The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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Conference Moderator: Dr. LuAnn McKinney, DVM, Diplomate ACVP
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CASE I – LMU Path 104/04 (AFIP 2974022)

Signalment: 1 year, female, Haflinger, *Equus caballus*, equine.

History: 48 hours after feeding a new batch of concentrates, two yearlings showed weakness, increased heart and respiratory rates, mucosal congestion and intense sweating. Both were treated symptomatically. They went down in sternal and eventually in lateral recumbency and died within 3 days. All other horses in the stable had refused the new feed and remained free of symptoms.

Gross Pathology: Myocardium pale and patchy, pulmonary edema.

Laboratory Results: Serum: CK 6431 IU/l; EHV I and IV negative. Analysis of a sample of the ration revealed heavy contamination with salinomycin (295mg/kg).

Contributor's Morphologic Diagnoses: Myocardium: Degeneration and necrosis, multifocal, with histiocytic and lymphocytic inflammation; subacute, moderate to severe (depending on location; histoslides from two different locations).
Skeletal muscle: Acute degeneration of single muscle cells.

Contributor's Comment: Salinomycin, monensin, lasalocid and narasin are ionophore antibiotics used in veterinary medicine mainly as coccidiostatics and growth promoters. Ionophores interfere with the membrane transport system for $Na+$ and $K+$, resulting in disturbances in intracellular calcium homeostasis. Mitochondrial failure, energy exhaustion and failure of calcium ion retrieval from the cytosol eventually lead to myofibrillar hypercontraction and degeneration (1). Typical findings in cases of ionophore antibiotic poisoning are degenerative cardiomyopathy and/or myopathy (2, 3).

Poisonings occur when target species are fed exceedingly high levels of ionophores due to incorrect mixture of premixes or when ionophore antibiotic containing feed is given to monogastric animals. Horses have a very low tolerance for the drugs (1, 3). The LD50 of salinomycin for horses is 0.6 mg/kg bodyweight (2). The ration in this case contained 295 mg/kg, which is even too high for the piglets it was intended for. The recommended dosage as a feed additive for piglets is 30-60 mg/kg feed and 15-30 mg/kg for fattening pigs (2).

AFIP Diagnosis: Heart: Myocardial degeneration and necrosis, subacute, multifocal, moderate, with histiocytic inflammation, Haflinger, equine.

Conference Comment: The contributor provides an excellent review of ionophore toxicosis. Attendees discussed the pathogenesis of ionophore toxicity and how ionophore antibiotics affect sodium, potassium and calcium homeostasis.

Briefly, ionophores are cation transporters that embed in plasma membranes and facilitate the movement of sodium and potassium ions from the extracellular fluid into the intracellular compartment. In response to the influx of sodium and potassium, hydrogen ions are exchanged to the extracellular fluid. Salinomycin, in particular, is a potassium ionophore that interferes with potassium transport across mitochondrial membranes, resulting in low intracellular energy production. The $Na+/Ca2+$ exchange mechanism may also be disrupted allowing a fatal accumulation of intracellular calcium (4). Calcium pumps are responsible for pumping cytosolic calcium into the sarcoplasmic reticulum in anticipation of the next action potential. Increased cytosolic calcium causes myofibril hypercontraction, ATP depletion and, ultimately, failed oxidative phosphorylation resulting in mitochondrial swelling, disruption and cell death (5).

Attendees discussed other causes of cardiomyocyte degeneration and necrosis in a variety of species. A brief list of causes and specific features of each follows:

- Nutritional myopathy (Vitamin E/selenium deficiency): generally more mineralization
- Exertional myopathies: Primarily affects type II fibers. Often results in a striped or streaking appearance of dead and dying myocytes mixed with less severely affected or more normal appearing myocytes
- Muscular dystrophy
- *Cassia* sp. (coffee senna) toxicity: also see diarrhea
- Gossypol toxicity: hepatopathy and cardiomyopathy
- Coyotillo toxicosis (*Karwinskia humboldtiana*): skeletal and cardiac muscle lesions in sheep and goats

- White snakeroot toxicity (*Eupatorium rugosum*): skeletal and cardiac muscle lesions in sheep, cattle, horses and swine
- Hairy vetch toxicosis (*Vicia villosa*): myocardial necrosis, with dermatitis, conjunctivitis, abortion, and granulomatous lesions in multiple organs

Readers are encouraged to review conference 7, case 2 from this year (2005-2006) for a case of Vitamin E deficiency in a brown pelican.

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CASE II – 05-24297 (AFIP 2984846)

Signalment: 8 week-old, male, Miniature Schnauzer/Poodle cross, canine

History: The puppy had a history of lethargy and anorexia. It developed seizures and incessant barking.

Gross Pathology: The stomach contained no ingesta. Intestinal contents were scant and normally formed. The liver was mildly swollen and yellow.

Laboratory Results: An FA test for canine distemper was negative. No bacteria were isolated from the lung, liver, spleen, and kidney. Normal flora was cultured from the intestine.

Histopathologic Description: The lung was congested. Diffuse mineralization of bronchiolar adventitia and alveolar septa was present. Many alveolar spaces were distended. Occasional septa were fragmented. They contained variable numbers ranging from a few to several macrophages and traces of proteinic material. In tissues not submitted, additional findings were severe hepatic lipidosis, brain edema, and mild pancreatitis.

Contributor's Morphologic Diagnosis: Pulmonary mineralization (metastatic calcification)

Contributor's Comment: Pathologic calcification is divided into dystrophic and metastatic on the presence of necrosis or normal tissue, respectively. Metastatic calcification is the result of persistent hypercalcemia. The causes of hypercalcemia are numerous. Some of the more common causes are hyperparathyroidism, vitamin D toxicosis, secondary hyperparathyroidism associated with severe renal disease, and cholecalciferol rodenticide toxicosis. The lesions occur primarily in the interstitial tissues of the lungs, kidney, stomach, and blood vessels. The deposits of calcium salts may be amorphous or crystalline. In the kidney, the deposits occur in basement membranes[1] .

Additional history obtained from the veterinarian and owner revealed that the bitch received four times the recommended dosage of a vitamin mineral supplement. The bitch and nursing puppy were both on premium quality rations. They were in confinement with no exposure to rodenticides. No lesions were observed grossly or microscopically in the thyroid, parathyroid and adrenal glands. Mineralization was detected in the gastrointestinal tract, kidney, or blood vessels. In other cases of hypervitaminosis D evaluated at our laboratory, the lung appears to be the primary site of mineralization. Confinement of mineralization to the lung may be vitamin D dose related. We have attributed sudden death in recently weaned puppies during stress of elective cosmetic surgery and routine examinations to severe lung lesions caused by hypervitaminosis D. We have also seen mineralization of lamina propria of the urinary bladder. The cause of death in this case was canine fatty liver syndrome. This is however a significant pulmonary lesion that could have resulted in respiratory insufficiency.

A presumptive diagnosis of hypervitaminosis D was made based on the history and laboratory findings. Hypervitaminosis D with resultant soft tissue mineralization and/or hypercalcemia has been reported in several species of domestic animals[2,3,4,5,6].

AFIP Diagnosis: Lung: Mineralization, interstitial, multifocal, moderate, with emphysematous change, intra-alveolar edema and histiocytosis, Miniature Schnauzer-Poodle cross, canine.

Conference Comment: As the contributor described, alveolar septa are frequently blunted and fractured and, in many areas, there is failure of the alveoli to collapse. Attendees discussed the mineralization of endothelial basement membranes resulting in alveolar edema and the compensatory removal of the excess fluid via phagocytosis by alveolar macrophages.

In this case, excessive vitamin and mineral supplementation are believed to have resulted in high levels of Vitamin D and a subsequent increase in intestinal absorption of calcium and phosphorus. Because of Mass Law interactions between serum calcium and phosphorus, ionized calcium concentration is reduced as a result of precipitation of calcium and phosphorus (7). The basement membrane of the alveolar septa is a common site for the deposition of calcium and phosphorus precipitate.

Other causes of hypervitaminosis D include the consumption of cholecalciferol containing rodenticides and the ingestion of plants containing vitamin D analogs (e.g. *Solanum malacoxylon*, *Cestrum diurnum*, and *Trisetum flavescens*). The margin of safety for vitamin D is narrow and most animals require only low amounts of supplementation.

Typical findings associated with acute, high dose exposure to vitamin D include gastric, small intestinal and myocardial hemorrhage. In cases of intermittent or chronic low dose exposure there is often mineralization of the lungs, kidneys, stomach and arteries. Chronic low dose exposure can also result in osteosclerosis.

Besides vitamin D toxicosis, there are many other causes of hypercalcemia. A short list of the more common causes encountered in veterinary medicine follows:

- Neoplasia (lymphoma, plasma cell myeloma, adenocarcinoma of the apocrine gland of the anal sac, tumors metastatic to bone)
- Primary hyperparathyroidism (hyperplasia, adenoma, adenocarcinoma) elevated levels of circulating parathormone cause increased intestinal absorption of calcium and phosphorus as well as increased renal activation of vitamin D
- Granulomatous inflammation (canine blastomycosis, bovine paratuberculosis)
- Hypoadrenocorticism (increased tubular resorption of calcium)
- Osteolytic lesions of bone
- Immobilization
- Metabolic acidosis

- Renal failure in horses (rarely canine renal failure associated with familial disease)

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CASE III – 05-12090 (AFIP 2984844)

Signalment: 7 day-old, female, Holstein, bovine

History: Several calves developed severe diarrhea at 2 to 3 days of age. This calf was submitted dead on arrival at the diagnostic laboratory. The calves were treated by the herdsman without an attending veterinarian. They received injectable cephalosporin and sustained release sulfamethazine boluses.

Gross Pathology: The eyes were sunken and there was marked loss of skin elasticity. The ventral portions of the cranial and middle lung lobes were consolidated. The abomasum was partially filled with fluid, clotted milk, and

remnants of medication boluses. Segmental congestion was present in the lower small intestine and colon. Intestinal contents were fluid and reddish brown.

Laboratory Results: No viruses were detected by direct electron microscopic examination of negatively stained feces. E. coli was isolated from the liver and it was resistant to sulfas and most antimicrobial agents.

Histopathologic Description: In the kidney, many collecting tubules near the corticomedullary junction were dilated and contained residual basophilic material often surrounded by degenerate and necrotic epithelial cells. Similar basophilic material was also present in large renal vessels. The material was not birefringent in polarized light. In sections of ileum and colon there were erosions and many denuded villi. Crypts were dilated and contain neutrophils and cellular debris. Blood vessels in the lamina propria were congested. Moderate depletion of gut associated lymphoid tissue was noted. The lung was characterized by acute aspiration pneumonia.

Contributor's Morphologic Diagnosis: Sulfonamide nephrosis

Contributor's Comment: Sulfonamide nephrosis is rare today with use of sulfonamides that are relatively highly soluble at the pH normally occurring in the kidney. In this case, the dosage of sulfamethazine was estimated to be 50% higher than recommended. Additionally, the calf was severely dehydrated and no replacement fluid therapy was used. This product is recommended for calves greater than one month of age that are ruminating.

Sulfonamide nephrosis is attributed to mechanical damage and local toxic effects.[1] In this case, material also crystallized in the renal vessels. This is interpreted to be a result of the severe dehydration and overdosage. Potentiated sulfonamides (not including sulfamethazine) have been associated with idiosyncratic drug reactions in dogs and humans. In dogs, fever, arthropathy, blood dyscrasias, hepatopathy, skin eruptions, uveitis, and keratoconjunctivitis have been reported.[2]

AFIP Diagnosis: Kidney: Tubular degeneration and necrosis, acute, multifocal, moderate, with intratubular amphophilic granular material, Holstein, bovine.

Conference Comment: The contributor provides an interesting case of tubular necrosis. Attendees discussed the cumulative effect severe dehydration, the calve's immature kidneys, and massive overdose may have played in the development of tubular necrosis. The moderator mentioned that sulfonamide precipitate is lost in processing and intreprets the amphophilic, granular material

found within the tubules as sloughed cellular, and perhaps mineralizing, debris forming clumps where the sulfonamide precipitate had once been.

Readers are encouraged to review conference 8 case 4 from this year (2005-2006) for another case of renal tubular necrosis in a rhesus macaque and a review of causes of renal tubular necrosis.

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CASE IV – UFSM-1 (AFIP 2992236)

Signalment: 18 month-old, castrated male, mixed breed, bovine

History: Twenty four yearling calves were placed in a 20-hectare paddock in November 2004. Fifteen hectares of this paddock were occupied by eucalyptus woods. Early in April of 2005, abundant amounts of the mushroom *Ramaria flavo-brunnescens* were growing in the soil within the eucalyptus woods (Fig. 1). The first calf became ill in mid-April and died in April 25, 2005. From this date up to May 5, 2005, 12 calves became sick. The clinical signs presented by the affected calves included anorexia, depression and marked drooling of saliva (Fig. 2); there was a striking smoothness of the dorsum of the tongue, ulcers in the in the dorsum of proximal third of the tongue and loss of the long hairs at the tip of the tail (Fig. 3). In some calves there were ulceration of the proximal third of dorsum of the tongue and others presented hyphema. Other signs variably present were loosening and loss of the hooves and of the corneal portion of the horns (Fig. 4). The clinical course ranged from 8 to 15 days after which affected calves either died or were euthanatized. Six of those were necropsied. Photographs were taken from multiple affected calves in this outbreak.

Gross Pathology: At necropsy, the calf of this report was in poor nutritional state and markedly dehydrated. Multiple small papillomas were distributed in the skin of the head and there was a focal screwworm lesion the labial commissure. Most of the long hairs of the tip of the tail were absent. Fat deposits were depleted and there was serous gelatinous atrophy of the fat in the coronary groove of the heart and around the kidneys. The dorsal surface of the tongue was smooth (atrophy of the lingual papilla) and there is a round ulcer (3 cm in diameter) in the dorsum of proximal third of the tongue (Fig. 5). In the final third of the esophagus the mucosa is ulcerated and covered by fibrinous exudate (Fig. 6). The esophageal lesion tapers off toward the more proximal parts of the esophageal mucosa.

Contributor's Morphologic Diagnosis: (1) Esophagus, fibrinonecrotic esophagitis, marked, with thrombosis and clusters of intralesional bacteria, mixed breed, bovine.

(2) Skin of the tip of the tail (slide not included) follicular telogenization and associated alopecia.

(3) Tongue (slide not included) ulcerative glossitis.

(4) Tongue (slide not included) atrophy of lingual papilla.

Etiological diagnosis: toxic esophagitis

Etiology and name of condition: Poisoning by the mushroom *Ramaria flavo-brunnescens*, "mal do eucalipto" (eucalyptus sickness).

Contributor's Comment: The mushroom *Ramaria flavo-brunnescens*, family Clavariaceae, grows exclusively in soils among Eucalyptus woods. It has been recognized in the south and southeast regions of Brazil[2] and Uruguay[7]. It is also widely distributed in North America and occurs in Australia and China, but is not well known in Europe[2]. In south Brazil, the growth cycle of the mushroom is from mid-autumn to early-winter (April-June), being especially abundant in the warm weather following rainy periods[7].

Spontaneously occurring outbreaks of poisoning due to the ingestion of *R. flavo-brunnescens* have been reported in cattle[7], sheep[6,7] and buffaloes[5] and the toxicosis was experimentally reproduced in cattle and sheep by oral feeding of the mushroom[3,6,8]. Due to the consistent association of the mushroom with Eucalyptus woods, the sobriquet "mal do eucalipto" (Portuguese for "eucalyptus sickness") was coined to designate the toxicosis[7]. The toxicosis was first documented in 1958[1], although its etiology was not established at that time.

The clinical signs and pathological aspects of the natural and experimentally-induced toxicosis in cattle were detailed elsewhere[7,8]. Affected cattle have anorexia, depression and marked drooling of saliva. A striking smoothness of the dorsum of the tongue due to atrophy of lingual papillae is characteristic. There is

often focal to multifocal fibrinonecrotic lesions at the margins of the tongue and similar linear lesions in the esophageal mucosa. Hyphema and corneal opacity lead to blindness in some cases. There is a loosening and loss of the hooves, of the corneal portion of the horns, and of the long hairs at the tip of the tail[7]. The clinical course ranges from 8 to 30 days after which affected cattle recover or death occurs[7,8].

Experimental trials in calves with reproduction of typical lesions have been done, but the lesions were of less intensity than the naturally-occurring disease[3,8]. In a study which was carried out to determine the morphological and pathogenic aspects of the epithelial changes occurring in the hooves, tail, horns and tongue of calves experimentally poisoned by *R. flavo-brunnescens*[3] the histopathologic changes were marked in those structures where hard keratinization occurred and where normally there is a high uptake of sulfur in the form of cystine during the keratinization process. Toxicosis appears to alter the metabolism of sulfur-containing amino acids in keratinocytes, particularly cystine, with resultant strength loss in the molecular structure of hard keratin and loosening of the hooves, hairs and horns, and flattening of the lingual filiform papillae. The lesions of cattle poisoned by *R. flavo-brunnescens* bare similarities to those of chronic selenium poisoning in cattle[4]. It is interesting to notice that in the first report of the disease[1] selenium toxicosis was suspected as a cause, although not confirmed.

Due to erosive and ulcerative lesions in the upper digestive tract, the disease has been confused with viral diseases such as BVD/mucosal disease and foot and mouth disease. The esophageal lesion such as the one of this report needs to be differentiated from those caused by uremia in cattle.

AFIP Diagnosis: Esophagus: Esophagitis, necrotizing, ulcerative, diffuse, severe, with chronic-active inflammation and intra-mucosal thrombi, mixed breed, bovine.

Conference Comment: The contributor provides a thorough summary and wonderful gross photographs of a very interesting toxicosis. Attendees struggled with tissue identification as the mucosa is entirely ulcerated in most sections and replaced by a necrotic coagulum. Discussion centered on what etiology could cause such widespread ulceration, especially since the entire lesion is at the same stage of development. Consideration was given to selenium and thallium toxicosis as well as bovine pestivirus (Bovine Viral Diarrhea-Mucosal Disease) and bovine aphthovirus (Foot-and-Mouth Disease). Some suspected ingestion of a caustic substance, such as an acid, due to the even distribution, stage, and severity of necrosis and ulceration.

As mentioned by the contributor, the hooves can loosen and, in fact, slough. Histological changes occur in the laminar epidermis of the hooves and consist of vacuolization of keratinocytes in the epidermal laminae and tight junctions between the stratum lamellar and stratum medium without increased thickness of either, suggesting absence or incomplete and irregular keratinization of the keratinocytes of the epidermal laminae. Deranged keratinization may induce separation of these two structures, resulting in hoof loss (3). For a more detailed review of the histopathologic changes associated with *Ramaria flavo-brunnescens*, readers are encouraged to review reference number 3.

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