The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

CONFERENCE 16
15 February 2006

Conference Moderator: Dr. Tabitha Viner, DVM, Diplomate ACVP
Smithsonian National Zoo
Washington D.C.

CASE I – 05-0027 (AFIP 2992349)

Signalment: 11-year-old, female common trumpeter (*Psophia crepitans*)

History: Possible exposure to snow and cold 01/26/05, with suspected frostbite. Treated symptomatically since 01/27/05. Right leg appears to have dry necrosis up to proximal metatarsus. Left leg appears to have necrosis of all toes to metatarsal-phalangeal joint, which is swollen. Due to poor prognosis, euthanasia performed on February 16.

Gross Pathology: There are very mild, scabbed abrasions bilaterally on the medial carpi. All four toes of the left leg are dry, hard, and interphalangeal joints are not movable. The left metatarsal-phalangeal joint is distended twice that of normal and the dorsal and lateral surfaces have several abrasions with crusted blood and erythema. The cranial half of the plantar surface is hard. The joints of the second and fourth digits move slightly, but those of the first and third do not articulate. All four toes of the right leg are dry, hard, and all interphalangeal and the metatarsal-phalangeal joints are not movable. The diameter of the distal ¾ of the tarsometatarsus is smaller than that of the proximal 5 cm and that of the left leg. The distal portion is dry and hard. The transition between the hard tissue and the normal proximal tissue is an irregular 0.5-1.0 cm band of swollen, whitish tissue.

Laboratory Results: Blood taken at clinical presentation showed an increased CPK (4930 U/L; ref range 793-1008 U/L). Subsequent bloodwork showed an increasing leukocytosis with a heterophilia, lymphopenia, and monocytosis, with decreasing hematocrit.

Histopathologic Description: Focally extensive bone necrosis. Large granuloma with necrotic debris and bacteria at center, surrounded by multinucleated giant

cells and macrophages with several fungal hyphae. Large area of infiltrate of lymphocytes, fewer heterophils. Multiple multinucleated giant cells dissecting under the necrotic layer of skin. Muscle atrophy, multiple ossified tendons. Viable skin with edema present below the necrotic epidermis in areas of slide.

Contributor's Morphologic Diagnosis: Leg (bone, skin, tendon): Necrosis, focally extensive, with secondary surface bacterial and yeast infection, common trumpeter (*Psophia crepitans*), gruiform.

Contributor's Comment: Cold injury is becoming increasingly more prevalent with the expanding epidemic of homelessness and the increased number of people involved in outdoor winter sports. Historically, military personnel were the people most susceptible to conditions promoting cold injury to the extremities. One manifestation of cold injury, trench foot, was seen commonly in the wars of the 20th century. This condition is caused by immersion of the feet in cold water at temperatures between 33 and 50 degrees Fahrenheit for longer than 12 hours (2). Another manifestation, frostbite, occurs when the tissues are exposed to temperatures below freezing. Ninety percent of human frostbite cases occur in the hands and feet and the pathophysiology of these changes is similar to that of thermal burns and ischemia/reperfusion injury (1).

Clinically, frostbite can be classified as superficial or deep (1,3). Only the skin and subcutaneous tissues are involved in superficial frostbite, whereas deep frostbite additionally involves the muscles, tendons and bones. The pathogenesis of frostbite involves three mechanisms that may take place simultaneously (1,3). One mechanism involves direct cellular damage by freezing. Ice crystals form in the tissues extracellularly which damage the cell membrane. This causes fluid to leave the cells resulting in cellular dehydration. As freezing continues, ice crystals form within the cells, expanding and causing the cells to burst.

Another mechanism involves the vascular response to cold. When body temperature begins to fall, vessels go through cycles of vasoconstriction and vasodilation until the temperature becomes so low that vasoconstriction becomes constant. This causes local hypoxia, acidosis and increased blood viscosity. This eventually leads to thrombosis of the vessels and ischemic injury to the tissues.

Local thrombosis and endothelial damage trigger the release of prostaglandins PGF2alpha and thromboxane A2. These potent inflammatory mediators precipitate further vasoconstriction and thrombosis. During rewarming, the tissue levels of these mediators increases. Subsequently, repeated freezing and thawing of tissues causes progressively more damage.

The result of these physiologic mechanisms of damage does not fully manifest until 22 to 45 days after the initial injury and can be seen as a distinct line of demarcation between hard, gangrenous tissue and viable tissue (3). The clinical course of frostbite in this common trumpeter took place over 21 days. Manifestation of visibly damaged tissue had progressively moved up the leg during this time. Additionally, histology showed that tissue that appeared to be viable was, in fact, affected and undergoing degenerative and necrotic changes.

AFIP Diagnoses: 1. Bone, skin and tendon, distal leg: Necrosis, coagulative, diffuse, common trumpeter (*Psophia crepitans*), avian.
2. Bone, proximal leg: Periosteal and endosteal new bone growth, focally extensive.
3. Skin, proximal leg: Epidermal hyperplasia and hyperkeratosis, diffuse, marked with focally extensive ulcer and superficial fungi.
4. Leg, proximal: Synovitis and cellulitis, heterophilic and histiocytic, multifocal, moderate.

Conference Comment: Attendees discussed the presence of necrotic bone and the lack of viable osteocytes within lacunae. There is periosteal and endosteal new bone growth within some sections of the less severely affected proximal bone. The presence of fungal hyphae within sections of the necrotic epidermis and dermis (best seen with GMS stain) was attributed to opportunistic invasion and growth following tissue death.

The contributor provides an excellent review of the pathogenesis of frostbite injury. Interestingly, birds with webbed feet have a vascular network, the rete mirabile, which transfers heat from the arteries in their legs and feet to the veins in an attempt to help prevent heat loss in the distal limbs and maintain the temperature of the feet closer to the ambient temperature.

As mentioned by the contributor, prostaglandins (PGF2alpha) and thromboxanes (TXA2) are responsible for the cycle of vasodilation and vasoconstriction that occurs in blood vessels undergoing cold injury. More specifically, PGF2alpha and TXA2 are arachadonic acid metabolites produced by the cyclooxgenase pathway. PGF2alpha is responsible for vasodilation and is an important potentiator of edema. TXA2 is a potent platelet aggregating agent and causes vasoconstriction (4).

Contributor: Smithsonian's National Zoological Park
http://nationalzoo.si.edu/default.cfm?ref=index.htm

References:
1. Murphy JV, Banwell PE, Roberts AHN, McGrouther DA: Frostbite: Pathogenesis and Treatment. J of Trauma: Injury, Infection and Critical Care, **48**:171-178, 2000
2. Pulla RJ, Pickard LJ, Carnett TS: Frostbite: An Overview with Case Presentations. J of Foot and Ankle Surgery, **33**:53-63, 1994
3. Reamy BV: Frostbite: Review and Current Concepts. JABFP, **11**:34-40, 1998
4. Kumar V, Abbas, AK, Fausto N: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 68-70. Saunders, Philadelphia, PA, 2005

CASE II – 04-0309 (AFIP 2992355)

Signalment: Northern water snake *(Nerodia sipedon)*, adult, female

History: It was in the exhibit with three cagemates. Apparent regurgitation observed one week previously.

Gross Pathology: The body of this northern water snake is in fair to poor nutritional condition as evidenced by scant amount of fat storages. There is a generalized marked pallor of all organs, oral cavity and muscle. The lungs are markedly pale and there are five, up to 6.0 x 0.3 x 0.3 cm, white, rounded parasites attached to the mucosa (Fig. 1). There are many, up to 6 x 1 x 1 mm, dark, C-shaped parasites with a thickened white wall in multifocal hepatic serosal areas surrounded by mild hemorrhage (Fig. 2). There is the same type of parasites in the renal parenchyma, in fewer numbers and with hemorrhagic areas up to 10 mm in diameter (Fig. 3). There is digested fish in the stomach. There is yellowish, soft digesta in the intestinal tract. The gallbladder is filled with green bile. The splenopancreas is diffusely pale. The thyroid is enlarged (12 x 8 x 3 mm). The brain is fixed with the head. The heart, bones, trachea, ovaries, adrenal glands, eyes, skin and ears are unremarkable.

Laboratory Results: Heart blood culture: Salmonella IV 45:g251
Parasite identification: *Kiricephalus coarctatus*

Histopathologic Description: Liver: Multifocally, there are numerous up to 12 mm in diameter cross section and oblique sections of pentastomid parasites that are expanding the hepatic capsule and parenchyma, and are surrounded by minimal inflammatory reaction composed of eosinophils, heterophils, lymphocytes, macrophages and hemorrhage. There is multifocal degeneration and necrosis with numerous macrophages and few eosinophils, heterophils and foreign body type multinucleated giant cells. Diffusely, there is mild fatty change.

Contributor's Morphologic Diagnosis: Liver, hepatitis, eosinophilic, subacute, mild, multifocal, with many pentastome nymphs, macrophages, lymphocytes, heterophils and diffuse lipid degeneration.

Contributor's Comment: This animal has pentastomes in several organs and probably was infected by ingestion of an intermediate host, such as a mouse or rat. Parasites are present within the lung, as a primary site (Johnson-Delaney, 1996) as evidenced in Fig. 4; and in the oral cavity (Fig. 5) due to the life cycle, where eggs are coughed up by the host, swallowed and passed in the feces (Johnson-Delaney, 1996). The parasites are found in the kidney, causing mild inflammation and edema (Fig 6), within the serosa of the oviduct causing hemorrhage (Fig. 7) and within the biliary duct with minimal inflammation.

The species is identified as *Kirecephalus coarctatus,* and this genus is the most common found in colubrids, such as this snake (Johnson-Delaney, 1996). The sepsis with Salmonella IV 45:g251 is probably a secondary bacterial infection due to the many organs affected by these parasites (Murray, 1996).

The infestation of this snake was probably caused by ingestion of the intermediate host, a mouse or rat. Crocodiles and lizards can be infected by ingestion of fish (Flach et al, 2000; Adams et al., 2001). Pentastomes have zoonotic potential as humans are incidental hosts.

AFIP Diagnoses: 1. Liver: Pentastome nymphs, multiple, Northern water snake (*Nerodia sipedon*), reptile.
2. Liver: Granulomas, multiple.

Conference Comment: The contributor provides a beautiful example of pentastome nymphs in the hepatic parenchyma of a snake. Attendees noted that the granulomas were scattered throughout the hepatic parenchyma but were not exclusively focused on the nymphs. Consideration was given to the possibility that the granulomas were preexisting in this snake from an unrelated inflammatory lesion.

Pentastomes, also known as "tongueworms", are a phylum of highly specialized arthropod parasites. Most pentastomes parasitize reptiles but a few genera occur in birds and mammals. The typical lifecycle of pentastomes starts with the adults in the respiratory tract of the reptile host where they mate and the female lays her eggs. The eggs are coughed up by the reptile, swallowed, move through the digestive tract and are expelled in the feces. In the environment, the eggs develop into larvae, are ingested by the intermediate host (usually a bird or rodent) and

develop into nymphs. The intermediate host is ingested by a reptile and the nymphs are released, burrow through the intestine and migrate to the lungs where they develop into adults. The larval stage of the parasite is capable of parasitizing a wide range of hosts (5,6,7).

Morphologic features which help identify pentastomes in tissue section include two pairs of hooks surrounding the mouth, striated musculature, a thin cuticle, and a multicellular intestine bordered by two acidophilic glands for most of the pentastome's length. The most characteristic and unique feature of pentastomes are the sclerotized openings in the body wall. The openings appear as eosinophilic rings when stained with H&E; however, they are black and easily visible when stained with Movat pentachrome stain (5).

A short list of pentastomes found in reptiles follows (7):

- *Armillifer* sp. in pythons and vipers
- *Porocephalus* sp. in boas and rattlesnakes
- *Kiricephalus* sp. in colubrid snakes
- *Sebekia* sp. in crocodilians
- *Raillietiella* sp. in lizards and snakes

Additionally, *Linguatula serrata* occurs in the nasal and paranasal sinuses of dogs and cats, where it causes bleeding, catarrhal inflammation, and some impediment to respiration (6).

For a simple algorithm to help identify parasites in tissue sections, readers are encouraged to review WSC 15, case 3, 2005-2006.

This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP.

Contributor: Smithsonian's National Zoological Park
http://nationalzoo.si.edu/default.cfm?ref=index.htm

References:

1. Adams, L, Isaza, R, Greiner, E. Fatal pentastomiasis in captive African dwarf crocodile hatchlings *(Osteolaemus tetraspis). J Zoo and Wildlife Med.* 2001; 32: 500-502.
2. Flach, EJ, Riley, J, Mutlow, AG, McCandlish, IAP. Pentastomiasis in Bosc's monitor lizards *(Varanus exanthematicus)* caused by an undescribed *Sambonia* species. *J Zoo and Wildlife Med.* 2000; 31: 91-95.
3. Johnson-Delaney, C. Reptile zoonoses and threats to public health. In: Mader, DR, *Reptile Medicine and Surgery.* Philadelphia, PA: WB Saunders, 1996: 27-28.

4. Murray, MJ Pneumonia and normal respiratory function. In: Mader, DR, *Reptile Medicine and Surgery.* Philadelphia, PA: WB Saunders, 1996: 403.
5. Gardiner CH, Poynton SL. An Atlas of Metazoan Parasites in Animal Tissues. Washington DC: Armed Forces Institute of Pathology; 1999.
6. Bowman DD: *Georgis'* Parasitology for Veterinarians, 6th ed. pp.232-233. W.B. Saunders, Philadelphia, PA, 1995
7. Nash H: Pentastomes: Respiratory Parasites of Reptiles. Available at: http://www.peteducation.com/article.cfm?cls=17&cat=1831&articleid=2754

CASE III – 6611/0411 (AFIP 2983550)

Signalment: Snowy owl, adult, unknown sex (*Nyctea scandiaca*).

History: There was an increase in mortality of captive owls in a zoological garden. Different species of the family Strigidae were affected. Formalin fixed organs (liver, spleen, kidney, lungs) of a snowy owl (*Nyctea scandiaca*) and the carcass of a long-eared owl were submitted for pathological examination.

Gross Pathology: The long-eared owl was in a moderate nutritional condition. The liver was green-brown, discoloured and contained multiple miliary yellowish-white foci. Similar lesions, but more confluent, were found in the spleen. The content of the intestine was fluid and of a dark reddish-brown colour. The lungs were congested and within the kidneys there was a moderate deposition of white, dry, crystalline masses.

Laboratory Results: Virology: Herpesvirus was detected in cell culture.
Microbiology: Unspecific.

Histopathologic Description: In the liver, there are multiple randomly distributed foci of coagulation necrosis. Hepatocytes near the foci show degeneration with pyknotic nuclei and swollen, homogenous, eosinophilic cytoplasm. Also in these areas few amphophilic intranuclear inclusion bodies (Cowdry type A) can be seen in hepatocytes. Only very few inflammatory cells (lymphocytes) are present. Vessels and sinuses are moderately congested.
Additionally, the spleen shows similar lesions but more severe with confluent foci of necrosis and intranuclear inclusion bodies in reticular cells.

Contributor's Morphologic Diagnoses: Liver, hepatitis, necrotizing, acute, multifocal and randomly distributed, moderate with intranuclear pale amphophilic

inclusion bodies in hepatocytes, snowy owl (*Nyctea scandiaca*), avian, etiology consistent with herpesvirus strigis infection.
Spleen (not submitted): Splenitis, necrotizing, acute, multifocal to coalescing, severe with intranuclear amphophilic inclusion bodies in reticular cells.

Contributor's Comment: The histopathological features are consistent with Hepatosplenitis infectiosa strigum, an infection with the herpesvirus strigis which was confirmed by cell culture.

The current taxonomy of the family of avian herpesviruses includes three subfamilies: alpha-, beta- and gammaherpesvirinae. Herpesvirus strigis is a betaherpesvirus. All avian herpesviruses are presently grouped into 11 different serotypes based on results of cross neutralisation tests. Herpesvirus strigis and herpesviruses from falcons and pigeons are serologically closely related (5). Herpesvirus strigis is a pathogen for several species of owls in the order Strigiformes. Natural infections have been observed in the Eagle Owl (*Bubo bubo*), Long-eared Owl (*Asio otus*), Great horned owl (*Bubo virginianus*) and the Snowy owl (*Nyctea scandiaca*). Experimental susceptibility was established in the Little Owl (*Athene noctua*) and the Tengelmalms Owl (*Aegolinus funereus*). Also the Old World Kestrel (*Falco tinnunculus L.*) was found susceptible to the virus. On the other hand the Tawny Owl (*Strix Aluco*) and the Barn Owl (*Tyto albo Scopoli*) seem to be resistant (2).

Owls affected by Hepatosplenitis infectiosa strigum show nonspecific signs of illness like apathy, lassitude and anorexia. Usually the birds die after two to five days of illness. In captivity mortality approaches 100 per cent and any birds surviving infection should be considered to be latently infected and likely to shed virus. Another supposed source of infection is pigeons which are fed to owls in captivity (1, 3, 4).

Gross pathological findings are numerous necrotic foci in the liver, spleen and bone marrow. Additionally diphtheroid lesions can be found in the mucosa of beak, oesophagus, proventriculus, and intestine. Histologically, these organs show necrotic lesions with intranuclear inclusion bodies of Cowdry Type A, particularly in hepatocytes and with lower frequency in reticular cells of the spleen (1, 4).

AFIP Diagnosis: Liver: Hepatitis, necrotizing, acute, random, moderate, with eosinophilic hepatocellular intranuclear inclusions, Snowy owl (*Nyctea scandiaca*), avian.

Conference Comment: The contributor provides an excellent review of herpesvirus infections in owls. In general, the alphaherpesviruses typically produce focal lesions in the skin and mucosa of the respiratory or genital tracts. An important characteristic of herpes virus infections is their ability to become latent with intermittent or persistent recrudescence and viral shedding. Alphaherpesviruses are typically latent in nerves; betaherpesviruses in secretory glands, lymphoreticular organs and the kidney; and gammaherpes in lymphoid tissue. Below is a list of avian herpesviruses and the diseases they cause:

- Avian alphaherpesviruses:
  - Avian HV1: Infectious laryngotracheitis
  - Avian HV2 (Gallid herpesvirus-2): Marek's disease
  - Psitticid HV1
    - Parrot herpesvirus
    - Pacheco's disease virus in psittacines
- Unclassified avian herpesviruses
  - Acciptrid HV1 (Bald eagle herpesvirus)
  - Anatid HV1 (Duck plague herpesvirus, duck viral enteritis)
  - Ciconiid HV1 (Black stork herpesvirus)
  - Columbid HV1 (pigeon herpesvirus)
  - Falconid HV1 (Falcon inclusion body disease)
  - Gruid HV1 (cranes)
  - Strigid HV1 (owl hepatosplenitis herpesvirus)
  - Phalacrocoraid HV1 (cormorant herpesvirus)
  - Perdicid HV 1 (bobwhite quail herpesvirus)
  - Budgerigar herpesvirus - reduced egg hatchability, no clinical disease in adults
  - Proliferative cutaneous pedal lesions in cockatoos and macaws also thought to be related to an unclassified herpesvirus

Additionally, the following viruses are known to cause hepatocellular intranuclear inclusion bodies in birds:

- Herpesvirus in parrots, pigeons, owls, hawks and ducks
- Adenovirus in chickens, goslings and bobwhite quail
- Parvovirus in geese
- Papovavirus in budgerigars (Budgerigar fledgling disease)

Contributor: Institut fuer Veterinaer-Pathologie, Universitaet Giessen
Frankfurter Str. 96, 35392 Giessen, Germany
http://www.vetmed.uni-giessen.de/vet-pathologie/

References:

1. Burtscher H. Die virusbedingte Hepatosplenitis infectiosa strigorum – 1.

Mitteilung: Morphologische Untersuchungen. Path. Vet. 1965; 2: 227-255.
2. Burtscher H , Sibalin M. Herpesvirus strigis: Host Spectrum and Distribution in infected Owls. J. Wildl Dis. 1975; 11: 164-169.
3. Forbes NA, Simpson GN. A review of viruses affecting raptors. Vet. Rec. 1997; 141: 123-126.
4. Gough RE, Drury SEN, Higgins RJ, Harcourt-Brown NH. Isolation of a herpesvirus from a snowy owl (Nyctea scandiaca). Vet. Rec. 1995; 136: 541-542.
5. Kaleta EF. Herpesviruses of Birds – a Review. Avian Pathol. 1990; 19: 193-211.

CASE IV – 47473 (AFIP 2994636)

Signalment: East African Bongo (*Eurycerus isaaci*), Third trimester fetus, male

History: A 3-year-old, primiparous East African bongo had dystocia and delivered, with assistance, a stillborn male, third trimester fetus on 24 February 2005. The dystocia was due to a left shoulder lock. The dam recovered uneventfully, and the placenta and fetus were submitted for necropsy.

Gross Pathology: Examined were the placenta and a third trimester, male, East African bongo fetus.

The placenta weighed 3,770 g (normal range 1,300 – 1,600 g) and had the normal number and arrangement of cotyledons for this species, which is between 125-150 cotyledons arranged in four rows. The cotyledons ranged in sized from 3.2 x 3.0 x 0.2 cm to 15.0 x 6.0 x 0.2 cm. The placenta had several abnormalities. The most striking lesion was four partially demarcated, variably sized (up to 975 g), irregular, smooth, fleshy, lobulated, red to purple masses that expanded and were covered by the intercotyledonary chorioallantoic membrane, which contained numerous small blood vessels (Figs. 1 and 2). Approximately 50% of the total cotyledons had diffusely tan maternal surfaces. The tissue at the chorioallantoic interface was thickened, firm, and translucent to opaque white. The intercotyledonary areas also were translucent to opaque with multifocal, thick, opaque, yellow to white foci.

The fetus (21.5 Kg BW wet, 79 cm crown to rump length) was wet, covered in dirt, had intact fetal hooves, and appeared flaccid and large. Remarkable findings besides the non-aerated lungs were limited to several connective tissue abnormalities. The scapular and humeral regions of the thoracic limbs had marked asymmetrical muscling, with the left limb larger than the right. Concurrent skeletal involvement was excluded by radiographic examination. The sternum was concave, and the ribs curved inward (pectus excavatum). The manubrium at the

left thoracic inlet had a 5.0 x 4.0 x 3.0 cm, firm, whitish grey to tan mass that was contiguous with the costo-sternal junction and had a similar consistency to cartilage. The trachea was flattened laterally throughout the entire length. A blind ended, 49.0 x 29.0 x 1.0 cm, smooth, tan, membranous mass filled one third of the abdominal cavity and was contiguous with the serosa of the forestomach (malformed omentum).

Histopathologic Description: The chorioallantois of the intercotyledonary placenta was expanded by multiple, poorly demarcated, variably sized, mesenchymal masses that were pleomorphic in cellular and stromal composition, of which the different morphologies were discrete or more often blended indiscernibly (Fig. 3) (this pleomorphism may be reflected by slight variations between slides). The more discrete regions had either moderate cellularity with scant vascularity (arbitrarily referred to as area #1) (Fig. 4) or had scant cellularity with pronounced vascularity (arbitrarily referred to as area #2) (Fig. 5). Area #1 was comprised of a pleomorphic population of spindle to stellate to oval shaped cells arranged in intersecting fascicles and bundles supported by minimal intervening, pale eosinophilic, fine fibrillar stroma. Cells sometimes assumed a storiform pattern or were contiguous with or whorled around vascular clefts, which variably contained erythrocytes. Most cells had distinct cell borders, tapering or blunt ends, a small to moderate amount of intensely eosinophilic, granular to amorphous, sometimes lightly vacuolated cytoplasm and a peripherally placed, thin to plump, hyperchromatic to open-face oval nucleus. Some cells had cytoplasmic cross striations, multiple nuclei, and central nuclear rowing. Area #2 was comprised of scattered oval to spindle shaped cells supported by a large amount of pale eosinophilic, fine fibrillar stroma. Cells sometimes were contiguous with and whorled around variably sized, usually blood-filled and often distended blood vessels, which infrequently contained unorganized fibrin thrombi. Cells had indistinct cell borders, a small amount of pale eosinophilic cytoplasm, and an oval to polygonal, basophilic nucleus with variably distinct fine chromatin stippling. Mitotic figures were rare. There were small, scattered foci of hemorrhage, and the stroma multifocally was expanded by edema.

In the chorionic villi and membrane of multiple cotyledonary and intercotyledonary regions, the stroma was replaced by sparsely cellular, pale eosinophilic connective tissue, and the surface was lined by low cuboidal epithelium. Some of these and other villi contained basophilic, granular material (mineralization) that partially to completely disrupted the villous stroma and epithelium. Some villi contained small numbers of scattered, non-degenerate neutrophils.

IHC: Pancytokeratin (AE1/AE3), CK 8/18 (UCD10.11), CK 7/8 (CAM 5.2), Mixed LMW>HMW CK (MAK 6), desmin, muscle-specific actin (HHF35), myoglobin, smooth muscle actin, CD 31 (PECAM), Von Willebrand factor VIII

Special stains: phosphotungstic acid-hematoxylin, Masson's trichrome

Contributor's Morphologic Diagnoses:

1. Placenta: developmental anomaly, probable stem cell tumor, with mild, multifocal fibrin thrombi
2. Placenta: moderate, multifocal, fibrosis and mild mineralization
3. Placenta: mild, multifocal, acute placentitis

Ancillary Findings in Fetus:

1. Thoracic limbs (humoral and scapular regions): skeletal muscle asymmetry (probable unilateral hypertrophy)
2. Sternum: pectus excavatum and focal cartilaginous and adipocyte proliferation
3. Omentum: diffuse malformation with adipocyte proliferation
4. Forestomachs: moderate, multifocal, mural adipocyte infiltration
5. Trachea: diffuse, lateral flattening

Contributor's Comment: On gross examination, the placental masses resembled chorangiomas, as described in humans.[1,2] Chorangiomas (chorioangiomas) are ill-defined, benign placental tumors that have been reported in humans and animals and are hypothesized to be of fetal blood vessel origin or a hamartoma of primitive chorionic mesenchyme. Chorangiomas can occur in the placenta, usually towards the fetal side, or the umbilical cord and can present as one to multiple, usually well-circumscribed, thinly encapsulated, fleshy, red masses that sometimes have tan foci indicative of infarction. Chorangiomas can have multiple histological appearances, depending on the contribution of vascular and stromal components and the degree of vascular engorgement, and sometimes assume a malignant appearance, but metastasis has not been reported. The appearance of the stromal components is not well described, but chorangiomas have been referred to as hemangioblastoma, fibroangiomyxoma, fibroma, myxoma, and pericytoma. In veterinary medicine, placental masses of similar description only have been documented in five bovine placentas, of which one case had concurrent fetal cutaneous and lingual hemangiomas.[3-5]

The gross similarity to chorangioma of the masses in this case, was not fully supported by findings on histology, special stains, and immunohistochemistry or by comparison of histology from this case with that from 21 human cases of chorangioma and with limited histological descriptions in the literature. Namely, the tumor in this case contained undifferentiated mesenchymal cells, endothelial cells, pericytes, smooth muscle cells, and skeletal muscle cells, of which the latter have not been described previously in chorangioma. An alternative diagnosis of hamartoma is not considered appropriate, because it implies the abnormal proliferation of tissue element(s) that normally are present at that site, which, in this case, would not include skeletal muscle cells. Neither is teratoma an appropriate term, because it implies the presence of cells from at least two, usually three different embryonic layers, and in this case, all cells were of mesoderm origin.

Thus, combining information of placental tumor morphology, immunophenotype, and embryogenesis (as described below), the placental developmental anomaly in this East African bongo is proposed to be a placental stem cell tumor. This proposal would suggest that there is an alteration in the local microenvironment or a dysregulation of autocrine- or paracrine-mediated proliferation and differentiation of dormant fetal multipotent cells into cells of endothelial, pericyte, smooth muscle, and skeletal muscle type. This leads to the question of whether this stem cell tumor represents a variant of chorangioma and therefore, whether chorangioma and chorangioma-like tumors as described in human and veterinary medicine actually are stem cell tumors of which the final cell type is dictated by the local microenvironment. It also raises the question of whether the presence of multiple musculoskeletal and omental anomalies noted in this East African bongo fetus represent additional regional anomalies in morphogenesis or have an unrelated pathogenesis. The additional histological findings of placental fibrosis and mineralization were non-specific alterations and are not uncommon findings in ruminant placentas in the experience of this institution. The inflammation was attributed to the prolonged parturition and considered insignificant.

Ancillary diagnostics included histochemical stains and immunohistochemistry (IHC). Phosphotungstic acid-hematoxylin (PTAH) confirmed the presence of cross-striations in some cells having morphologic features suggestive of skeletal muscle (Fig. 6). Masson's trichrome stain was consistent with IHC results as discussed subsequently.

The IHC panel included markers for epithelium, specifically pancytokeratin (AE1/AE3) and low molecular weight keratins (CAM 5.2, MAK 6, UCD10.11), mesenchyme (vimentin), endothelium (CD31-PECAM and Von Willebrand factor (VWF) VIII), and muscle (desmin, muscle-specific actin-HH35, myoglobin, α-smooth muscle actin, and skeletal myosin). For each antibody, the external and internal controls were appropriately immunoreactive, except in the case of myoglobin, and were weakly immunoreactive in the case of UCD10.11. Internal controls were of normal tissue from this fetus, including non-affected placenta, liver, skeletal muscle, lymph node, peripheral nerve, and adipose tissue. To facilitate interpretation of IHC, cells within the mass were divided into three main regions: blood vessel endothelium, blood vessel wall and pericytes, and interstitial cells. Table 1 provides a summary of results for antibodies immunoreactive with cells in the mass. Overall, immunophenotypic characterization showed the masses had endothelial, smooth muscle, skeletal muscle, undifferentiated muscle, and fibrous components. Additionally, it showed that area #1 often contained less mature cells than area #2 and contained skeletal muscle cells, unlike area #2.

During embryogenesis, embryoblastic-derived mesenchyme and vessels contribute to the formation of the placenta.[6] Later in placental development, hemangioblastic

progenitor cells differentiate from local fetal mesenchyme and develop into fetal capillaries and hematopoietic stem cells and subsequently are involved in villous differentiation and placental maturation. Fetal mesenchyme is derived from progenitor cells in the somites, which are condensations of mesoderm that later become compartmentalized into the dermatomyotome and sclerotome. Cells from the dermatomyotome can remain undifferentiated or can become endothelium (also from the sclerotome), epidermis, muscle (skeletal, cardiac or smooth muscle), muscle satellite cells, adipose, and cartilage.[7] Determination and differentiation of the progenitor cells occur through a complex cascade of events that involves a network of signaling proteins and transcriptional factors to mediate the sequential transcription of distinct genes during different stages of development. The regulatory factors have a paracrine or autocrine affect, so that cell determination and differentiation is determined by the signals it receives from neighboring cells.

Recent studies suggest that at least some satellite cells of skeletal muscle are not derived from somite cells but from the endothelium and/or pericytes of embryonic vessels, such as the dorsal aorta.[7] Cells competent to generate satellite cells can express both myogenic markers, including desmin, and endothelial markers, including CD31 (PECAM) and α-smooth muscle actin, but not VWF VIII. They can differentiate, depending on the local regulatory factors, into skeletal muscle during embryonic and postnatal growth and regeneration, into vascular components, or perhaps into other mesenchyme. Cells competent to generate satellite cells migrate to their destination, likely through the circulation, early in development. These cells, however, do not acquire the competence to differentiate until later in development and upon maintenance of interaction with neighboring cells. These progenitors may remain demonstrable only in the bone marrow; therefore, they may be related to or representative of the multipotent mesenchymal cells that have been identified in the bone marrow and are capable of producing chondroblasts, adipocytes, skeletal muscle, osteoblasts, and possibly endothelial cells.[7,8] Thus, it is possible that the placental masses and perhaps fetal anomalies in this bongo are due to alterations in the local regulatory factors that control determination and differentiation of fetal multipotent cells.

Antibody	Area #1: Cellular, Scant Vascularity			Area #2: Vascular, Cell Poor		
	Vessel Endothelium	Vessel Wall and Pericytes	Interstitial Cells	Vessel Endothelium	Vessel Wall and Pericytes	Interstitial Cells
Vimentin	All	All	Most	All	All	All
CD 31	All	Negative	Negative	Most	Negative	Negative
VWF VIII	Most	Negative	Negative	All	Negative	Negative
Desmin	Negative	Few	Few-Half	Negative	All	Rare-Few
MSA	Negative	All	Most	Negative	All	Rare
SMA	Negative	All	Half	Negative	All	Half-Most
Skeletal Myosin	Negative	Negative	Rare	Negative	Negative	Negative

Table 1: Comparison of the presence and relative number of immunoreactive cells of the blood vessel endothelium and wall (including pericytes) and the interstitium of areas #1 and #2. The terms refer to the subjective assessment of the number of immunoreactive cells in that region of tissue. MSA: muscle specific actin; SMA: α-smooth muscle actin.

AFIP Diagnoses: 1. Placenta (per contributor): Atypical mesenchymal proliferation with striated muscle differentiation, East African bongo (*Eurycerus isaaci*), ruminant.
2. Chorioallantois: Fibrosis, multifocal, mild, with mineralization, and focal villar coagulative necrosis.

Conference Comment: The contributor provides a very interesting case, gross photographs, diagnostic criteria and a detailed write-up on the differentials considered. Attendees were surprised to find skeletal muscle cells ("strap cells") in the section of placenta and debated their origin. Most agreed that they may have come from aberrant fetal mesoderm that localized to the placenta.

Attendees discussed embryonal rhabdomyosarcomas as another neoplasm in which skeletal muscle cells are found. Embryonal rhabdomyosarcomas can arise in a variety of sites which do not normally contain skeletal muscle. Two types can be identified histologically: the round cell type and those composed predominantly of

primitive myotubes. Although rare, the identification of strap-like cells with cross striations (best recognized with PTAH stain) supports rhabdomyocyte differentiation (9). Additionally, immunohistochemistry for desmin or myoglobin and other muscle markers can also be used to establish muscle differentiation of the tumor (10).

The origin of all fetal organs can be traced back to the primary germ layers which originate from the inner cell mass of the developing embryo. Below is a simple chart that identifies the three layers of the inner cell mass and the tissues derived from each germ layer (11):

Inner cell mass:

Ectoderm

- Epidermis, hair, hooves
- Nervous system

Mesoderm

- Somites, muscle tissues (smooth, striated, cardiac)
- Circulatory organs, heart, blood and lymph vessels
- Connective tissue (bone, cartilage, ligaments and tendons)

Endoderm

- Glands, liver
- Inner lining of digestive tract

Contributor: Zoological Society of San Diego, Department of Pathology
http://cres.sandiegozoo.org/staff/div_pathology.html

References:

1. Benirschke K: Recent trends in chorangiomas, especially those of multiple and recurrent chorangiomas. Pediatr Developm Pathol **2**:264-269, 1999
2. Bernirschke K, Kaufman P: Benign tumors. *In*: Pathology of the Human Placenta, 3rd ed., pp. 709-718. Springer-Verlag, New York, 1995
3. Corcoran CJ, Murphy EC: Rare bovine placental tumour – a case report. Vet Rec **77**:1234-1235, 1965.
4. Karlson AG, Kelly MD: Choriohemangioma of the bovine allantois-chorion. J Am Vet Med Ass **99**:133-134, 1941
5. Kirkbride CA, Bicknell EJ, Robl MG: Hemangiomas of a bovine fetus with a chorioangioma of the placenta. Vet Pathol. **10**:238-240, 1973
6. Bernirschke K, Kaufman P: Early development of the human placenta. *In*: Pathology of the Human Placenta, 3rd ed., pp. 49-56. Springer-Verlag, New York, 1995
7. De Angelis L, Berghella L, Coletta M, Lattanzi L, Zanchi M, Cusella-De Angelis MG, Ponzetto C, Cossu, G: Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. J Cell Biol **147**:869-877, 1999

8. Planat-Benard V, Silvestre JB, Cousin B, Andre M, Nibbeling M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Penicaud L, Casteilla L: Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation **109**:656-663, 2004
9. Meuten DJ: Tumors in Domestic Animals, 4th ed., pp. 351-353. Iowa State Press, Ames, IA, 2002
10. Meuten DJ, Everitt J, Inskeep W, Jacobs RM, Peleteiro M, Thompson KG: Histological Classification of Tumors of the Urinary System of Domestic Animals, 2nd series, fascicle 11, pp. 33-34. Armed Forces Institute of Pathology, Washington D.C., 2004
11. Hafez ESE: Reproduction in Farm Animals, 5th ed., pp. 245. Lea & Febiger, Philadelphia, PA, 1987

Signature Authenticated by Approve
Approved by: Carl I Shaia,
on:Sunday, 19 March, 2006 at 10:23:31

Carl I. Shaia, 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.