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CASE I –06-12468 (AFIP 3026808).

Signalment: Adult, female, mule deer (*Odocoileus hemionus*).

History: Animal was captured by net gun, blindfolded and transported to a staging area where it was anesthetized and received a tonsillar biopsy and a radio collar. The anesthesia was reversed and the animal was then transported back to the original capture site where it was released. Seven days later it was noticed to be severely lame in the hind limbs and was euthanized by gunshot.

Gross Pathology: Prior to necropsy it was noted that the lower hind limbs could be flexed without resistance from the extensor muscles. Deer was in poor body condition with no internal stores of fat. There was marked subcutaneous hemorrhage about the lower aspects of both tibiae. The entirety of both gastrocnemius muscles were swollen, dark red, necrotic, friable and torn from the insertions. Other muscles appeared unremarkable except for the extensor carpi radiali, tongue and deep pectoral muscle, which were streaked with areas of pallor. In addition, the extensor carpi radiali were edematous and tightly swollen within the fascial sheaths.

Histopathologic Description: Approximately 60-80% of the myofibers are degenerative or necrotic in the examined sections. Muscle fibers are characterized by sarcoplasmic hypereosinophilia and flocculation, loss of cross-striations, and nuclear pyknosis, karyorrhexis and karyolysis (necrosis) and varying stages of degeneration (myocyte swelling, pallor). Necrotic myocytes are separated, surrounded and replaced by a low to moderate number of macrophages, lymphocytes and rare neutrophils, hemorrhage and edema. Occasionally

macrophages infiltrate individual myofibers. Mineralization is not noted in the examined section. A low number of *Sarcocystis* sp. cysts are present.

Contributor's Morphologic Diagnosis: Skeletal muscle: Degeneration and necrosis, multifocal to coalescing, severe, subacute with lymphohistiocytic myositis and interstitial hemorrhage.

Contributor's Comment: Capture myopathy (exertional rhabdomyolysis) as a disease syndrome is an important cause of morbidity and mortality of handled wild animals. The syndrome is characterized by damage to both skeletal and cardiac musculature and has been seen in a great variety of species including mammals (herbivores and carnivores) and birds. Lesions are associated with extreme exertion and shock related to pursuit, immobilization, restraint, handling and transport. Clinical signs associated with this disease syndrome are varied and include depression, reluctance to stand and ataxia. These signs may be displayed immediately following the triggering episode or may be delayed in their appearance for a period of days or weeks.[1]

The pathophysiology of capture myopathy is reported to be related to both vasogenic-neurological shock and metabolic acidosis.[2] Handling stimulates discharge of the sympathetic nervous system and subsequent release of catecholamines. Exhaustion of the sympathetic nervous system leads to decreased vascular tone, pooling of blood in viscera, decreased venous return and decreased cardiac output. Inadequate nutrient delivery and increased demand for energy caused by exertion rapidly depletes cells of their readily available ATP stores. In order to meet their metabolic requirements cells begin anaerobic metabolism which in turn leads to the accumulation of lactic acid and subsequent metabolic acidosis. This exacerbates the already compromised cardiac output and systemic blood pressure. Decreased function of the sodium-potassium pump due to inadequate ATP levels leads to sodium influx and cellular swelling. Increased intracytoplasmic calcium levels activate numerous enzymes and cause the progression from cellular injury to cell death.[1,2] Release of intracellular potassium can lead to acute cardiac arrest. If animals survive the initial stages, the release of large amounts of myoglobin coupled with hypoxia can lead to renal tubular damage and animals may die subsequent to acute renal failure.

Treatment is problematic in that efforts to administer treatment may induce or compound capture myopathy. Traditional attempts at treatment have involved antioxidant administration and free radical scavengers. Dantrolene sodium has been used to treat humans with malignant hyperthermia and in some cases of capture myopathy. The basis for this is to attempt to prevent calcium release from the sarcoplasmic reticulum. Its use is hampered by expense and the difficulty in administration of intravenous fluids to wild animals in a field situation.[2]

AFIP Diagnoses: 1. Skeletal muscle: Degeneration and necrosis, multifocal to coalescing, severe, with histiocytic inflammation, satellite cell proliferation, and interstitial hemorrhage, mule deer (*Odocoileus hemionus*), cervid.

2. Skeletal muscle: Sarcocysts, multifocal, few.

Conference Comment: The contributor provides a concise summary of the pathophysiology of capture myopathy. Acquired myopathies generally fall under one of three categories: toxic (e.g., Gossypol, ionophore, and *Cassia* sp.), nutritional (e.g., vitamin E/selenium deficiencies), and exertional (e.g., azoturia, tying-up, porcine stress syndrome, capture myopathy).

Predisposing factors for capture myopathy include species, method of capture, high environmental temperature, physical condition of the animal, and nutritional status of the animal. Species with high metabolic rates are more susceptible to capture myopathy. More aggressive methods of capture cause a greater incidence of capture myopathy. High environmental temperatures do not allow for heat dissipation generated by physical exertion and stress. Deficient levels of vitamin E and selenium predispose to a higher incidence of capture myopathy with more severe clinical signs.[2]

Renal lesions in exertional myopathy are associated with renal ischemia, secondary to shock, and myoglobin. Histomorphologic features include tubular epithelial swelling, degeneration, and necrosis; orange to red granules in tubular epithelial cells; granular, myoglobin, and proteinaceous casts; tubular regeneration; and interstitial edema.[1]

Clinical pathology abnormalities in cases of exertional myopathy include increases in CK, AST, LDH, ALT, BUN, and Cr; hyperphosphatemia; hyperkalemia; myoglobinemia; myoglobinuria; and a stress leukogram.[1,3]

Conference attendees discussed the concepts of monophasic and multiphasic skeletal muscle lesions. Monophasic lesions are all in the same stage of degeneration, regeneration, or necrosis, and imply a single insult (e.g., a single dose of a toxin or a strenuous episode). Multiphasic lesions are in multiple stages of degeneration, regeneration, or necrosis and imply an ongoing insult such as chronic or intermittent toxin exposure or vitamin E/selenium deficiency.

Muscle injuries can also be classified as reversible (metabolic, toxic, nutritional) or irreversible (heat, intense inflammation, infarction). The myofiber basal lamina

remains intact and viable satellite cells remain in reversible muscle injury allowing for regeneration. In irreversible muscle injury, large areas of satellite cells are destroyed, and healing occurs by fibrosis. If the insult to muscle disrupts the myofiber basal lamina but does not damage the satellite cells, attempts at regeneration are ineffective resulting in the formation of muscle giant cells.[4]

Attendees also discussed differential diagnoses for skeletal muscle necrosis in various species to include the following: Exertional rhabdomyolysis, equine polysaccharide storage myopathy (EPSSM), nutritional myopathy (vitamin E/selenium deficiency), ischemic myopathy due to anesthesia, plant toxicity (*Cassia* sp., ionophore toxicity (monensin), clostridial myositis (malignant edema, botulism), malignant hyperthermia-like syndrome, and *Streptococcus*-associated myopathy.

Readers are encouraged to review WSC Conference 7/ Case II from the 2005-2006 academic year for a case of hypovitaminosis E in a brown pelican.

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CASE II - A06-12006-1a (AFIP 3026261).

Signalment: 11-mo-old, male, Rocky Mountain elk, *Cervus elaphus nelsoni*.

History: Two farm-raised elk on pasture stopped coming to the feeder 3 weeks before presenting for lameness and thickening of the limbs distal to the elbows and hocks. Pulmonary nodules were evident radiographically in both elk and a radiographic diagnosis of hypertrophic osteopathy was made for metacarpal and metatarsal bones. Tuberculosis was suspected and the elk were euthanized.

Gross Pathology: In both elk, the carpal, metacarpal, tarsal, metatarsal bones, and the radii, ulnae, and tibiae had smooth thickening of cortical bone, particularly on dorsal and proximal aspects. Areas of bone lysis, sometimes filled with caseous exudate, were present in the left metatarsus and a distal phalanx of one elk.

Both elk had encapsulated pulmonary nodules that occupied most of the parenchyma in one animal. The nodules contained abundant caseous exudate. Tracheobronchial, retropharyngeal, mediastinal, and prescapular lymph nodes were variably enlarged up to 15 cm in diameter and contained caseous exudate. Caseated nodules up to 4 mm in diameter were also in the myocardium, kidney, and spleen of the elk with lytic and caseated bone lesions; this elk also had lingual ulcers.

Laboratory Results: Clinical pathologic abnormalities included mild anemia and hyperglobulinemia. *Aspergillus fumigatus* was cultured from lung of both elk post mortem. Surveillance testing for chronic wasting disease was negative on formalin-fixed brain. Cultures of lung and lymph node were negative for *Mycobacterium*. Virus isolation from pooled tongue and spleen was negative. Fecal flotation and Baermann funnel technique for lungworms were negative.

Histopathologic Description: Pulmonary granulomas are encapsulated and contain myriad hyphae. Fungal hyphae are 5-8 μm thick with parallel walls, septate, and dichotomously branched. They form palisades at the periphery of granulomas and are more disorganized and fragmented in the center. Fungal hyphae in lung and in other tissues were strongly positive by immunohistochemistry for *Aspergillus* spp. A band of degenerated neutrophils and eosinophils with fewer epithelioid macrophages and rare multinucleated giant cells surrounds the caseous centers of granulomas. Degenerated leukocytes and fungal hyphae are also observed in bronchioles and small bronchi. Some affected airways are ectatic or expand into early (nonencapsulated) granulomas. Interlobular septa are expanded by fibrous connective tissue. Fibrosis of interalveolar septa is evident in some lobules; in these lobules, alveolar spaces are often filled with fibrin that is mixed with neutrophils, macrophages and fibroblasts.

The bony section is prepared from a cross-section of the large metatarsal bone. The periosteum is markedly thickened by parallel and perpendicularly oriented trabeculae of immature bone with abundant osteoblastic activity and much less osteoclastic activity.

Contributor's Morphologic Diagnoses: 1. Granulomatous pneumonia with intralesional fungal hyphae.

2. Periosteal new bone formation (periosteal hyperostosis), metatarsal bone.

Contributor's Comment: Fungal granulomas were also found histologically in tongue, lymph nodes, myocardium, kidney, and bone marrow. Histologic findings in both elk were consistent with pulmonary and systemic aspergillosis and periosteal hyperostosis (hypertrophic osteopathy).

Pulmonary aspergillosis is the most common fungal infection observed in farmed elk in our laboratory. The source of infection in this case was undetermined, but inhalation of spores from contaminated feed was suspected.

Hypertrophic osteopathy has been described in humans, numerous domestic species and in one roe deer from Germany.[1,2,3] The pathogenesis is poorly understood, but hypertrophic osteopathy is usually associated with space-occupying lesions in the thorax, neoplastic or inflammatory processes in the lung, and neoplasms of abdominal organs, particularly the urinary bladder.[1,2] Hypertrophic osteopathy in our case was most likely the result of numerous pulmonary granulomas.

AFIP Diagnoses: 1. Lung: Granulomas, multifocal and coalescing, with myriad hyphae, Rocky Mountain elk, *Cervus elaphus nelsoni*, cervid.

2. Bone, metatarsus (per contributor): Hyperostosis, periosteal, diffuse, severe.

Conference Comment: *Aspergillus* spp. are opportunistic pathogens that cause serious disease in debilitated or immunocompromised animals or in animals on prolonged antibiotic treatment. Transmission occurs by inhalation of spores resulting in pneumonia. *Aspergillus* commonly invades blood vessels and can spread hematogenously to various organs. The necrotizing vasculitis can lead to thrombosis and infarction. Some species of *Aspergillus*, such as *A. flavus* and *A. parasiticus*, produce highly toxic and carcinogenic aflatoxins. The Aspergillus species most commonly associated with disease in animals include *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus*.[4]

Histologically, the hyphae are 3-6 um wide, parallel walled, regularly septate, with dichotomous acute angle branching. Spores usually do not form in tissues, but can be seen on surfaces exposed to air (air sacs, trachea).[4] Conidial heads or fruiting bodies are composed of a golden-brown dome-shaped terminal vesicle covered by phialides from which chains of conidia are produced.[5]

Conference attendees briefly discussed lesions caused by *Aspergillus* sp. in various species. Aspergillosis is most common and severe in young chicks and turkey poults that become infected by inhaling spores in contaminated bedding resulting in pneumonia/air sacculitis/tracheitis ("brooder pneumonia"). Captive penguins are especially susceptible. In mammals, aspergillosis is seen in most species with mycotic dermatitis, keratitis, and pneumonia being the most common manifestations. However, dissemination to other organs can occur.

Hypertrophic osteopathy occurs in humans and in domestic animals with the dog being most commonly affected. Periosteal new bone formation is usually confined to the diaphyseal region of the distal limbs with the radius, ulna, tibia, and metatarsals most commonly involved. The bones of the upper limbs and phalanges are relatively spared. Lesions typically regress if the inciting cause is removed. Causes of hypertrophic osteopathy in the dog include:[1,6,7]

1. Endocarditis
2. *Dirofilaria immitis*
3. Rhabdomyosarcoma of the urinary bladder
4. Esophageal granulomas and tumors associated with *Spirocerca lupi*
5. *Hepatozoon americanum*
6. Intrathoracic neoplasia or inflammation

Hypertrophic osteopathy is associated with ovarian neoplasms in the horse.[1,6]

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CASE III –2 (AFIP 3026185).

Signalment: Age unknown (adult), Female, European brown hare, *Lepus europaeus*.

History: The hare came from a group of 250 that developed multiple cutaneous tumors out of 1000 farmed game hares.

Gross Pathology: Skin tumors were 1-3 cm in size, often alopecic and ulcerated (Fig. 2) and most commonly located on the ears (Fig. 3) and limbs (Fig. 4). On cut section, lesions had a homogeneous, white surface (Fig. 5). After 4 to 6 weeks the lesions showed progressive regression in most animals. In some cases, the tumors detached from the skin surface leaving bleeding ulcers that evolved into alopecic scars.

Laboratory Results: Electron microscopy from tissue samples identified typical biconcave poxviral particles (Fig. 1). The virus was isolated, injected in embryonated eggs and produced typical lesions in chicken embryos (white-yellowish pocks). EM from these lesions confirmed the presence of poxviral particles in the embryos.

Histopathologic Description: Epidermis is characterized by erosions, intracellular edema, and serocellular crusts (not in all sections). Dermo-epidermal detachment is also evident (not in all sections). Dermis and adnexa are almost completely substituted by an unencapsulated, poorly demarcated neoplasm extending to the deep borders. The tumor is composed of irregular to interlacing bundles of atypical, variably sized, spindle cells embedded in minimal fibrous stroma

containing elevated numbers of capillaries. Cells have generally indistinct cell borders, abundant clear cytoplasm often containing bright, irregular, amorphous to granular, PAS positive (Fig. 6) cytoplasmic eosinophilic poxviral inclusions. Inclusions are occasionally present in the follicular epithelium (not in all sections). Mitoses range from 0-2 per HPF. Among neoplastic cells anisokaryosis and anisocytosis are prominent and bi- to multinucleated giant cells are present.

Contributor's Morphologic Diagnosis: Haired skin, dermis: dermal fibroma with intracytoplasmic eosinophilic poxviral inclusions, European brown hare, *Lepus europaeus*.

Contributor's Comment: Leporipoxviruses are implicated in the development of Myxomatosis and Shope fibromas in rabbits and fibromatosis in hares.[1] Recently, a form of mucocutaneous dermatitis in Mountain hares (*Lepus timidus*) from Finland, once supposed to have a *Treponema* spp. aetiology seems also to be caused by a leporipoxvirus.[2] The viruses in all these diseases seem to be transmitted by biting insects/arthropods and or direct contact.

Fibromatosis (poxviral hare fibromas) is a disease described in wild and reared game hares.[1,3,4] The disease is caused by a leporipoxvirus which is antigenically related to the Shope fibroma virus of rabbits.[1] Fibromatosis is associated with high morbidity but low mortality and is characterized by single to multiple protruding, dermal tumors mostly located on ears and legs.[3] In 2003, the re-emergence of fibromatosis in farmed game hares has been reported in Italy.[3]

Hare fibromas are grossly and microscopically similar to lesions described in cottontail rabbits with Shope fibroma.[1] Both diseases are characterized by the development of cutaneous, usually benign tumours, characterized by proliferation of dermal fibroblasts, epithelial hyperplasia and intracytoplasmic eosinophilic inclusions within both epithelial and mesenchymal cells.[1] In adult rabbits, the tumors are localized and are characterized by spontaneous regression[1] whereas in neonatal rabbits and immunocompromised adults they may become invasive and may develop into malignant fibrosarcomas. Rabbits bearing Shope fibroma generally develop a cell-mediated immune response and virus-neutralizing antibodies with cytotoxic and cytolytic activity whose kinetics parallel the tumors' development and regression.[5] Extracts of Shope fibroma tumours have been found to contain a second virus, antigenically virtually identical to SFV, but with a different behavior demonstrated *in vitro* and *in vivo* experiments.[1,6] This virus has been called Malignant rabbit fibroma virus.[1,6] Malignant rabbit fibroma virus represents a lethal tumorigenic rabbit poxvirus derived from a recombination between Shope fibroma virus and Myxomavirus.[1,6] Malignant rabbit fibroma virus induces fibroma-like tumours that disseminate extensively and do not regress. In these lesions inclusion bodies are not detectable. MRV severely reduces both T

and B cell function with immunodepression.[6] This feature seems to account for its aggressive behavior.

Leporipoxvirus is a member of the Poxviridae family, which comprises a large family of double-stranded DNA viruses that are able to infect both vertebrates (Chordopoxvirinae) and insects (Entomopoxvirinae). After cell infection, contrary to other DNA viruses, Poxviruses do not enter the nucleus of infected cells but establish a virus factory in the cytoplasm that represents the site of viral transcription and DNA replication. Distinct viral inclusions localized within endoplasmic reticulum start to be evident 6 hours post-infection. With the beginning of virus assembly, endoplasmic reticulum disappears and viral antigens can be found throughout the cytoplasm and the cell membrane.

Poxvirus genome encodes the majority of the enzymes required for its own replication and is mostly autonomous from host functions compared to any other animal virus group. The Poxvirus genome consists of two groups of genes with different locations: the first group consists of genes that are required for viral transcription, genome replication and assembly of progeny virions (essential genes). Essential genes are clustered within the central region of the linear genome. The second group of genes are not essential for growth *in vitro* but are required for the replication of the viruses in their natural hosts since they define the host range, tissue specificity and virulence.[7] This second group includes genes that encode epidermal growth factor like (EGF-like) substances. The EGF-like factors of three poxviruses have been isolated: vaccinia growth factor (VGF), Myxoma virus growth factor MGF) and, Shope fibroma virus growth factor (SFGF).[8] SFGF is a broad-specificity ligand that activates all ErbB-1 containing receptor combinations, VGF binds primarily to ErbB-1 homodimers, and MGF only to heterodimes of ErbB-2 and ErbB-3. The growth factors of the three Poxviruses display unique patterns of specificity to ErbB receptor tyrosin kinases. Although viral growth factors bind to the respective receptor with an affinity that is up to 1000-fold weaker than that of the homologous mammalian ligand, their proliferative signals are more intense than their mammalian counterparts.[8] As an additional mechanism, most poxviruses are able to inhibit apoptosis. Shope fibroma virus has been demonstrated to inhibit apoptosis via binding to the host DNA.[7]

AFIP Diagnosis: Haired skin: Atypical mesenchymal proliferation, dermal, focally extensive, marked, with epithelial ballooning to reticular degeneration, epithelial and mesenchymal eosinophilic cytoplasmic inclusion bodies (hare fibroma), European brown hare (*Lepus europaeus*), lagomorph.

Conference Comment: The contributor provides an excellent overview of hare fibromatosis, Shope fibroma virus of rabbits, and malignant rabbit fibroma virus.

Conference attendees briefly reviewed how to distinguish Shope fibroma virus lesions from those of Myxoma virus. Typical gross findings associated with myxomatosis include multiple, subcutaneous, mucoid to gelatinous masses, especially on the face and around body orifices. Additionally, mucopurulent conjunctivitis and subcutaneous edema are observed.[9,10] Gross findings associated with Shope fibromatosis include circumscribed firm flattened nodules primarily on the legs and feet that may also occur on the muzzle, periorbital, and perineal areas. Metastasis to abdominal viscera and bone marrow may occur in young rabbits. Key histomorphologic features of myxomatosis include proliferation of large, stellate mesenchymal cells ("myxoma" cells) separated by a loose myxomatous matrix. The epithelium overlying the masses may be hyperplastic and/or degenerate (ballooning degeneration). Large eosinophilic intracytoplasmic inclusions may be present in epithelial cells of the epidermis or conjunctiva. Lymphoid depletion of the spleen is also a common finding. Key histomorphologic features of Shope fibromatosis include proliferation of fibroblasts with a mixed inflammatory cell infiltrate. Epithelial hyperplasia is also present with rete pegs projecting into the fibroblastic mass. In contrast to myxomatosis, eosinophilic intracytoplasmic inclusion bodies can be found in the fibroblasts and the epithelial cells of the epidermis overlying the mass.[10]

Attendees also discussed squirrel fibroma virus (Leporipoxvirus) which is also believed to be transmitted by biting arthropods. Gross lesions range from solitary to numerous firm cutaneous nodules over the entire body and/or marked epidermal thickening around the eyes and ears. Internal organs such as the lungs, lymph nodes, liver, and kidney may also be involved. As with rabbit fibroma virus, the lesions often spontaneously regress. The microscopic lesions resemble those of rabbit fibroma virus with proliferation of spindle cells, epithelial hyperplasia, and large prominent eosinophilic intracytoplasmic inclusion bodies in mesenchymal and epithelial cells.[11]

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CASE IV - S-64564 (AFIP 2812385).

Signalment: Dog, *Canis familiaris*, Beagle, male, 5.5 months of age.

History: A male beagle dog arrived with its cohorts from an established vendor and was held in routine quarantine. Standard prophylactic procedures by the vendor included routine vaccination (canine distemper, adenovirus, parainfluenza, parvovirus, Leptospirosis, Bordetella, and rabies) and deworming (ivermectin and pyrantel pamoate). After receipt and quarantine for 1 week, this dog and its cohorts received deworming followed several days later by re-immunization. One day following re-immunization, this dog was unexpectedly observed in lateral recumbency. The dog was hypothermic, cyanotic, and dyspneic, with blood in the nose and mouth. The dog died while blood was being drawn for diagnostic assessments (CBC and blood culture).

Gross Pathology: The carcass had blood staining about the muzzle, mouth, and nostrils. The thoracic cavity contained approximately 50 mL of red sanguinous fluid. The pericardium was thickened and reddened, with adhesions to the diaphragm. Thymic lymph nodes were mottled and firm. The trachea contained excess pink froth and the lung lobes had multiple to locally extensive, red to dark, often firm foci.

Laboratory Results: A CBC on the blood from this moribund dog had slightly elevated erythroid parameters (RBC, Hgb, Hct) and leukopenia with a degenerative left shift. Multiple lung and tracheal swabs, lung tissue, and thoracic fluids were submitted for aerobic and anaerobic bacteriology. *Escherichia coli* (*E. coli*) was isolated from a specimen of lung tissue, from the lung surface, pleural fluid, and trachea, and from blood culture. This *E. coli* isolate was typed as O type 6 with cytotoxic necrotizing factor 1. *Streptococcus* Group G was also isolated from lung surface, pleural fluid, and trachea. Viral isolation and florescent antibody microscopy for adenovirus and parainfluenza virus in lung specimens were negative.

Contributor's Morphologic Diagnosis: Lung, hemorrhagic pneumonia

Contributor's Comment: *Escherichia coli,* of the bacterial family Enterobacteriaceae, is a normal inhabitant of the lower intestinal tract of all warm-blooded animals, is a well-known enteric pathogen, is present in most cases of canine pyometra, and is a common urinary tract pathogen.[3] Recently, this organism has also been reported to be the most commonly isolated bacteria from dogs with lower respiratory tract disease.[1] In contrast, this organism is rarely identified in cases of human pneumonia.[2] The α- and γ-hemolytic Streptococci can be isolated from the lower respiratory tract of normal dogs, which makes their implication as primary respiratory pathogens problematic.[1]

Although *E. coli* O type 6 has been sporadically reported from veterinary diagnostic case materials, this type is most often associated with urogenital tract infections. To our knowledge, this is the first isolation of an *E. coli* O type 6 from a case of fatal hemorrhagic pneumonia in the dog.

AFIP Diagnosis: Lung: Bronchopneumonia, necrotizing, acute, multifocal, severe, with hemorrhage, and myriad bacteria, Beagle, canine.

Conference Comment: The surface antigens of *E. coli* on which serotypes are based include the following:

1. **O (somatic)** – determined by the sugar side chains on the lipopolysaccharide molecule
2. **K (capsular)** – polysaccharide
3. **H (flagellar)** – proteinaceous
4. **F (fimbrial)** – proteinaceous; adhesive function

E. coli are classified into one of three general categories: (1) gastrointestinal commensals, (2) intestinal pathogenic strains, and (3) extraintestinal pathogenic strains. Most strains of *E. coli* are gastrointestinal commensals and do not typically cause disease in healthy immunocompetent animals. The extraintestinal pathogenic strains are further subdivided based on specific pathogenic mechanisms and include enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroaggregative, and diffusely adherent *E. coli*. The extraintestinal pathogenic *E. coli* have been associated with urinary tract infections, meningitis, septicemia, and pneumonia in humans and animals. In dogs, extraintestinal pathogenic *E. coli* is most frequently implicated in urinary tract infections and has also been associated with mastitis, pyometra, otitis, prostatitis, skin disease, and cholecystitis.[3,4]

Serotypes O4 and O6 have been isolated from dogs with hemorrhagic pneumonia. Both serotypes have the virulence factors *alpha* hemolysin and cytotoxic necrotizing factor 1 (CNF1) which are frequently identified in infections caused by extraintestinal pathogenic *E. coli*. Cytotoxic necrotizing factor producing strains are also referred to as necrotoxic *E. coli*.[4]

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