The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2004-2005

CONFERENCE 12
12 January 2005

Conference Moderator: LTC Mark Mense, DVM, PhD, MBA, DACVP
Department of Veterinary Pathology
The Armed Forces Institute of Pathology
Washington, DC 20306

CASE I – 04-514 (AFIP 2941213)

Signalment: 6 year-old Rhodesian Ridgeback, neutered male, dog (*Canis familiaris*).

History: This dog initially presented with a chief complaint of vomiting. Over a period of several days its condition declined, and it developed lingual ulcers. Six days after initial presentation the dog developed tachypnea, a cough and excessive salivation. It fatigued easily and developed cyanosis after exercise. Therapy included fluids, antibiotics and oxygen via a nasal line. The dog's condition worsened over the next day. Due to its declining condition the dog was euthanatized. A postmortem examination was carried out at the veterinary hospital (on the day following the death of the animal) and tissues were submitted to the Veterinary Diagnostic Laboratory at Oregon State University for examination.

Laboratory Results: Chemistry panels showed elevated lipase, at > 6000. BUN and creatinine were not elevated. Multiple blood gases were performed over the last 3 days before euthanasia. Despite therapeutic oxygen insufflation pO2 fell from initial values of 170 mm Hg to 69, while pCO2 rose from initial values of 42.4 to 64 mm Hg.

Contributor's Morphologic Diagnosis: Diffuse pulmonary fibrosis with hemorrhage and edema and multifocal hyperplasia of type II pneumocytes.

Contributor's Comment: No gross description of lesions was available. A full set of tissues was submitted for histopathologic examination. Lesions were confined to the lung and kidney, which are presented in this submission.
In the lung there is diffuse hemorrhage and pulmonary edema, with alveoli containing eosinophilic proteinaceous fluid. Macrophages are present in many

alveoli. There is diffuse thickening of alveolar walls and many alveoli are lined discontinuously by large, hypertrophied cells, presumably hyperplastic type II pneumocytes. There is increased collagenous tissue on the pleural surface. Pleural mesothelial cells are hypertrophied and most appear to be ciliated. There is vascular congestion and some pulmonary vessels are thrombosed (in some sections). Trichrome stains reveal moderate, diffuse interstitial fibrosis. In the kidney there is diffuse congestion and scattered acutely necrotic tubules within the cortex.

This is one of seven cases presented to the Veterinary Diagnostic Laboratory within a period of about one week with similar clinical histories. All affected dogs had visited a particular park in Portland, Oregon. All had been seen to either ingest some material or to have vomited shortly after the visit. All developed gastrointestinal signs and progressive dyspnea. Some had elevated BUN and creatinine levels early in the clinical course, although that was not true for this case. Based on clinical signs and histopathology a presumptive diagnosis of paraquat intoxication was made. This was confirmed by the detection of paraquat in the urine of some of the cases.

Paraquat (1,1′-dimethyl-4,4′-bipyridillium dichloride; CAS # 1910-42-5) is a contact herbicide and its use is restricted in the US. Despite its restricted use, poisoning of pet animals remains a problem and in this series of cases is presumed to have been malicious.

The lung is the primary tissue affected in paraquat toxicity.[1] However, other tissues, including the kidney, liver and thymus also may be affected. In this series of cases lesions were consistently present in both lung and kidney. Experimentally paraquat produces pulmonary edema and hemorrhage with necrosis and loss of both type I and type II pneumocytes. Alveolar capillary endothelium is spared. These lesions may be followed by hyperplasia of type II cells. The extent of loss of pneumocytes and, thus, the capacity for regenerative hyperplasia of type II cells are dose dependent. In time diffuse interstitial fibrosis develops. The pulmonary lesions of paraquat toxicity are not unique and are similar to those induced by other agents that cause diffuse alveolar injury.

The targeting of the lung in paraquat toxicity is explained by its selective uptake in that tissue. Uptake is an energy dependent process that can occur against a concentration gradient. It utilizes the same biological uptake mechanisms that transport other polyamines, such as putrescine and spermidine. Toxicity is considered to result from cyclic oxidation-reduction reactions of the paraquat molecule, which result in the generation of large quantities of reactive oxygen species, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH′). Toxicity is dependent on molecular oxygen and tissue damage is

probably mediated, at least in part, by lipid peroxidation. Depletion of cellular NADPH may also play a direct role in toxicity.

Lesions in this case are consistent with paraquat toxicity. The discontinuous appearance of the hyperplastic type II pneumocytes is most likely explained by the dose received by this dog. Severe depletion of type II cells by the toxin would be expected to result in "patchy" hyperplasia. Renal lesions are relatively acute compared to those in the lung and the relative contributions of direct paraquat renal toxicity and hypoxia are not clear. The presence of cilia, or rudimentary cilia, on pleural mesothelial cells has been reported in hyperplastic, metaplastic or neoplastic conditions affecting these cells.

AFIP Diagnoses: 1. Lung: Pneumonia, interstitial, hemorrhagic, diffuse, moderate, with type II pneumocyte hyperplasia, multifocal interstitial fibrosis, and emphysematous change, Rhodesian Ridgeback, canine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, minimal, with glomerular sclerosis.

Conference Comment: The contributor provides a thorough overview of paraquat toxicity in dogs. Conference attendees also considered other differential diagnoses for pulmonary edema and hemorrhage including warfarin toxicity, oxygen toxicity, nitrogen toxicity, ionizing radiation, and a neoplastic induced clotting deficiency.

Both paraquat and diquat are bipyridyl herbicides that have caused numerous deaths in humans and various animal species. Intoxication involves either carelessness or malicious poisoning and has been reported in cattle, sheep, horses, pigs, poultry, dogs, rabbits, and fish. Clinical signs and lesions vary with the species, dose received and route of administration.[2]

The most common route of administration for most species is ingestion. Cattle often present with neurological signs including dullness, incoordination, and staggering that progress to prostration, convulsions, coma and death. Oral lesions have also been reported in horses after grazing on freshly sprayed pasture. The lesions described in poultry and swine are similar to those seen in dogs, with dyspnea and pulmonary edema noted in both species. In sheep, intravenous administration of paraquat resulted in a dose-dependent decrease in glomerular and tubular function. The lesions in fish include massive desquamation of gill epithelial cells.[2]

In humans, accidental or intentional ingestion is the most common route of exposure; however, inhalation and dermal exposure have also been reported.

Acute toxicity results in severe damage to all organ systems and death within 24 hours. Chronic toxicity results in progressive pulmonary fibrosis and pulmonary edema resulting in dyspnea and asphyxia.[3]

Contributor: Oregon State University, Veterinary Diagnostic Laboratory, 105 Magruder Hall, Corvallis, Oregon
http://www.vet.orst.edu/

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CASE II – 98-1713 (AFIP 2694678)

Signalment: Tissue from a bovine.

History: Four of 21 cattle died suddenly during July 1998, while one recovered on a small farm close to Bloemfontein in the Free State Province of South Africa. When the farm was visited two days after the mortalities occurred, *Cestrum* spp. plants were found in an adjacent camp to which the cattle had gained access through a break in the fence. The tops of the plants had died off due to the effects of frost but at the bottom of the plants there were tufts of luscious green foliage which showed evidence of having been browsed.

Necropsies were performed on 2 of the 4 animals by the provincial state veterinarian and a range of organ specimens from the second case were submitted in unbuffered formalin to the Onderstepoort Veterinary Institute for histopathological examination. The plant involved was identified as a *Cestrum* sp. at the local herbarium.

Gross Pathology: Both of the animals necropsied presented with multiple hemorrhages throughout the serosal surfaces as well as on the endo- and epicardium, swollen and friable livers with marked hepatic lobular accentuation, hemorrhage and edema of the wall of the gall bladders, and colonic and cecal hemorrhages.

Contributor's Morphologic Diagnoses: 1. Necrosis and hemorrhage, coagulative, severe, acute, liver with hepatocyte vacuolation and presence of intracellular and loose intrasinusoidal segrosomes, midzonal, severe, acute.
2. Bile ductular proliferation, subacute, portal, moderate.

Contributor's Comment: As the tissues were fixed in unbuffered formalin, they became very fragile and several moderate to severe artifactual lesions are present in many sections:

- Acid hematin pigment accumulation
- Tears along edges
- Folds
- Presence of pink fluid in lumens of larger vessels
- Tearing and unraveling of walls of larger vessels

The liver revealed extensive periacinar coagulative necrosis and hemorrhage. Midzonal hepatocytes were extremely swollen and vacuolated, and many contained intracytoplasmic, single or multiple bright eosinophilic droplets of varying size, presumably cytosegrosomes. A few segrosomes were also present loose within the sinusoids in this region.

The history of ingestion of *Cestrum* spp. plants and the macroscopical- and histopathological changes of multiple hemorrhages and an acute hemorrhagic hepatosis correspond to the lesions described for acute to subacute cases of *Cestrum* poisoning.[1,2] The most likely differential diagnoses for this lesion in South Africa include acute seneciosis and acute algal toxicity.[1] No *Senecio* spp. plants could be found on the property and algal toxicity was unlikely in this incident as no algal blooms were noted in the stream from which the cattle drank, nor were mortalities reported in other groups of cattle drinking from the stream, either up- or downstream from the affected farm.

Species of the genus *Cestrum* are evergreen, ornamental shrubs which have been imported into South Africa from South America where they occur indigenously. In Africa suspected cases of poisoning of livestock due to *C. laevigatum, C. parqui* and *C. aurantiacum* have been reported from South Africa, Zimbabwe, Kenya and the island of St. Helena. In South Africa, outbreaks of poisoning by *C. laevigatum* have traditionally occurred in the Kwazulu-Natal Province.[1] The outbreak described above is one of 3 outbreaks attributable to *Cestrum* spp. toxicity, all of which occurred in the Free State Province of South Africa, an area not traditionally associated with *Cestrum* toxicity in South Africa. The *Cestrum* spp. are known for their ability for rapid establishment and spread, particularly along river banks and in recent years it appears that in South Africa, these plants have spread beyond the Kwazulu-Natal Province northwards into the Free State and Gauteng Provinces despite all 3 species having been proclaimed as weeds in South Africa.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, centrilobular and midzonal, diffuse, acute, severe, bovine.

Conference Comment: Hepatoxicity may be acute or chronic, but often is somewhere in between, and the agent's effect is usually a function of dose-rate. Nonetheless, agents that most often cause acute hepatotoxicity in cattle include: blue-green algae, *Cestrum* sp., cocklebur, poison peach, and sawfly larvae. Agents that most often cause chronic hepatotoxicity in cattle include: aflatoxin, pyrrolizidine alkaloids, sporidesmin, lantana, and nitrosamines.

There are several species of the genus *Cestrum*. In the United States, pathologists are most familiar with *Cestrum diurnum* as a cause of enzootic calcinosis in cattle. The species known to be hepatotoxic include: *C. parqui*, *C. laevigatum*, and *C. aurantiacum*, all of which cause similar hepatic disease. However, in the field, speciation within the genus is often uncertain due to hybridization.

Cestrum spp., the ink-berry plants, cause acute hepatotoxicity in South America, southern and central Africa, and Australia. Cattle are most frequently affected; however, sheep, goats, and fowl are susceptible. The young leaves and unripened berries are the most toxic parts of the plant. The toxin, an atractyloside, has been recently identified. The histological lesions are consistently centrilobular (periacinar) and are identical to lesions caused by poison peach (*Trema aspera*) and cocklebur (*Xanthium pungens*).

Contributor: Onderstepoort Veterinary Institute, Pathology Section, P.O. Box 12502, Onderstepoort, South Africa
http://www.arc.agric.za/institutes/ovi/main/divisions/path2.htm

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CASE III – S04-66 (AFIP 2948647)

Signalment: One-year-old, male, mixed-breed dog, canine.

History: The dog had clinical signs of gradual weight loss, vomiting, anorexia, and was fed with commercial dog food for several months.

Gross Pathology: The submitted samples were formalin fixed kidney, stomach and spleen. Prominent, white, powder-like deposits were observed on the kidney sample. The other samples had no significant gross lesions.

Laboratory Results: Moderate increases in WBC (15.2×10^3 /μl), neutrophils (95% of WBC), markedly increased BUN (407 mg/dl) and creatine (18.3 mg/dl).

Contributor's Morphologic Diagnosis: Kidney: Interstitial nephritis, fibrosing, lymphocytic, diffuse, severe, subacute, with severe tubular necrosis, mineralization, intratubular and pelvic oxalate crystals, canine.

Contributor's Etiologic Diagnosis: Oxalosis associated nephrotoxicosis.

Contributor's Comment: The lesions in the kidney were characterized by prominent interstitial fibrosis, crystal accumulation, atrophy, calcification, and mild lymphocytic infiltration. The outline of the kidney was rough. Multifocally renal tubular lumina had accumulation of light yellow crystals. These crystals were round with many lines radiating from their center and had a birefringent appearance with polarized light. Affected tubular epithelium was degenerate to necrotic with mineral deposition on some tubular basement membranes and in the tubular epithelium. Some glomerular atrophy was also noticed. The interstitium had prominent fibrous connective tissue proliferation. The Alizarin red S stain was positive in the mineralized areas. Histopathologically, this case has marked renal tubular injury, prominent connective stroma proliferation and combined with the results of clinical pathology, renal failure is suspected clinically. The finding of renal tubular lumina with radially arranged crystal deposition could be induced by many causes; the most probable is calcium oxalate deposition. In a recent outbreak (March 2004) estimated at 3000 cases of canine renal failure in Taiwan, there was no sex, age or breed predilection. The ages of affected dogs ranged from several months to adult. The owners of the dogs told clinicians that their pets only ate a certain brand and type of commercial dry dog food. After eating this dog food for several days, dogs were presented with various degrees of renal failure. Finally, most of the affected dogs had gradual weight loss.

Oxalate is a simple organic carboxylic acid, which is excreted as calcium oxalate by many fungi. Oxalate also occurs as byproducts in various plant tissues, in urine,

and in mantles of certain bivalves. In dogs, the most common cause of nephrotoxicosis is exposure to ethylene glycol. Ethylene glycol is rapidly absorbed from the gut, with peak plasma concentration achieved 2 to 3 hours after ingestion. It is predominantly metabolized in the liver in the following sequence: ethylene glycol to glycoaldehyde to glycolate to glyoxylate. Glyoxylate is converted to several final metabolites, including oxalate, glycine, and formate. Oxalate then binds with calcium and forms a soluble complex which is filtered by glomeruli. As water is reabsorbed by the tubules and the pH of the filtrate decreases, calcium oxalate precipitates and forms crystals. This results in nephrosis, and hypocalcemia if enough calcium is complexed. Other sources of oxalate may come from plant origin foods. Plants which may contain toxic amounts of oxalate are *Halogeton glomeratus* (halogeton), *Sarcobatus vermiculatus* (greasewood), *Rheum rhaponticum* (the common garden rhubarb), *Oxalis cernua* (soursob), *Rumex* spp. (sorrel and dock). In tropical and subtropical areas, certain grasses that are cultivated widely (genera of *Cenchrus*, *Panicum* and *Setaria*), accumulate large amounts of oxalate, and have been associated with renal oxalosis in cattle and sheep and with skeletal disease in the horse, the latter due to conditioned calcium deficiency.

The fungi *Aspergillus niger* and *A. flavus* can produce large quantities of oxalates on feedstuffs. Large doses of ascorbic acid have caused oxalate nephrotoxicosis in humans and in a goat; ascorbic acid is a metabolic precursor of oxalate. Primary hyperoxaluria, a rare inherited metabolic condition, occurs in humans, cats and perhaps dogs (Tibetan spaniels). Pyridoxine (vitamin B_6) deficiency and methoxyflurane anesthesia can also cause renal oxalosis.

Calcium oxalate is precipitated in the renal tubules during the process of elimination. A fatal outcome may occur from renal insufficiency and uremia after the earlier symptoms have abated. Conversely, recovery is possible, with blood urea levels slowly subsiding after about one month. Cystitis and urethritis may be a part of this syndrome.

AFIP Diagnosis: Kidney: Tubular degeneration, necrosis, and loss, diffuse, moderate, with interstitial fibrosis, and multifocal tubular mineralization and oxylate crystal deposition, mixed-breed, canine.

Conference Comment: The contributor provides a thorough overview of the most common causes of oxalosis, as well as the pathophysiology of calcium oxalate deposition in the kidney. As mentioned by the contributor, ethylene glycol is the most common cause of oxalosis in dogs and cats.

Intoxication with ethylene glycol is usually seasonal and coincides with the changing of antifreeze solutions in the spring and fall. Many antifreeze solutions are composed of up to 95% ethylene glycol. Toxicosis is common due to the sweet taste and very low minimal lethal dose (1.5 ml/kg for cats and 6.6 ml/kg for dogs). Ethylene glycol itself is of low toxicity. It is rapidly absorbed from the gastrointestinal tract and most is excreted unchanged in the urine. Only a small percentage is metabolized in the liver to the primary toxic metabolite glycolic acid (glycolate).[1,5]

The clinical signs of depression, ataxia, and osmotic diuresis develop within a few hours of ingestion of ethylene glycol. The nervous signs are due to the effects of aldehydes and severe metabolic acidosis, which develops due to accumulation of lactic acid, glycolate, and glyoxylate. Pulmonary edema, tachypnea, and tachycardia develop sequentially over the next 12 hours and are likely due to the systemic effects of the osmotic diuresis. If the animal survives for 1-3 days after ingestion, acute renal failure develops as a result of renal tubular damage caused by glycoaldehyde, glycolic acid, glyoxylic acid, and oxalate. The oxalate precipitates with calcium in the renal tubular lumina resulting in intrarenal obstruction, and tubular epithelial degeneration and necrosis. Using polarized light, the microscopic identification of large numbers of birefringent crystals in renal tubules is virtually pathognomonic for ethylene glycol toxicity in dogs and cats.[1,5] Alizarin red S stain will stain calcium oxalate crystals red at a pH of 7.0, but not at a pH of 4.2. In contrast, calcium phosphate and calcium carbonate stain red at a pH of both 7.0 and 4.2. In addition, calcium oxalate can be confirmed by its insolubility in 2M acetic acid, since both calcium phosphate and calcium carbonate are soluble.[6]

Addendum: It was recently brought to our attention by Dr. Wayne Corapi at Texas A&M University College of Veterinary Medicine that the intratubular crystals in this case are histomorphologically similar to the melamine-containing crystals recently identified in the kidneys of cats and dogs that were fed pet food on the Menu Foods recall list manufactured between December 3, 2006 and March 6, 2007. The similarities between the March 2004 outbreak of an estimated 3,000 cases of canine renal failure in Taiwan associated with a specific brand and type of commercial dry dog food and the March 2007 pet food-associated nephrotoxicity in the United States are striking. In both cases, pets that were fed a specific brand of food died of renal failure. Lesions in both outbreaks were characterized by marked tubular degeneration and necrosis, with many intratubular birefringent crystals. Melamine-containing crystals are round, pale-brown, and birefringent with many lines radiating from their center as seen in this case. Conversely, oxalate-containing crystals are smooth and plate-like with a blue tinge due to a prismatic effect when polarized. Additionally, histochemical stains performed at the AFIP are consistent with melamine-containing crystals. The Alizarin red S stain is staining

mineralized areas as stated by the contributor. Specifically, it is staining areas of calcium phosphate deposition and not the melamine-containing crystals. Upon reviewing this case and performing special stains, we concur with Dr. Corapi and believe this is a case of pet food-associated nephrotoxicosis with melamine-containing crystals.

Contributor: Animal Technology Institute Taiwan, Division of Animal Medicine, P.O. Box 23, Chunan, Miaoli, Taiwan

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CASE IV – UFSM-2 (AFIP 2940460)

Signalment: 7 year-old, castrated male, crossbred, bovine (*Bos taurus*).

History: The owner reported that for the last 60 days this draft ox had chronic bloat, regurgitation of food and loss of weight. The rumen had been punctured several times during this period to alleviate the bloat. At clinical examination the mucous membranes were pale, there was marked bloat, and subcutaneous emphysema extended from the ruminal area to the shoulder, lumbar and cervical areas on the left side. This ox was from an area where squamous cell carcinoma (SCC) of the upper digestive tract is endemic in cattle. There was heavy infestation of bracken fern (*Pteridium aquilinum*) in the pasture where this ox was

kept. A clinical diagnosis of SCC of the upper digestive tract was made and the ox was euthanatized due to a poor prognosis.

Gross Pathology: Depletion of body fat deposits and pale mucous membranes were observed at necropsy. The extensive subcutaneous emphysema of the left side of the body was interpreted as being secondary to the ruminal punctures. Small papillomas (2-5 mm in diameter) were present in the surface of epiglottis, soft palate and pharynx. There was 1 liter of serous, clear yellow, partially coagulated fluid in the peritoneal cavity. The rumen was markedly distended by gas. The distal portion of the esophagus was distended (approximately 3 times its normal diameter), firm and contained a dark green plug of packed ingesta (grass) in its lumen. The ruminal contents were markedly dry and a mass, formed by multiple nodules (some edematous), protruded from the ruminal mucosa. The ruminal wall, just at and immediately distal (8 cm) to the esophageal entrance, was thickened (approximately 5 cm) and its cut surface was firm, gray-white, and speckled with yellow (keratinization) and dark brown (necrosis) foci. This mass caused stenosis of the ruminal entrance. There was hypertrophy of the muscular layer of the walls of rumen and reticulum. Clear translucent, gelatinous edema was noted in the abomasal folds and multiple nodules due to *Oesophagostomum* sp. were present in the wall of the small intestine. There was atrophy of the left liver lobe and a dry, friable (sand-like) gallstone was located within the gallbladder.

Contributor's Morphologic Diagnoses: 1. Rumen, squamous cell carcinoma, well differentiated, with dystrophic mineralization, bovine, 7-year-old, castrated male, crossbred, bovine.
2. Rumen, squamous epithelial papillomas, bovine, 7-year-old, castrated male, crossbred, bovine (slides not included).
3. Pharynx and esophagus, squamous epithelial papillomas, bovine, 7-year-old, castrated male, crossbred, bovine (slides not included).

Contributor's Comment: *Pteridium aqulinum* (bracken fern) is a plant of worldwide distribution that causes intoxication in livestock in several regions of the world. Bracken fern is one of the more important toxic plants for cattle in Brazil, causing severe losses in the southern and southeastern regions. The plant also grows in other Brazilian regions including the states of Bahia, Amazonas, Acre, Mato Grosso and Pernambuco.[8]

There are three clinical manifestations of the poisoning by bracken fern and they were recently reviewed.[8] When cattle graze large amounts of the plant (between 10 and 30 g/kg/bw/day or more) for relatively short periods of time (weeks to few months, generally until the weight of ingested plant equals the weight of the animal) bone marrow aplasia develops, which results in an acute, usually fatal, clinical disease characterized by fever, hemorrhagic diathesis, thrombocytopenia

and neutropenia. When cattle ingest less than10 g/kg/bw/day for longer periods (one year or more), a chronic disease characterized by intermittent hematuria is observed. This form is known as enzootic hematuria and is related to the development of tumors in the urinary bladder. Hemangioma is frequently found in these cases but several other types of benign and malignant neoplasms may occur.[7] Enzootic hematuria eventually leads to chronic anemia and death. As in this case, the development of SCC in the upper digestive tract of cattle is the third clinical manifestation related to the ingestion of bracken fern.[8] The occurrence of SCC in the digestive system of cattle is rare[6] and is virtually not observed in cattle grazing pastures where bracken fern is absent.[8] The clinical course associated with the SCC of the upper digestive tract in cattle is rather chronic (months to years) and the deleterious effects of the tumor are mainly mechanical and related to the interference with feeding and rumination. This leads to extreme malnutrition. Affected cattle are usually 5 years-old or older. It is presumed that the development of the SCC occurs when cattle ingest small amounts of bracken fern for extended periods (years) of time. The neoplasm occurs in one or more of the following anatomical sites in the bovine digestive tract:[5,8] base of the tongue, esophagus, cardia and rumen. Clinical signs include coughing, regurgitation of food, bloat, diarrhea and progressive loss of weight which eventually culminate in death. The clinical signs vary depending on the location of the tumor, for example, coughing is related to tumors located in the base of the tongue and as in this case, bloat is related to tumors located in the esophagus or cardia. Metastasis occurs in some cases, mainly to the regional lymph nodes and lungs. However, SCC of the rumen may metastasize to the liver through the portal circulation.

A recent survey carried out by our lab that included necropsies of 14 cattle with SCC of the upper digestive tract (yet unpublished data) revealed multiple neoplasms in 57% of the cases and a single mass in 43%. The main affected anatomical sites were base of tongue (5/14), pharynx (3/14), epiglottis (6/14), proximal esophagus (3/14), distal esophagus (2/14), middle esophagus (3/14), entrance of the rumen ("cardia") (3/14) and rumen (2/14). Metastasis were observed in 50% of the cases and were identified in retropharyngeal (4/14), mediastinal (2/14), gastric/ruminal (3/14), hepatic (1/14), paravertebral (1/14) and mesenteric (1/14) lymph nodes; in the lungs (1/14) and in the liver (1/14). Usually a few or several papillomas were observed in the proximities of the malignant masses (SCC). Histological evidence of transition between benign (papilloma) and malignant (SCC) growths were occasionally found. The consistent finding of papillomas in the sites where SCC develop led to the suspicion that bovine *Papillomavirus* (BPV) has a role in the pathogenesis of bracken fern associated SCC in the upper digestive tract of cattle and this was later confirmed.[3,4]

There are six subtypes of BPV (BPV-1-6) that induce lesions with specific characteristics and distributions. SCC of the upper digestive tract in cattle is

associated with prolonged ingestion of bracken fern and concomitant infection with BPV-4; whereas BPV-2 is associated with bladder tumors and enzootic hematuria in cattle feeding on bracken fern invaded pastures.[3,4] The BPV induced papillomas are benign growths that occasionally undergo malignant transformation due to genetic or environmental factors. In cattle, infection of the upper digestive tract with BPV-4 leads to the formation of papillomas which eventually regress (within approximately one year) due to a host-derived cell mediated immune response.[2] However, in cattle grazing bracken fern, which contains immunosuppressants, the papillomas persist for longer periods and may be transformed to carcinomas.[4] *In vitro* studies indicate that bracken fern is the co-carcinogen of BPV-4 in the pathogenesis of the SSC of the digestive tract in cattle. In addition, the flavonoid quercetin, a well known mutagenic compound of bracken fern, synergizes with BPV-4 in malignant transformation of papilloma cells.[3] The combination of increased viral BPV-4 transcriptional activity, the failure of cell arrest at G1 and the malfunction of the tumor suppressor protein p53 are thought to be the events contributing to transformation of the cell.[1,3] In fact, a strong epidemiological correlation between bracken fern consumption, high incidence of persistent papillomas, and SCC of the upper digestive tract has been noted. The progression from papilloma to SCC was experimentally reproduced in cattle fed bracken fern.[4]

In sheep, bracken fern causes progressive retinal degeneration referred to as bright blindness and in horses and pigs it has been described as causing a nervous disease due to thiamin deficiency.[5] However despite the high incidence of bracken fern poisoning in cattle, none of these conditions in sheep, pigs or horses were documented in Brazil. The feeding of horses with high amounts of bracken fern failed to induce the neurological disease (Gava, personal communication).

Tumors of the digestive tract are reported in humans consuming the crosiers and rhizomes of bracken fern. In southeastern Brazil, a case-control study showed a 3.4 and 3.45-fold increased relative risk respectively for developing esophageal and gastric cancer in people who ingested bracken fern. Ptaquiloside, another carcinogenic substance isolated from bracken is present in the milk of cows fed this plant and it has been demonstrated that milk from these cows causes tumors in mice.[5]

AFIP Diagnosis: Rumen (per contributor): Squamous cell carcinoma, crossbred, bovine.

Conference Comment: The contributor provides a thorough overview of bracken fern toxicosis and the three clinical syndromes recognized in cattle, which are dependent on the dose and duration of ingestion. As mentioned by the contributor,

bovine *Papillomavirus* (BPV) has a role in the pathogenesis of bracken fern associated SCC in the upper digestive tract of cattle.

Many viruses, both DNA and RNA, can cause neoplasia in humans and animals. The AFIP comments on conference 5, case III (October 2004) includes a list of common virally induced neoplastic diseases. At the cellular level, one of two consequences may follow infection with potentially oncogenic DNA viruses. If the infection is productive, the cells produce infective virus particles and the cells are lysed in the process. If the infection is non-productive, the cells survive and may be transformed, with introduction of specific gene sequences or gene products. Oncogenic DNA viruses contain specific viral oncogene products that are responsible for neoplastic transformation. The viral oncogene products are often virus specific with particular host cell targets. From the list below, it is apparent that many oncogenic DNA viruses share common mechanisms of cellular transformation mediated by their specific oncogene protein products. A prominent shared mechanism is the interaction with and functional inactivation of the tumor-suppressor gene products, Rb and p53, both of which play critical roles in processes responsible for cellular homeostasis, such as the cell cycle and apoptosis.[9]

Virus	Viral Oncogene Product	Cellular Target
Adenovirus	E1A (289aa)	Rb
	E1A (243aa)	Rb
	E1B (495aa)	p53
	E1B (175aa)	unknown
Polyomavirus	Large T-antigen	Rb
	Middle T-antigen	src
	Small T-antigen	unknown
SV40	Large T-antigen	Rb, p53
	Small T-antigen	unknown
Papillomaviruses		
BPV-1	E5	PDGF receptor
HPV-16	E6	p53
	E7	Rb

Contributor: Universidade Federal de Santa Maria, Departamento de Patologia, Santa Maria, RS, Brazil

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gnature Authenticated by Appro
Approved by: Shelley P Honnold,
dnesday, 09 February, 2005 at 19

Shelley P. Honnold, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.