49th AAVLD Diagnostic Pathology Slide Session

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October 14, 2006

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Meeting coordinators:
    Lead: Carol Lichtensteiger, University of Illinois
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CNS Toxoplasmosis in Two African Crested Porcupines (*Histrax cristata*)

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Jamee B. Moorman[1], Matti Kiupel[1]

Toxoplasmosis due to *Toxoplasma gondii* is a ubiquitous protozoan parasite whose only definitive hosts are felids. However, numerous intermediate hosts including domestic and wild mammals and birds have been reported.

In this case report, we describe infection in captive African crested porcupines (*Histrax cristata*). Two adult porcupines shared an exhibit, with the male exhibiting chronic neurologic signs of head tilt and ataxia but not progressing. While the female developed acute neurologic signs including head tilt, circling and weakness. The female porcupine failed to respond to therapy, condition continued to deteriorate, and she was euthanized.

Gross necropsy revealed no lesions. Formalin-fixed specimens were submitted to the DCPAH, Michigan State University. The brain had multifocal areas of inflammation and malacia affecting both the cerebrum and cerebellum. There was marked lymphoplasmacytic perivascular cuffing, with lesser numbers of neutrophils and eosinophils admixed. Several 15 – 60 μm diameter protozoal cysts were associated with the inflammatory lesions.

Immunohistochemical staining with antibody against *T. gondii* was strongly positive. PCR on formalin-fixed brain was negative for *T. gondii*; however, PCR was positive on fresh brain.

This case illustrates the typical histologic appearance of CNS toxoplasmosis, and points out the importance of feral cat control at zoologic parks to prevent the spread of this protozoal disease to valuable zoo species.

References

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Histopathological and Immunohistochemical Findings
In a Case of Equine Rabies

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A 10 year old Standardbred gelding had a history of neurologic signs characterized by slight alteration in mentation and behavior for 24 hours duration. The following day, the animal had a temperature of 102.5 degrees Fahrenheit and neurologic signs had progressed to ataxia of all four limbs and hyperexcitability when handled. The animal was euthanized and presented for necropsy to the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH).

No significant abnormalities were noted on gross post-mortem examination.

Histopathologic examination revealed perivascular cuffing in the cerebellum and brain stem. Extending from the proximal spinal cord to the cauda equina there was perivascular cuffing, multifocal gliosis, satellitosis, and perivascular hemorrhage. There was also multifocal random hemorrhage and paravertebral ganglioneuritis in the cauda equina.

Rabies virus was detected with direct fluorescent antibody examination, and immunohistochemistry revealed antibody staining in the Purkinje cells and in neurons of the brain stem, spinal cord, and cauda equina. When present, rabies microscopic lesions are typically most severe in the hippocampus, brain stem and gasserian ganglia[1]; the histologic lesions in this horse were most severe in the cauda equina.

Out of 152 horses submitted to the DCPAH for rabies examination between January 2001 and June 2006, four were positive for rabies. Of these four, one was diagnosed in May 2002, whereas, three were diagnosed from February to June 2006. The Zoonotic Diseases Epidemiologist of the Michigan Department of Community Health (MDCH) reported "the MDCH has detected 23 cases of rabies out of 986 animals tested [from January 1, 2006] through June 26, 2006. Four of these animals were horses. Over the same time period in 2005, nine positive animals, [none of which were horses], were detected out of 965 submissions. This year is unusual not only in the number of positive animals detected in the first half of the year, but also in the fact that four horses have been found to be positive for rabies, the most in a single year since 1999 when there were three."

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West Nile Virus Encephalitis and Equine Herpesvirus Pneumonia
In an 11-Month-Old Alpaca

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Signalment and Clinical History: An 11 month old male alpaca (*Lama pacos*) with a history of muscle tremors, progressive ataxia and recumbency which was unresponsive to treatment was euthanized and submitted dead for necropsy to the DCPAH.

Gross and Microscopic Lesions: Intraluminal white foam was present throughout the length of the trachea. The left lung was diffusely congested and the right lung had focally extensive congestion of the dorsal lung field. No abnormalities were noted within the brain or spinal cord. Microscopically, sections of brain and spinal cord were characterized by mild, multifocal lymphoplasmacytic perivascular cuffing, mild multifocal gliosis, mild diffuse congestion, mild multifocal axon degeneration and mild multifocal lymphoplasmacytic meningitis. In addition, there was ménage melanosis. Sections of lung had regional multifocal intra-alveolar mixed inflammation primarily composed of macrophages. In affected areas, there was also a perivascular infiltration of mixed inflammatory cells, and regions in which the macrophages were degrading or consuming the bronchiolar epithelium.

Morphologic Diagnosis:
1.) Brain: mild lymphoplasmacytic perivascular meningoencephalitis
2.) Spinal cord: mild lymphoplasmacytic perivascular meningomyelitis with mild diffuse congestion, mild gliosis and mild axon degeneration
3.) Lung: multifocal granulomatous alveolar pneumonia and bronchiolitis

Laboratory Findings:
1.) Brain: West Nile Virus polymerase chain reaction (PCR) positive
2.) Lung: Equine Herpes Virus type 1 PCR positive

Discussion: This alpaca had central nervous system lesions consistent with West Nile Virus (WNV) infection, and infection was confirmed by PCR. WNV is a member of the Flaviviridae family and was first recognized in the Western Hemisphere in 1999. This virus has since been isolated from many species including humans, horses and new world camelids such as llamas and alpaca. New world camelids, like humans and horses, are susceptible to the fatal encephalitic form of the disease, though like with humans and horses, not all infected animals develop central nervous system disease.

Interestingly, in addition to the WNV infection, lung tissue from this alpaca was positive by PCR for Equine Herpes Virus type 1 (EHV-1). EHV-1, a member of the Herpesviridae family, is a common cause of abortion and encephalitis in horses. EHV-1 infection has been previously described in alpacas and llamas as a cause of encephalitis and ophthalmitis, though this is the first known report of EHV-1 pneumonia in a new world camelid.

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Nonepitheliotropic Cutaneous B cell Lymphoma in a Dog

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Lymphoma of the skin is rare in all veterinary species, but most commonly reported in dogs and cats. Cutaneous lymphomas are subdivided into epitheliotropic and nonepitheliotropic varieties. The epitheliotropic form originates in the skin and does not spread to other tissues until late in the disease. The nonepitheliotropic forms may be an expression of multicentric lymphoma or, if present in isolation, may be considered a variety of solitary or extranodal lymphoma. Subcutaneous lymphoma may present with multiple lesions.[1] Epitheliotropic lymphomas have been reported to be T cell lymphomas based on CD3 staining. [1] The majority of nonepitheliotropic cutaneous lymphomas are of T cell lineage in dogs.[2]

A 9-year-old spayed female terrier mix-breed dog was presented to the Veterinary Medical Teaching Hospital at Kansas State University (KSU) with one month history of multiple cutaneous or subcutaneous masses. On physical examination the dog was bright and alert. Six masses were located cutaneously or subcutaneously in the neck, shoulder, cranial aspect of right stifle, and left ventral thigh. The masses were moderately firm and movable. Regional lymph nodes palpated as normal.

Fine needle aspirations (FNA) of the masses were performed and cells were stained with modified-Wright stain for microscopic examination. The preparations had high nucleated cellularity and consisted of pleomorphic round cells occurring individually. The cells had moderate degree of anisokaryosis with large nuclei (up to 25 μm in diameter), finely stippled chromatin and multiple, variably sized nucleoli (up to 8 μm in diameter). Cytoplasms were light to moderate blue, homogeneous and contained variable numbers of small, sharp, clear vacuoles. Mitotic figures were frequent. Other cells included occasional macrophages and neutrophils. A cytologic diagnosis of malignant round cell neoplasia was made.

Excisional biopsy samples obtained from the masses were fixed in buffered formalin, embedded in paraffin, and sections were cut (4μ) and stained with H&E. Histologic examination revealed a dense random distribution of anaplastic cells in the dermis and subcutis. Individual cells had an abundant amount of eosinophilic cytoplasm, indistinct cell borders, and large vesicular nuclei with prominent, pleomorphic nucleoli. Many nucleoli were similar in diameter to erythrocytes. Five to ten mitotic figures were observed per high power field. The histologic findings were most consistent with a lymphoma.

Immunocytochemical staining for CD antigens performed on the unstained FNAs from the masses identified expression of CD20 with weak staining for MHC II. Immunohistochemical staining performed on formalin-fixed sections of the masses identified expression of CD45, CD79a and vimentin. Cells dissociated from a needle aspirate of tissue were labeled with a panel of monoclonal antibodies to CD antigens and analyzed by flow cytometry. The neoplastic cells expressed CD45, CD18, CD79a and MHC II. Collectively, based on the fact the neoplastic cells expressed lineage specific and lineage associated B cell antigens (CD79a, CD20, and MHC II), but lacked of T cell antigens, the subcutaneous masses in this dog were determined to be of B cell lineage. The absence of CD21 and surface IgM expression together with expression of CD20 suggested an immature B cell phenotype.

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Botryomycosis in a Mixed Breed Dog

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A 5 year old male mixed breed dog was presented dead to the DCPAH. This dog was reportedly dehydrated. The owners had taken him to a veterinarian on two occasions. However, the dog died overnight while at the clinic.

At necropsy, the dog was thin and 5 to 10 % dehydrated. The abdominal cavity was filled with approximately 1 liter serosanguineous fluid which had multiple pale yellow, 1-2 mm long, soft particles suspended in it. The omentum and mesentery were diffusely thickened, friable, dark red to black and had hundreds of the pale yellow particles adhered to them.

Microscopically, the expansile omental mass consisted of a well vascularized admixture of fibrous connective tissue with infiltrates of large numbers of histiocytes and fewer lymphocytes and plasma cells. This admixture of cells almost totally effaced the normal adipocytes. Randomly disseminated in the granulomatous mass were irregular foci of pyogranulomatous inflammation. Centrally located in many of these pyogranulomas were dense aggregates of gram positive coccoid bacteria and fewer filamentous gram negative bacteria, which were embedded in an eosinophilic homogenous material (Splendore-Hoeppli material). These aggregates referred to as sulphur (or botryomycotic) granules are consistent with the soft, yellow particle detected in the abdomen on gross evaluation. There was proliferation of similar granulomatous tissue on the capsule of the spleen, liver, kidney and adrenal gland, as well as on the serosa of the stomach, small intestine and large intestine.

The intense inflammatory intra-abdominal response in this dog with the formation of botryomycotic granules is most consistent with botryomycosis. *Aeromonas* sp., *Enterococcus* sp. and rare *Staphylococcus intermedius* were isolated from the intrabdominal botryomycotic grains. Botryomycosis, also referred to as bacterial pseudogranuloma, granular bacteriosis, granuloma pyogenicum, actinophytosis or bacterial pseudomycosis, is a poorly understood granulomatous bacterial infection. The most significant distinguishing feature is that the bacteria involved in the infection do not spread throughout the tissue but instead aggregate and form large granules which resemble the sulphur granules of actinomycosis. Botryomycosis is more commonly reported in humans where it occurs as a cutaneous or visceral form. The pathogenesis of botryomycosis is unclear but it is speculated that genetic factors and immunodeficiency may be involved. The visceral form, such as occurred in the dog in this case, is usually a chronic disease that is non-responsive or poorly responsive to treatment. The bacteria usually associated with botryomycosis are staphylococci, streptococci or gram negative enteric bacilli. Specific agents include *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus* spp., *Pseudomonas auruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Nisseria mucosa*, *Actinobacillus lignieresii*, *Bacteroides* spp. And *Proprionobacterium acens*. The process of granule formation is not well understood.

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A Case of Cutaneous Phaeohyphomycosis (*Bipolaris spicifera)* in a Horse

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An 18-year-old Quarter Horse was presented to large animal clinic of VTH of UGA with a five year history of nodular dermatosis. Initially, animal owner observed solitary dermal nodular lesion (approximately 1-2 cm in diameter). During the following five years numerous similar lesions progressively appeared on the entire body of the horse. Nodules were well-circumscribed, round to irregularly shaped, multifocal, fibrotic and erosive to ulcerated and measured between 0.5 – 8 cm in diameter. Multiple biopsy samples were taken; wet samples and formalin fixed samples were submitted for microbial culture and histopathologic examination, respectively.

Histologically, the haired skin samples revealed multifocal to coalescing inflammatory nodules within superficial and deep dermis. Inflammatory zones were consisted of a large number of viable and degenerate neutrophils, macrophages, plasma cells, scattered Langhans-type and foreign-body multinucleated giant cells, lymphocytes and rare eosinophils. These cells surrounded a core of brownish fungi, eosinophilic necrotic debris mixed with hemorrhage. Fungal hyphae were 5-10 μm wide, septate, irregular, rarely branching with thin, brownish, nonparallel walls. Several macrophages and giant cells contained fungal organisms or conidiophores. Occasionally, there were multiple non-pigmented hyphae. Transverse sections of hyphae measured about 7-10 μm in diameter, with 2-3 um-thick brown walls and central basophilic protoplasm. Peripherally, there were a few hemosiderin-laden and erythrophagocytosed macrophages. Often, inflammatory nodules were encompassed/separated by dense collagenous fibrous connective tissue. Overlying epidermis exhibited mild to moderate parakeratosis, acanthosis, pigmentary incontinence and few pustules. A diagnosis of nodular pyogranulomatous dermatitis with intralesional dematiaceous fungi was made. Fungal culture from wet tissue samples yielded septate, pigmented hyphae with conidiophores. Presumptive etiologic diagnosis was made as "cutaneous bipolariasis". Culture sample was further confirmed and speciated at Fungus Testing Laboratory, UTHSC-San Antonio as *Bipolaris spicifera*. Aerobic bacterial culture of biopsy sample yielded no bacteria.

Bipolaris spicifera infection is one of the causative agents of cutaneous phaeohyphomycosis in horses. Other pathogenic dematiaceous fungi include: *Alternaria alternata, Exserohilum rostratum* and *Cladosporium* spp.[1] These fungi are saprophytic and found in decomposing plant debris and soil and considered as opportunistic pathogens in animals and people. Clinically, these fungi can cause superficial dermatitis and deep mycoses of multiple organ systems in immunocompetent animals and people. Rarely, fungemia in immunosuppressed children have been recorded .[2] Fungal dermatitis caused by *Bipolaris* spp., is rare in horses.[3] Frequently infection is established via wound contamination. Combined infection of *Bipolaris spicifera* and *Torulopsis glabrata* in a dog and nasal granuloma due to *Bipolaris* spp., in South African cattle have been reported.[4,5]

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Clear Cell Adnexal Carcinoma in a Dog

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A four-year-old, castrated male, Labrador retriever dog had a cutaneous mass on its left shoulder.

Histologically, this deep dermal/subcutaneous mass was expansile and partially-encapsulated. The mass was composed of multiple nests or lobules of polyhedral cells with distinct cell boundaries, moderate to abundant, clear to pale, reticular or fibrillar cytoplasm with multiple, variable sized (up to 15 micrometer in diameter), vacuoles with feathery borders.

Tumor cells had a round to ovoid, anisokaryotic nucleus with finely stippled chromatin and 1 or 2 distinct nucleoli. Mitotic index was 0 to 1 per hpf with occasional abnormal mitotic figures. Mononuclear or multinucleated (up to 4 nuclei) giant cells were uncommon. Lobules of neoplastic cells were separated by moderate to abundant collagenous or fibrovascular trabeculae, which sometimes were infiltrated by macrophages, lymphocytes, and plasma cells. Multifocally within trabeculae there were clusters of spindloid cells that resembled dermal papillae. Rarely, neoplastic cells had fine or coarse, dense basophilic cytoplasmic granules (keratohyalin). There was rare focal necrosis and/or mineralization.

Microscopic findings in this case were consistent with clear cell adnexal carcinoma. This name has been recently proposed for a canine cutaneous neoplasm with characteristic morphologic features: lobular, nodular, and nest-like architecture; a component of epithelioid cells with clear cytoplasm; follicular dermal papilla-like structures; low mitotic index; nuclear pleomorphism. Previously this tumor was diagnosed as clear-cell hydradenocarcinoma and follicular stem cell carcinoma.

Periodic acid Schiff positivity and failure of staining with oil red O of cytoplasmic vacuoles distinguishes clear cell adnexal carcinoma from sebaceous carcinoma. Balloon cell melanomas neither form nests nor contain follicular dermal papillae-like structures. Clear-cell basal cell carcinoma lacks follicular dermal papilla-like structures. Immunohistochemical profiles of clear cell adnexal carcinoma include coexpression of keratin and vimentin, frequent positivity for S-100, and infrequent reaction for Melan A. Clear cell adnexal carcinoma reportedly can be cured by surgical excision, has low metastatic (2 of 26) and recurrence (1 of 26) rates despite the atypical and pleomorphic appearance of neoplastic cells.

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Granulomatous sebaceous adenitis in a Weimaraner

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A 2-year-old castrated male Weimaraner dog presented with generalized pruritic dermal nodules that resembled hives in July 2005. Skin lesions were partially responsive to glucocorticoid therapy. However, as lesions waned or resolved over the following 3 months, pruritus persisted and patchy alopecia developed where nodules had been. Serum chemistry values were within normal limits. Two excisional skin biopsy specimens from the lateral aspect of the thorax and thigh were submitted to the Animal Disease Diagnostic Laboratory in November 2005.

Three sections of one skin biopsy and two of the other were histologically similar. Lesions consistent with urticaria were not present. The epidermis had orthokeratotic hyperkeratosis. Follicles were in various developmental stages, but infundibula and ostia were often filled with lamellar keratin. Secondary follicles were atrophied. Lymphocytes and plasma cells with few other leukocytes infiltrated the perifollicular dermis. Apocrine sweat glands were unremarkable, but no sebaceous glands were observed in any of the five sections. The most striking feature was the formation of parafollicular granulomas, either at or inferior to the isthmus. These granulomas often had an amorphous eosinophilic center with fat globules surrounded by epithelioid macrophages and small lymphocytes with focally prominent neutrophils.

Morphologic diagnosis was parafollicular granulomatous dermatitis with destruction of sebaceous glands. The absence of sebaceous glands in all sections, the parafollicular location of the granulomas, and the lipid content of the granulomas prompted a diagnosis of granulomatous sebaceous adenitis. No infectious agents were detected in additional sections stained with Fite's acid fast, Brown and Brenn Gram's or PAS stains.

Sebaceous adenitis is an uncommon, poorly understood cosmetic skin disease in dogs with strongest breed predilection in Standard Poodles, Samoyeds, Akitas, German Shepherds, Belgian Shepherds, and Vizslas. Lesions usually develop in early adulthood, affecting the trunk, head and pinnae, but frequently becoming generalized. By the time of biopsy, sebaceous glands have often disappeared.   Other salient histologic features include epidermal and follicular hyperkeratosis and variable, but often minimal, parafollicular inflammation at the isthmus. Vizslas and other short-coated breeds (including Weimaraners [http://www.natural-akita.com/JPTeez/html/sa.html]) tend to develop nodular lesions with patchy alopecia and prominent parafollicular granulomas.

The Weimaraner of this report had granulomatous sebaceous adenitis like that described in Vizslas. Pruritus in sebaceous adenitis may indicate secondary pyoderma, so the dog was treated temporarily with enrofloxacin.   Skin condition improved with benzoyl peroxide shampoos, topical application of 50% propylene glycol in water, mineral oil application followed by Palmolive or Ivory soap shampoo, and oral capsules containing safflower oil, borage seed oil, fish oil and vitamin E.   The dog is currently maintained only with the fatty acid/vitamin E supplement.

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Vetch Poisoning in Adult Angus Cows

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An 880 lb. adult Angus cow was submitted for necropsy to the California Animal Health and Food Safety Laboratory System (CAHFS), Davis. Eight cows died within a week. All cows were on free range pasture and all cows affected had calves. Heifers on the same range were not affected and bulls on an adjoining pasture were not affected. Clinical signs which included dehydration, weight loss and alopecia over the head, neck, trunk and limbs developed over a week and all cows that developed clinical signs died. Histopathologic lesions included multifocal, eosinophilic and lymphohistiocytic myocarditis; multifocal eosinophilic, lymphocytic and granulomatous interstitial nephritis; severe, diffuse, lymphocytic and granulocytic adrenalitis; and moderate, diffuse, lymphohistiocytic and eosinophilic dermatitis. Hepatitis, interstitial pneumonia, enterocolitis and splenitis were also seen on microscopic examination of tissues. A diagnosis of vetch poisoning was made based on histopathology and history of exposure to vetch pasture.

In 2000, a cluster of three separate cases of vetch poisoning in cattle was diagnosed at CAHFS. Vetch (*Vicia benghalensis*) from the associated pastures in three different regions of northern California was collected and examined for plant diseases. A plant disease, leaf spot/stem spot of vetch was diagnosed on the vetch and the etiologic agent, *Ovularia* sp. was isolated from the vetch associated with lesions on the stems and leaves. In the current case, the plant disease was identified in the pasture with the cows but not the bulls. *Ovularia* was grown on plates and then inoculated onto grain. Inoculated grain and alfalfa hay was then fed to two adult beef cows that had been exposed to vetch pasture the previous year over a 24 day period. No clinical signs developed.

The pathogenesis of vetch poisoning is unknown. The reason why vetch that is commonly used as forage becomes poisonous is unknown. The inflammation associated with vetch poisoning suggests animals develop a hypersensitivity reaction but it is apparently not an individual animal hypersensitivity since multiple animals are typically affected and clusters of cases occur certain years with no apparent cases other years. In California, clusters of cases were diagnosed in 1990, 2000, 2005 and 2006. The epidemiology suggests environmental conditions may play a major role, and environmental conditions can be highly tied to plant diseases. Leaf spot/stem spot of vetch caused by *Ovularia* has been identified in many of the outbreaks seen in California. Cows fed *Ovularia*-inoculated grain did not develop clinical signs but as in some other plant toxicoses, the poisoning may occur as a result of response of the plant to the plant fungus. Further studies are needed to determine if *Ovularia*-infected vetch will elicit clinical signs and lesions in cattle.

Porphyromonas Septicemia in a Rabbit

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During late 2005 summer the Animal Disease Diagnostic Laboratory received a dead juvenile rabbit for necropsy that belonged to a group of six. This rabbit died with nose bleed as the sole clinical sign. Another rabbit showed similar clinical signs but was still alive at the time of the submission. Considering the type of clinical signs and the diagnosis of rabbit hemorrhagic disease (RHD) in a rabbit farm in Southern Indiana earlier that year, a diagnostic protocol for exotic animal diseases was followed.

Grossly, the external nares were covered with blood. No vaginal or rectal discharge was observed. The lungs were diffusely congested and edematous. The right cranial and both caudal lung lobes were slightly firm and dark red. The liver was slightly enlarged, had irregular tan to red areas on its surface and marked lobular pattern. The state veterinarian was contacted and samples of spleen, lung, and liver were collected and shipped overnight to APHIS Foreign Animals Diseases (FAD) Laboratory to rule out calicivirus infection.

Microscopically, the lung had multifocal to focally extensive alveolar necrosis associated with hyaline membranes, edema and free red blood cells. Neutrophils infiltrated interalveolar septa in multiple areas. Several medium to large-sized pulmonary vessels were occluded with thrombi composed of fibrin and degenerated leukocytes. Some affected vessels had thickened hyalinized walls or neutrophilic infiltration (leukocytoclastic vasculitis and fibrinoid necrosis). Large aggregates of Gram-negative filamentous bacteria were present within thrombi, alveolar capillaries and alveolar spaces. The heart had multiple foci of inflammation and myocardial necrosis associated with Gram-negative filamentous bacteria. There was massive centrilobular to paracentral (and sometimes panlobular) coagulative hepatocellular necrosis with minimal inflammation. Numerous leukocytes, mostly neutrophils, were present within sinusoids. Lymphocytes were present within portal areas.

Histologic findings were consistent with septicemia by filamentous bacteria morphologically compatible with *Fusobacterium necrophorum (F. necrophorum)*. Liver lesions were attributed to profound hypoxia secondary to pulmonary and cardiovascular changes. APHIS FAD Laboratory analysis was negative for RHD virus. *Porphyromonas assacharolytica* was cultured from lung. This anaerobic bacterium, closely related to *Fusobacterium* sp., causes Lemierre's syndrome in humans. This syndrome is characterized by oropharyngeal infection with anaerobic bacteria leading to septic thrombophlebitis and disseminated abscesses. Lemierre's syndrome has been reported in a rabbit with *F. necrophorum* stomatitis. The rabbit in our case had necrotizing glossitis, but bacteria were not observed in this lesion. The present case had a clinical history and gross findings highly suggestive of RHD and highlights the importance of ancillary tests to rule out exotic diseases. NOTE: Included with the slide are one or two sections of rabbit liver with natural RHD infection (separated by a black line at the bottom of the slide).

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A Case of Canine Influenza

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A five year old male intact Fox Wire Haired Terrier was submitted to the CSU Diagnostic Laboratory for post mortem examination with a brief and nonspecific clinical history of doing poorly and progressively worsening over the course of 7 days ultimately resulting in death. This clinical course occurred while the dog was kenneled in a facility in Littleton, Colorado. No further specific clinical signs were provided and there was no indication of therapeutic intervention. At necropsy, the animal was reported to be in good body condition. Gross findings were limited to the respiratory system consisting of multiple, random, regionally extensive areas of redness and firmness (consolidation) within the pulmonary parenchyma and a moderate increase in mucinous, exudative material within the trachea and mainstem bronchi. The remainder of the post mortem exam was unremarkable. Microscopic examination of the pulmonary tissue revealed a severe, generalized, necrosuppurative bronchopneumonia with bronchitis, bronchiolitis, and mild to moderate bronchiectasis with numerous intralesional bacterial colonies.

Primary differentials at the time of necropsy included canine distemper virus (CDV), canine influenza virus (CIV), and *Bordetella bronchiseptica*. Canine adenovirus 2, *Mycoplasma* sp., canine parainfluenza virus 2, canine herpesvirus, and bacterial pneumonia were also considered. Fresh pulmonary tissue was submitted for polymerase chain reaction (PCR) and bacterial culture. PCR results for CDV were negative however PCR results for CIV were positive. Bacterial culture yielded a 3+ growth of Beta Hemolytic *Streptococcus* and 1+ growth of *Staphylococcus intermedius*. No *Bordetella sp.* was isolated. Immunohistochemistry for influenza on formalin fixed paraffin embedded tissue is pending.

Canine influenza virus (type A) is an emerging canine pathogen first isolated in 2004 from a Florida Greyhound racetrack and determined to be closely related to the H3N8 equine influenza virus. Since that time, canine influenza outbreaks have occurred at tracks in eleven other states (Alabama, Arizona, Arkansas, Colorado, Iowa, Kansas, Massachusetts, Rhode Island, Texas, West Virginia, and Wisconsin). Numerous states have also confirmed infection in pet dogs. Clinical signs associated with canine influenza infection occur in two forms; 1) the mild form, which can persist for 10 to 30 days despite therapy, consisting of a soft, moist cough, with or without nasal discharge and a low grade fever, and 2) the more severe form resulting in pneumonia, fever, and lung lobe consolidation. As clinical signs may resemble those associated with the more common and well recognized agent *Bordetella bronchiseptica* (kennel cough) cases of canine influenza infection may go unrecognized clinically. Thus, it may ultimately become the responsibility of the diagnostic pathologist to recognize this emerging pathogen as a potential cause of disease and to submit the appropriate samples for ancillary diagnostics to confirm infection.

Currently, the most reliable and sensitive ante-mortem test to confirm infection is paired serum titers to demonstrate the presence of viral antibodies. Acute samples should be collected at time of presentation and convalescent samples collected 10 to 21 days thereafter. In a post-mortem setting, viral isolation and PCR from infected tracheal or pulmonary tissue have been determined as reliable diagnostic modalities. It is important to note however that these tests have only been demonstrated as reliable on fresh or frozen tissue whereas formalin fixed tissue may yield false negative results. Immunohistochemical analysis for canine influenza utilizing monoclonal anti-H3 antibodies has been demonstrated on formalin fixed tissue however is not currently an available or readily accessible routine test.

In summary, canine influenza should be considered as a differential for any dog with coughing, fever, nasal discharge, depression, and or evidence of pneumonia. In the ante-mortem state, paired serum titers should be recommended while, if the animal has expired, fresh tissue samples of lung and or trachea should be collected for PCR and or virus isolation. Reported microscopic lesions consist of tracheitis, bronchitis, and bronchiolitis, with or without suppurative bronchopneumonia, necrosis, and hemorrhage. A definitive diagnosis of canine influenza however, can not be deduced on histopathology alone. Additionally, lesions subsequent to secondary bacterial infections may be superimposed upon and obscure those induced by the canine influenza virus further complicating microscopic interpretation. Supplemental diagnostic modalities, as discussed, are necessary and should be pursued appropriately for any suspect case of canine influenza.

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Tyzzer's Disease (Clostridium piliforme infection) and probable copper toxicity in a lamb

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Clinical history: A 3 week-old Dorset Horn male lamb, one of a group of 20 lambs outdoors, was found collapsed and jaundiced with oral and ocular haemorrhages. The ewes were reported to be very well fed.

Necropsy observations: The necropsy examination was performed by the practicing veterinarian. He reported confluent proliferative lesions on rostral tongue, blood stained pleura, swollen liver with a mottled nodular surface and mucoid and blood stained small intestinal contents. There was dark discolouration of kidney and the urine was very dark/black.

Histopathology: Liver: Severe necrotising hepatitis characterised by extensive multifocal to coalescent areas of hepatocellular coagulative and lytic necrosis associated with fragmenting leucocytes, together with cholangiolar hyperplasia and fibroplasia. Faintly staining bacilli in hepatocytes adjacent to necrotic areas and bile plugging of canaliculi are also present. A Warthin Starry stain confirms presence of abundant intracellular slender bacilli; these also stain moderately in a Giemsa preparation. A rhodanine preparation reveals sparse scattered cytoplasmic granular staining in intact hepatocytes. Kidney: Moderate acute nephropathy involving prominent hyaline protein droplet formation particularly in proximal convoluted tubules and prominent intensely eosinophilic homogeneous casts in tubular lumina. No staining detected in a rhodanine preparation.

Laboratory results: Bacteriology: profuse growth of *E. coli* on routine cultures of liver, kidney, lung & spleen. Real time PCR for *Clostridium piliforme* DNA: Liver: positive. Electron microscopy: parapox virus particles detected in lingual lesion.

Biochemistry

Test	Result (lamb)	Reference Range	Units
Copper (Kidney*)	2110	125-650	µmol/kg DM
Copper (Liver*)	908	300-8000	µmol/kg DM

* The identity of the tissue analyzed was confirmed by histological examination.

Comment: The staining characteristics of the intracytoplasmic bacteria and their location at the margin of necrotizing lesions are characteristic of Tyzzer's disease caused by *Clostridium piliforme*, in accordance with the detection of *C. piliforme* DNA in liver. The published reports of Tyzzer's disease in ruminants involve calves, in which hepatic fibroplasia and jaundice/hemoglobinuria were not recorded.   The presence of stained granules in hepatocytes in the rhodanine preparation indicates lysosomal storage of copper, although liver copper levels are at the low end of the reference range. It is possible that the presumptive hemolytic anemia may relate to release of copper from necrotic hepatocytes, leading to hemolytic anemia and elevated renal copper levels. The absence of histochemically detectable copper in kidney is consistent with a cytosolic location of copper which suggests recent accumulation. No other case of copper poisoning has been reported in the flock and a possible extrinsic source of copper has not been identified. Alternative explanations for lysosomal accumulation of copper include an intrinsic abnormality of copper metabolism and reduced biliary excretion of copper.

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Cocklebur Toxicosis in Beef Calves

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Signalment and history: A 10 month old steer was presented to the University of Illinois Veterinary Diagnostic Laboratory for necropsy. This calf was in a group of 45 beef calves on pasture in early winter. The calves had been processed 2 weeks prior, at which time they had been aggressive to people entering the pasture. On the morning of presentation, four calves were down, and two died. The owners were concerned that a relative might be poisoning their calves.

Gross lesions: The liver was grossly normal, except that there was a 13 x 9 x 6 cm cyst with a thick, red, gelatinous wall and filled with translucent, red fluid extending from its free edge between the right and quadrate lobes. The rumen contained approximately 40 liters of ingesta, approximately 5% of which was composed of cockleburs. Cockleburs were also entangled within the fur.

Histologic lesions: Throughout the liver, centrilobular to midzonal hepatic cords, comprising approximately 60% of the area of the sampled section, were affected. Cords were distorted due to variably shrunken, hypereosinophilic hepatocytes, which often contained pale (karyolytic), darkly basophilic (pyknotic), or fragmented (karyorrhectic) nuclei. Hepatocytes near the edges of affected were frequently enlarged, and occasionally binucleate.

Discussion: Cockleburs are toxic to all animals due to the presence of a number of toxic components, but signs and lesions are thought to be due primarily to the activity of carboxyatractyloside. This compound bears structural similarities to adenosine diphosphate (ADP), and as a result, it is able to bind adenosine nucleoside translocase, which is responsible for transporting ADP into mitochrondria. Carboxyatractyloside binds tightly to the translocase, preventing ADP from returning into the mitochondria for rephosphorylation.

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Systemic Histiocytosis in a Bichon Frise: Proposal of a Macrophage-Subtype

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Signalment and clinical history: A 9-year-old male-neutered Bichon Frise presented to the referring veterinarian with a history of lethargy. Harsh lung sounds were auscultated bilaterally, and pale mucous membranes were observed. The patient went into respiratory arrest during the clinical exam and died.

Gross and microscopic findings: Necropsy revealed severe pericardial and pleural effusion, and the heart and diaphragm were mottled white-pink throughout. Histopathology revealed dissection, displacement, and effacement of many planes of myocardial and diaphragmatic muscle fibers, as well as gastric and urinary bladder smooth muscle bundles, by partial whorls to sheets of many epithelioid macrophages (CD18, MAC387 and lysozyme-positive; CD3, CD79, S100, smooth muscle actin and desmin-negative) that are often organized around arterioles and are surrounded by low numbers of neutrophils, eosinophils, lymphocytes, and plasma cells. Muscle fibers in the heart and diaphragm are observed undergoing varying stages of degeneration. There was a single, small (0.75 cm diameter) nodular infiltrate of similar histiocytes organized around an arteriole in the lung. The liver was severely congested. No bacterial, fungal, or protozoal organisms were observed following the use of special stains, and aerobic culture of the heart, stomach, and urinary bladder was negative for bacteria.

Morphologic diagnosis: Reactive (systemic) histiocytosis.

Discussion: The canine proliferative histiocytic disease "complex" offers ample opportunity for diagnostic investigation[1]. The term histiocyte is often used to describe either myeloid dendritic antigen presenting / Langerhans cells or macrophages, with antigen presentation and myeloid ancestry as the common thread. There are three broad categories of canine proliferative histiocytic diseases, which include one reactive disease (reactive histiocytosis - cutaneous or systemic form) and two neoplastic diseases (cutaneous histiocytoma, and malignant histiocytosis / histiocytic sarcoma - localized or disseminated form). Both lymphomatoid granulomatosis and malignant fibrous histiocytoma share some microscopic features with several of the canine proliferative histiocytic diseases, although lymphomatoid granulomatosis and malignant fibrous histiocytoma / anaplastic sarcoma with giant cells are composed of CD3-positive lymphocytes and mesenchymal cells, respectively.

We came to the diagnosis of systemic histiocytosis in our case based upon the perivascular distribution in several organs and the associated mixed inflammatory cells. Systemic histiocytosis is believed to be a reactive process, and a manifestation of immune system dysregulation which responds to immunosuppressive therapy[2]. We confirmed the infiltrating cells in our case to be of macrophage, not dendritic antigen presenting cell origin following an immunostaining panel (positive staining for CD18, MAC387 and lysozyme, and negative staining for CD3, CD79, S100, smooth muscle actin, and desmin). While both dendritic antigen presenting cells and macrophages are known to immunostain positive for CD18 and MAC387, dendritic antigen presenting cells reportedly do not contain immunoreactive lysozyme.[3] To our knowledge, this is the first report of demonstrated macrophage differentiation in a case of canine systemic histiocytosis.[4] Here, we propose the creation of a macrophage-subtype of systemic histiocytosis.

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Polymyositis in a domestic ferret

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A 9-month-old intact female ferret had a 6 day course of illness that included the following clinical signs: initial anorexia, weakness and weight loss; severe depression, inability to hold its head up for prolonged periods; fluctuating temperature between 101-106° F; nasal and ocular discharge; the owner also reported a focal blister on the tongue and small "bumps" on the lower eyelids. The referring veterinarian reported that the ferret also developed increased respiratory effort and passed soft green stool during the 6 day treatment period, during which her condition declined slowly prior to spontaneous death on May 1, 2006. Radiographs were unremarkable, hematocrit was 46%, ALT was 78 u/L, and bilirubin was 0.2 mg/dl. The clinical condition including fever was unresponsive to treatment that included intravenous fluids, parenteral enrofloxacin and dexamethasone, orally administered metronidazole and pepto bismol, and force feeding. The diet was a commercial product acquired from a pet store, and the animal was not vaccinated. Three other ferrets in the household remained unaffected.

The ferret weighed 710 grams and was mildly dehydrated at necropsy. No oculonasal discharges were noted despite the history given. A focal, 5 mm diameter zone of acute hemorrhage was noted on the ventral aspect of the tongue. Numerous small (1-2 mm) raised tan nodules were present in the subcutaneous tissue of the ventral intermandibular region, and these extended along fascial planes adjacent to the cervical trachea. Multiple ecchymoses were observed in subcutaneous fat and beneath the pleura of several lung lobes, which were congested. Approximately 3 cc of amber clear fluid was in the pleural cavity. The spleen was markedly enlarged, pink and had a fleshy consistency. The liver was diffusely pale orange and firm. Microscopic lesions consisted of multifocal and coalescing infiltrates of neutrophils within sections of heart, esophagus and multiple sections of skeletal muscle. Macrophages and lymphocytes infiltrated some of these tissues, also; in some regions inflammatory cells were mixed. Lesions ranged from mild to severe and included muscles of the tongue, both eyelids, face, esophagus, heart, thigh, and superficial muscles beneath the skin. Fragmentation, atrophy and loss of myofibers and regeneration of myofibers were also observed. Both thyroids glands contained pyogranulomatous inflammation that extended into adjacent fascia. Marked hepatic lipidosis and marked splenic extramedullary hematopoiesis and megakaryocytic hyperplasia were noted.

Differential diagnosis for clinical signs and inflammatory lesions included canine distemper virus (CDV), Aleutian disease virus (ADV), FIP, septicemia, toxoplasmosis, neosporosis and sarcocystosis. CDV was discounted by negative immunofluorescence results. No bacteria were detected by gram stains of several muscle sections. The owner declined further diagnostic testing.

Signalment, clinical signs, nature and distribution of microscopic lesions are consistent with a diagnosis of Disseminated Idopathic Myositis, also referred to as myofasciitis, polymyositis and suppurative myositis. This is an emerging syndrome of young ferrets (5-24 months) that was not recognized prior to 2003 and is of unknown origin, although an immune-mediated response has been suggested in the etiopathogenesis[1,2,4]. At this time, there is no reported successful treatment and most cases are fatal. Criteria for diagnosis include inflammation in the esophagus, heart and at least one other skeletal muscle[5,6]. Approximately 80 cases have been documented according to the American Ferret Association as of February 2006, and about 40 cases had been confirmed by histopathology[2]. The AFA has a reporting form available online to report other documented cases[3].

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Rickets in a Llama

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In March of 2006, a 7-month-old, male llama was presented to the Large Animal Clinic, Western College of Veterinary Medicine because of a 2- to 3-month history of lameness and increasing amounts of time spent in recumbency.

The findings of a CBC and serum chemistry analysis were consistent with muscle injury and insufficient ingestion or absorption of phosphorus and magnesium. Radiographs revealed osteopenia, irregularly widened physes, and pathological fractures of the femoral heads.

Given the severe changes in the skeleton, poor prognosis for return to normal, and financial considerations, the llama was euthanized and submitted for necropsy. Lesions in the skeletal system included the following: i) ribs that were easily bent or folded; ii) curvature or bowing of multiple long bones; iii) focal retention of hyalin cartilage in the metaphyses deep to the physes, and in the epiphyses deep to the articular cartilage of long bones (femora, humeri, radii, and tibias); iv) long bones with expanded medullary cavities and thinned cortices; v) and compression fractures of the neck of the femoral heads and the heads of the humeri.

Microscopic examination of the bones helped to characterize the nature of the lesions detected grossly. There was irregular and segmental thickening of physes in the limb bones characterized by abnormal accumulation of chondrocytes in the zone of hypertrophy. Invasion of vessels from the metaphyses into this cartilage was disorganized. Deeper in the metaphyses, separated from the physes, were occasional islands of unmineralized cartilage.

Diagnoses: 1. Rickets, 2. Osteomalacia, 3. Osteopenia 4. Chronic pathologic fractures

Rickets can be caused by deficiencies of vitamin D, calcium, or phosphorus. Although the cause of the lesions was not confirmed in this case, hypophosphatemic rickets due to vitamin D deficiency has been described in growing llamas and alpacas raised in areas of high latitude, and crias born in the fall and winter are at higher risk. Inadequate endogenous production is thought to play a major role in the vitamin D deficiency. Saskatoon, Saskatchewan is located at about 52 degrees North. For comparison, Lima, Peru is located at about 12 degrees South. The presence of osteopenia would suggest the animal was also not receiving adequate calcium and was hyperparathyroid.

In people, the reemergence of rickets has also been associated with impaired dermal synthesis in countries of extreme latitude, extensive coverage of the skin with clothing, and the use of sunscreens.

Metastatic mast cell tumor in the liver of a cat

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A 14 year old spayed female domestic short-haired cat presented with a history of vomiting and a splenic mass. Surgical biopsies of liver, pancreas and spleen were submitted for assessment. The submitted slide is a portion of the section of the liver biopsy.

Histologically, diffusely within the section, the liver architecture is obscured by small sheets of round cells within the sinusoids and vessels. These cells have distinct cell borders, abundant faintly granular eosinophilic cytoplasm, a large round to oval eccentrically placed nucleus with granular chromatin and variably distinct nucleoli. There is two-fold anisocytosis and anisokaryosis. No mitotic figures are seen. The surrounding hepatocytes have many small vacuoles within their cytoplasm. Duffy's stain highlights the granules within the neoplastic cell population.

Samples from the spleen show almost complete effacement of the normal architecture by neoplastic cells similar to those seen within the liver. Small remnants of normal lymphocyte aggregates of the white pulp are the only remaining identifiable remnants of normal splenic architecture. Based on this histopathology and results of special stains a diagnosis of splenic mast cell tumor (MCT) was made. Unfortunately, no further follow up was available on this case and the clinical outcome is unknown.

Visceral MCTs in cats are generally divided into two types, intestinal and splenic. Unlike cutaneous MCTs in cats which are generally benign, visceral MCT are usually malignant and the prognosis is considered poor. Splenic MCTs are one of the two most common neoplastic causes of splenomegaly in cats (lymphoma being the other). No breed or sex predilection is reported and this tumor has not been associated with positive feline leukemia status.

Metastatic disease, as in this case, is common with the liver reported as being a site of metastasis in 90% of cases. Other reported common sites of metastasis are, in decreasing order of incidence, the visceral lymph nodes, bone marrow, lung and intestine. Peripheral mastocytosis has been reported to occur in up to 40% of cases.

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Pancreatic Lithiasis in a Holstein Cow

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Signalment and Clinical History: A 9 year old Holstein cow from small dairy herd was submitted alive for necropsy to the Diagnostic Center for Population and Animal Health, Michigan State University. According to the history, several cows in the herd (around 22) had died within the last 6 months. The visiting veterinarian reported a general low body score, low milk yield (40 pounds/head), and high numbers of cows with milk fever in the herd. He determined that the non-fiber carbohydrate level in the food was significantly too high at 44% and most likely responsible for the described problems. After correcting the food composition the herd stabilized and the milk yield increased dramatically (65 pounds/head). However, the owner was concerned about mycotoxins in the forage based on a feed analysis that had indicated yeast growth and aflatoxins (levels were considered average by an expert).

Gross Description: The submitted cow did not demonstrate any of the described clinical signs, was in good nutrition and adequate hydration and in fact weighed approximately 2000 pounds. The lesion of interest from which tissue samples were submitted was localized in the pancreas. Throughout the pancreas there were multiple, variably sized (5-15 cm) circumscribed hard masses. On closer examination, large numbers of white calculi ranging in size from 1 to 10 mm in diameter as well as white sabulous material were found to occlude the lumen of multiple distended pancreatic ducts. Calculi were round to rhomboid and had either a smooth or roughened surface. The wall of the affected ducts was thickened and fibrosis surrounded some of the larger ducts. All other organs were grossly unremarkable.

Microscopic Description: The submitted slides contain sections of pancreas. Intraductual calculi made tissue processing difficult and most sections will not contain calculi that were lost during processing. In sections of pancreas there was marked ductular ectasia with intraductular calculi. Affected ducts were surrounded by marked fibrosis. In most ectatic ducts lining epithelial cells were lost and there were small numbers of lymphocytes and plasma cells subjacent to the lumen. Surrounding pancreatic ducts was a moderate to marked proliferation of dense connective tissue (fibrosis) with occasional trapped atrophic exocrine acini. There was also multifocal fat necrosis, and mild lymphoplasmacytic interstitial pancreatitis.

Morphologic diagnosis: Pancreas: ductular ectasia with intraductular calculi and marked periductular fibrosis and lymphoplasmacytic pancreatitis and multifocal lobular atrophy and fat necrosis.

Mineral analysis: Tissue mineral analysis of samples of liver demonstrated no abnormalities and showed all trace mineral concentrations within normal ranges. Using mass spectroscopy the composition of pancreatic calculi was determined to be $CaCO_3$ (calcite).

Discussion: Pancreatic calculi rarely occur in animals, but have most commonly been observed in cattle. According to some studies in Europe, one in 12,000 bovines will likely be affected with females older than 4 years of age being at higher risk. Cows reared on silicon-rich soil are more likely to develop this condition. According to most published data, pancreatic stones in cattle are composed of about 95% $CaCO_3$ (calcite). The physical and chemical characteristics of the stones described here were similar to those previously published data. Pancreatic juice contains large amounts of bicarbonate and the interaction with Ca^{2+} is critical in the pathogenesis of stone formation. Recent attention in human pancreatic lithiasis has focused on the role of pancreatic stone protein (Reg protein/lithostathine), which may prevent the harmful activation of protease precursors in the pancreatic juice and may also contribute to stone formation. A similar protein has been found in cattle. The clinical significance of this condition in cattle is unknown, but most cases have been described as isolated necropsy or slaughter house findings. In one study of 169 cases of bovine pancreatic lithiasis digestive disorders with diarrhea were most frequently encountered.

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Placental Chlamydiosis in a goat

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A placenta from a Boer goat of unknown age with a history of previous stillbirths and abortion was submitted to the UGA necropsy service. On gross examination, there were multiple variably sized yellow plaques on the placenta. There was congestion around the plaques. Amniotic fluid was clear and yellow. Microscopically, the allantoic endoderm had multifocal areas of mineralization that were surrounded by moderate numbers of degenerate neutrophils and a few macrophages. Immunohistochemistry and FA results revealed numerous *Chlamydophila* spp. organisms in the placenta. The final diagnosis in this case was severe necrotizing placentitis with intra-lesional *Chlamydophila* spp.

The family *Chlamydiaceae* comprises two genera, *Chlamydia* and *Chlamydophila*. *Chlamydia* species infect only mammals (rodents, humans, swine). Host specificity for *Chlamydophila* species is not that rigid, including for amphibians, reptiles, birds, and mammals. Except for *Chlamydophila pneumoniae* the rest of the *Chlamydophila* species infect animals. *Chlamydophila spp.* are obligate, gram negative, intracellular organisms that are susceptible to some antibiotics much like bacteria. They have a two-stage developmental cycle including an extracellular infectious elementary body and an intracellular vegetative reticulate body. *Chlamydophila spp.* causes enzootic abortions during the third trimester of pregnancy in sheep and goats. *Chlamydophila spp.* enter via the digestive tract, respiratory tract, or hematogenous spread to the placenta. The bacterium first localize within the mononuclear cells in the endometrial stroma and the epithelium next to the intercaruncular areas with an increased neutrophilic response in that region. *Chlamydophila spp.* infection starts from the maternal side and then colonizes the fetal placenta. *Chlamydophila spp.* replicate in the fetal placenta (trophoblasts) unlike the efficiently controlled maternal placenta. Trophoblasts do not induce the expression of tryptophane degrading enzyme allowing the bacterium to replicate (using tryptophane). There is lack of expression of MHC I expression rendering the trophoblasts resistant to CD8 cell attack. *Chlamydophila abortus* causes abortions in goats and has a zoonotic risk. In addition to abortions, *Chlamydophila spp.* causes a spectrum of diseases in a variety of hosts. Other infectious agents which cause necrotizing placentitis in goats include *Toxoplasma gondii, Campylobacter spp., Listeria spp., Salmonella spp.* and *Coxiella burnetti.*

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