifestation in cattle. The abortion form is occasionally seen. The septicemic form, which is usually seen in neonatal calves, may be a post parturient manifestation of the abortion syndrome, causing weak, septic calves. Many mammals, some birds, and some cold-blooded animals are susceptible to septicemic listeriosis; however, the disease is very rare in adult ruminants, though it has been described in ewes. Tissues affected in the few described ruminant cases include liver, lymphoid tissue, and intestine, as with this cow.

Listeria monocytogenes is ubiquitous and is carried asymptomatically in the intestine of carrier animals, causing clinical disease only occasionally. The enteritis noted in this case is expected with clinical septicemic listeriosis, as invasion is reportedly from the intestine. We would expect involvement of the portal triads to be most pronounced, as ascending infection via portal circulation is the mechanism of systemic spread. Lesions in the lymph nodes and spleen, as well as bacteremia, all features documented in this case, are also seen in described cases, and would be expected, as the infection makes use of the circulatory and lymphatic systems to spread systemically. Mammary infection, usually subclinical and often the source of human disease through contaminated dairy products, was not described in this case.

For the purpose of determining the source of an outbreak of listeriosis in humans, strain serotyping is performed at a few laboratories. Thirteen separate serotypes are reported for this species. The serotype of *L. monocytogenes* was not identified in our case because this cow was the only individual affected. A predisposing cause, such as corticosteroid use or concurrent debilitating disease, was not reported with this case of listeriosis.

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04-21986

STEVEN RUSHTON
Rollins Animal Disease Diagnostic Laboratory. Raleigh, North Carolina

Signalment: Twelve week old mixed breed pig

History: This pig was presented to diagnostic laboratory for two day history of melena and sudden death.

Gross examination: Marked mesenteric lymphadenopathy is identified, and there were multifocal to coalescing beige nodules within the cortices. The kidneys are moderately enlarged with similar granular beige nodules in the cortex.

Histopathological examination:
Lymph node: Marked effacement of the follicular architecture is present, and follicles are replaced by large numbers of epithelioid macrophages along with multinucleated giant cells, lymphocytes and moderate numbers of eosinophils. Occasional macrophages contain small numbers of round, 1-2 micrometer deeply basophilic “grape-like” inclusions within the cytoplasm.
Kidney: Large numbers of multifocal to coalescing aggregates of epithelioid macrophages, admixed with occasional multinucleated giant cells and eosinophils have displaced the normal interstitial and periglomerular areas within the cortex and medulla. Similar deeply basophilic inclusions are also identified within the cytoplasm of the macrophages.
Liver: Occasional small aggregates of epithelioid macrophages and occasional eosinophils are located within the connective tissue of the portal tracts.

Morphologic diagnosis:
Lymph node and Kidney; lymphadenitis and nephritis, granulomatous and eosinophilic, severe, multifocal to coalescing with intracytoplasmic basophilic inclusions.
Liver; hepatitis, granulomatous, mild, multifocal.

Immunohistochemistry: Histiocytes of all three organs contain abundant, positive staining within the cytoplasm for Porcine Circovirus-2 and for Porcine Reproductive and Respiratory Virus. Occasional endothelial and renal tubular epithelial cells also stain for both viruses.

Comment: Porcine Circovirus-2 is associated with and the primary cause of postweaning multisystemic wasting disease (PMWS) in swine and is often associated with concurrent Porcine Parvovirus and PRRS infection. Immune activation and concurrent infection with Porcine Parvovirus is associated with increased numbers of virus antigen-positive cells and therefore increased severity of PMWS lesions caused by PCV-2.

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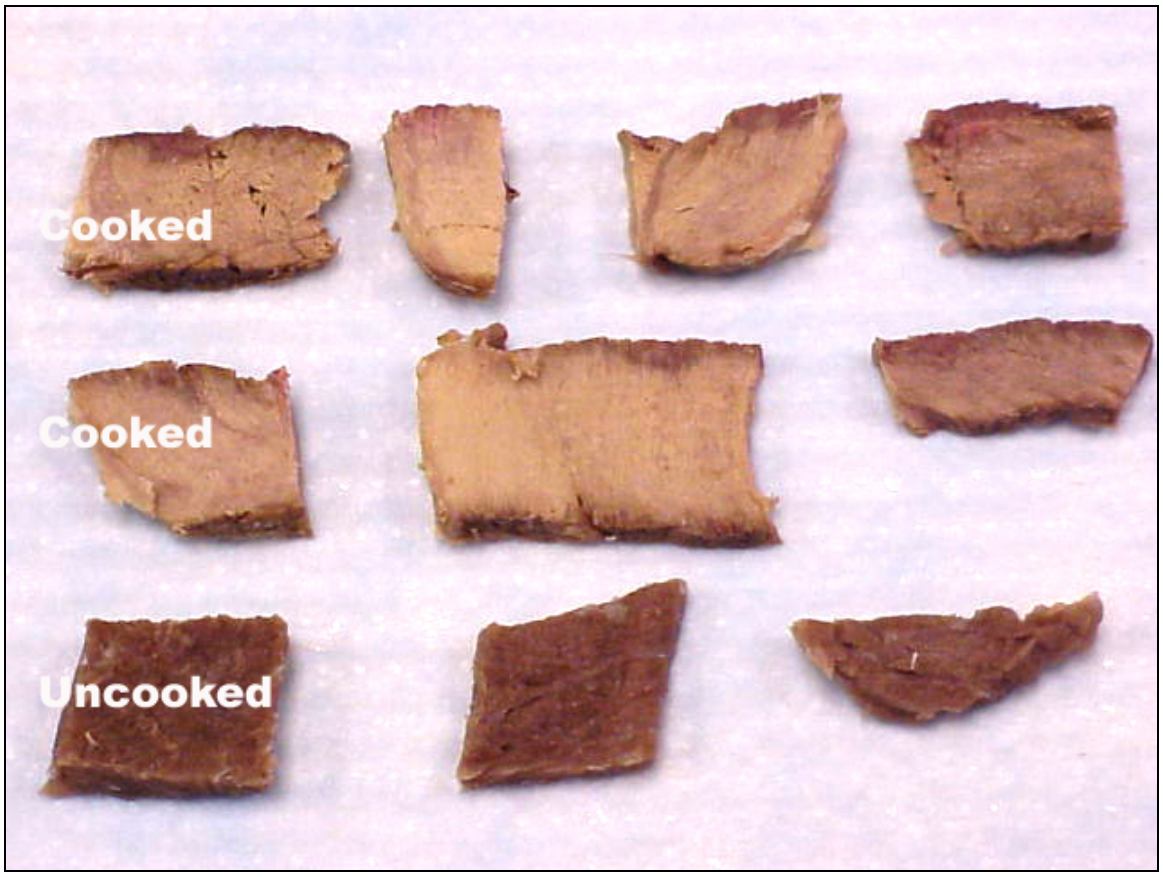
03-21307
MYOPATHY IN COOKED ELK STEAKS?

Tanya D. Graham
South Dakota State University

Several uncooked and cooked elk steaks were submitted to the diagnostic lab for examination. The owner had observed multiple pale pink spots within the cooked steak and reported that these pink spots remained pink, even after thorough cooking.



Histological examination of multiple sections of cook and uncooked steak revealed multifocal areas of myofiber hypertrophy with an associated increase in interstitial fibrosis. The hypertrophied myofibers were hyalinized and lacked cross-striations. In some sections of both cooked and uncooked steaks there were intramyofiber protozoal organisms compatible with Sarcocystis sp. These cysts were not associated with the hypertrophied myofibers. Immunohistochemistry for Toxoplasma gondii and Neospora sp. was negative.

E. coli was cultured from 3 out of 14 sections of cooked and uncooked steak. In the absence of any inflammation, this is believed to be a post mortem contaminate. No evidence of myositis, vasculitis, or neoplasia was observed in the sections examined.

Immunohistochemistry performed on cooked sections of steak was unrewarding. Immunohistochemical staining of the uncooked steak is shown below (Table 1).

Stain	Cooked Steak (Normal)	Cooked Steak (Hypertroph	Uncooked Steak (Normal)	Uncooked Steak (Hypertroph)
Myoglobin*	No staining	No staining	Positive	Negative
Actin*	No staining	No staining	Weak Positive	Negative
Smooth muscle actin	No staining	No staining	Negative	Negative
Desmin	No staining	No staining	Positive	Negative
Masson's Trichrome	Purple-Red	Red	Red	Blue

The only reported case of capture myopathy in Elk involved three acute to subacute cases, in which hemorrhage, edema, myofiber fragmentation and attempts at myofiber regeneration were the main features observed histologically. In the present case, the changes are chronic and there is minimal evidence of individual myofiber regeneration. These fibrous bands of connective tissue may be the results of fibrosis following myofiber injury such as transport/capture myopathy or they may be idiopathic collagen bundle hypertrophy. In either case, why the fibers did not cook the same as the adjacent myofibers is a mystery.

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