

The Armed Forces Institute of Pathology  
Department of Veterinary Pathology  
WEDNESDAY SLIDE CONFERENCE  
2006-2007

CONFERENCE 25  
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**CASE I** – V05-03814 (AFIP 3032063)

**Signalment:** Unknown age, female Alpaca, *Lama pacos*.

**History:** The owners of this alpaca were 'out of town.' Another person was feeding, watering, and serving as caretaker of the herd of 12 alpacas. No reportable abnormalities had been noticed by the substitute caregiver, until going to feed the group and one of the alpacas was found dead. There was no evidence of a struggle where the alpaca was found. A local veterinarian performed a necropsy and submitted multiple unfixed tissues to the laboratory.

**Gross Pathology:** The liver had multiple caseous abscesses throughout, varying from 0.5 to 2.0 cm diameter. Lung tissue had multiple abscesses throughout all lobes ranging from 0.3 cm to 2.0 cm diameter. Lymph node and thymus exhibited multiple abscesses. The myocardium contained multiple abscesses in both the free walls and the interventricular septum, up to 0.7 cm diameter. Kidney cortices had multiple 0.1 to 0.2 cm foci. Multiple firm nodules were present in the spleen and the abomasal lymph node was 3.0 cm diameter.

**Laboratory Results:** *Coccidioides immitis* was isolated from lung and liver samples. Identification and/or isolation of acid fast bacteria were negative from lymph node and liver samples.

**Histopathologic Description:** Multifocal abscesses were present throughout the liver, lungs, multiple lymph nodes, heart, spleen, adrenal glands and kidneys. These abscesses were characterized by central necrosis rimmed by cellular debris and neutrophils, with an outer rim of histiocytes. Fungal organisms were observed within these lesions. The fungal structures varied in size from 15 to 45 microns

diameter, presenting as spherules with a 2 micron thick, refractile/double contoured capsule. The fungal spherules contained numerous 3-4 micron diameter basophilic bodies, considered to represent endospores.

**Contributor's Morphologic Diagnosis:** Lung: Pneumonia, multifocal, necrotizing, neutrophilic and histiocytic (granulomatous/pyogranulomatous), with moderate to marked patchy coalescing hemorrhage, edema and intralesional fungal spherules (*Coccidioides immitis*).

**Contributor's Comment:** This case represented a widely disseminated presentation of *C. immitis* infection. Follow up history with this herd of alpaca revealed that a second alpaca died within a few days of this case death. Because of this and the apparent efficient dissemination of the organism, and further because of the reported high susceptibility of the llama (a close relative to alpacas) to Coccidioidomycosis, we are proposing that the alpaca very likely shares this same susceptibility to infection and probably widespread dissemination of *C. immitis*. This is certainly not a sufficient number of cases to make a definitive broad assumption, but will add to the data that may accumulate as the alpaca is introduced and flourishes in areas to which the species is naïve and in which *Coccidioides immitis* may exist and/or be endemic (discussed later below). The abundant organisms present, in even small sections of affected tissue, in this case provides an excellent example of the variable morphology of *C. immitis* that may not always be afforded in some of the other animal species in which Coccidioidomycosis may be diagnosed.

Coccidioidomycosis is a systemic mycosis caused by the dimorphic fungus *Coccidioides immitis*. In soil, the fungus exists in a mycelial (saprophytic) phase while in tissues or body fluids, the fungus exists as a spherule (parasitic) phase. Spherules are large structures with a capsule filled with endospores.<sup>6</sup> The mycelial phase of *C. immitis* is found only in a specific ecologic region, the Lower Sonoran life zone in North America, Mexico, and Central and South America. This region includes the arid or semi-arid areas of the southwestern United States (California, Arizona, Utah, Nevada, parts of southwest Colorado, New Mexico, and southwestern Texas), northwestern Mexico, Argentina, Colombia, Guatemala, Honduras, Paraguay, Venezuela, and probably Bolivia.<sup>4</sup> Natural infection is found in many mammalian species, at least 22 species of free ranging and captive wild animals, as well as humans.<sup>1</sup> Domestic animal species reported to be susceptible to *C. immitis* infection include horses, cattle, sheep, pigs, dogs, cats and non-human primates.<sup>2,3,11</sup> Disseminated coccidioidomycosis amongst animals has been reported to occur in dogs, cats and non-human primates.<sup>2,3</sup> However, as this report and others have demonstrated, New World (South American) Camelids, such as the llama, are extremely susceptible to *C. immitis* infection and commonly develop widely disseminated disease.<sup>4,8</sup> In this case we demonstrate the apparent high

susceptibility and disseminated disease due to *C. immitis* in the alpaca. Alpacas are New World Camelidae in the same genus as llamas.<sup>7</sup> Alpacas are smaller than llamas and valued as a producer of finest quality wool.<sup>10</sup>

Infection by *C. immitis* is mainly by inhalation of arthroconidia present in wind borne dust, frequently resulting after dust storms.<sup>4,6,7</sup> Local traumatic inoculation and infection can occur, resulting in a fluctuating abscess, but is rare and dissemination from such a focus would be very unusual.<sup>3,6</sup> After inhalation, the arthroconidia transform into spherical endospores, which subsequently enlarge and undergo internal cleavage to form large spherules containing hundreds of endospores, a process taking several days.<sup>6</sup>

Spherules subsequently rupture, releasing endospores and the cycle becomes repetitive. Arthroconidia and endospores can be engulfed by macrophages, but are not killed, resulting in spread through the circulation within macrophages to other sites of localization. Dissemination commonly occurs to bones, skin, abdominal viscera, heart, genital tract and eyes. The bones and eyes are two of the most common sites of dissemination in animals.<sup>1,3,6,7</sup> Bone involvement accounts for one of the more commonly described clinical signs in dogs, that being a shifting leg lameness. Other signs commonly described in disseminated coccidioidomycosis of dogs include: persistent and/or fluctuating fever; anorexia and weight loss; weakness; localized peripheral lymphadenopathy; draining skin lesions, keratitis, uveitis and acute blindness.<sup>7</sup> Microscopic lesions are typically pyogranulomatous, more neutrophils are associated with arthroconidia and endospores, while more epithelioid macrophages and giant cells are observed in association with spherules.<sup>3,6</sup> In this case of disseminated coccidioidomycosis in an alpaca, histopathological lesions tended to be similar in location and character.

Diagnosis of coccidioidomycosis can be made with serological testing using agar gel immunodiffusion and complement fixation techniques. Culture from exudates, body fluids or tissue can be utilized, but requires specialized handling and equipment and can potentially result in human exposure, unless unscrupulous laboratory technique is used. Direct observation of the organism from body fluids or exudate is possible and histopathological identification of the organism from fixed tissue using H&E staining and enhancement of identification by special stains such as Gomori's methenamine silver (GMS) or periodic acid-Schiff (PAS). The size of the spherule, presence of endospores and presence of a double contoured wall characterize the spherules of *C. immitis* compared to other potential fungal pathogens.

Treatment in animals can be successful with long term antifungal systemic therapy (Ketaconazole, Itraconazole, Fluconazole or Amphotericin B).<sup>2,6,7</sup>

Coccidioidomycosis is not a true zoonosis, in that transmission from animals to man does not occur. This is because the infective form, arthroconidia, is not produced in tissue or body fluids. The relationship of animal infection to human infection is that exposure to the same environmental source of infection can be common. One exception could be the possibility of inhalation of endospores by humans performing necropsy procedures upon infected animals.<sup>4</sup>

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**AFIP Diagnosis:** Lung: Pneumonia, granulomatous, multifocal, moderate, with diffuse edema and numerous fungal spherules, etiology consistent with *Coccidioides immitis*, pacifica (*Lama pacos*), camelid.

**Conference Comment:** The contributor provides a complete overview of *Coccidioides immitis*. The two most common organs affected by *C. immitis* with systemic dissemination are bone and eye. As pointed out by the contributor, the arthrospores are the infective form. Endospores are not infective. *C. immitis* is a primary pathogen capable of infecting immunocompetent hosts and may also be an opportunistic pathogen in contrast to the opportunistic fungi (*Aspergillus* sp., *Candida* sp., *Zygomycetes*) in which immunodeficiency is necessary for infection. Cell-mediated immunity is more important than humoral immunity in containing/clearing the fungus and for resistance to reinfection.<sup>1,2,5</sup>

As pointed out by the contributor this case provides an excellent example of the variable morphology of *C. immitis*. Sporangia ranging from 30-200 um in diameter containing numerous 2-4 um endospores (mature spherules) and immature spherules ranging from 5-30 um in diameter containing flocculent material are present in the slides provided.<sup>1,2,5</sup> Splendore-Hoeppli material surrounds spherules in some sections. Many conference participants commented on the striking multinucleated giant cells present in this case.

Conference participants reviewed the histomorphologic features of the systemic mycoses, the fungi/algae that endosporulate, and cell-mediated immunity.

Fungi/algae that reproduce by endosporulation include:

1. *Chlorella* sp.
2. *Prototheca* sp.
3. *Coccidioides immitis*
4. *Rhinosporidium seeberi*
5. *Batrachochytrium dendrobatidis*

Readers are encouraged to review WSC 1 / Case I, 2006-2007 – a case of *Blastomyces dermatitidis* in the eye of a cat and WSC 21 / Case I, 2006-2007 – a case of *Cryptococcus neoformans* in the lung of a dog.

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**CASE II – 05-25021 (AFIP 3026812)**

**Signalment:** 10-year-old castrated male, Cocker Spaniel dog (*Canis familiaris*).

**History:** Dog presented for a 5.0 to 6.0 cm in diameter, ulcerated mass on the fifth digit of the left forepaw. The mass had grown rapidly in four months. Fine needle aspiration cytology was performed. The affected digit was amputated and submitted for histologic examination.

**Gross Pathology:** The amputated fifth digit of the left forepaw with an approximately 4.5 x 4.5 x 4.0 cm firm, partially haired, ulcerated mass was received. On cut section the mass was homogeneously white.

**Laboratory Results:** Fine needle aspiration cytology revealed a poorly cellular preparation comprised of clusters and individual round to polygonal cells with distinct to indistinct cell borders and a moderate nuclear to cytoplasmic ratio. The nucleus was eccentric, round with coarsely clumped to reticular chromatin. The cytoplasm was moderate and blue. Some cells possessed a perinuclear clear zone, interpreted to be a Golgi apparatus. Anisokaryosis and anisocytosis were moderate. There were occasional binucleate and rare multinucleated forms. The cells stained positively with methyl green pyronine. There were scattered macrophages and occasional multinucleated giant cells were seen. Clusters of sebocytes were also present. Free erythrocytes and platelet clumps were abundant. There were clumps of cellular debris and occasional nondegenerate neutrophils. The cytological diagnosis was a discrete/round cell tumor and a cutaneous plasmacytoma was considered most likely.

In formalin-fixed, paraffin-embedded sections of the digital mass tumor cells stained positively with methyl green pyronine. The tumor cells also exhibited variable levels of staining with anti-CD79a and anti-CD45RA and did not stain with CD18. Some of the tumors stained moderately with anti-IgG.

**Histopathologic Description:** Extending from the limits of the hair follicles is an expansile, pseudoencapsulated mass that comprises large coalescing deposits of an amorphous, homogeneously pink, slightly isotropic material; multinucleated giant cells; packets of individual round to polygonal cells; thick collagen fibers, spindled cells and scattered adipocytes. Cell borders for the round to polygonal cells are distinct. The nuclear to cytoplasmic ratio is moderate to high. The nucleus is centric to eccentric, round to oval with coarsely stippled chromatin and usually a single nucleolus. The cytoplasm is scant to moderate and blue. Most cells possess a paranuclear clear zone. Cellular features of malignancy include: bi-, tri- and multinucleation (up to 5 nuclei per cell) often with nuclei of unequal size; moderate anisocytosis and anisokaryosis; megalocytosis and karyomegaly. Mitoses are

present (2 to 4 per 40X objective field). There are individual macrophages with bi-, tri- and multinucleated forms. The multinucleated giant cells are partially or totally engulfing the collections of amorphous, homogeneously pink, slightly isotropic material, interpreted to be amyloid. This material, when stained with Congo red, has apple green birefringence with polarized light and this birefringence was retained following treatment with potassium permanganate, consistent with immunoglobulin-derived or primary amyloid composed of lambda light chains.

**Contributor's Morphologic Diagnosis:** The histological diagnosis was a digital, cutaneous (extramedullary) plasmacytoma with intralesional, primary amyloid and granulomatous inflammation.

**Contributor's Comment:** Cutaneous plasmacytomas have been recognized in dogs and cats. The incidence of cutaneous plasmacytomas has been reported to be 1.5% of all canine skin tumors.<sup>1</sup> In most instances canine cutaneous plasmacytomas develop as solitary lesions, but multiple masses may occur and may be numerous in some cases. Cocker Spaniels appear to be predisposed to this tumor.<sup>1,2</sup> Tumors occur frequently in at or near areas of chronic immune stimulation. Common locations are the pinnae, lips, digits, chin and oral cavity.<sup>1-4</sup>

Approximately 10% of canine cutaneous plasmacytomas show varying degrees of amyloid production.<sup>1</sup> Primary or immunoglobulin-associated (AL) amyloid is derived from immunoglobulin light chains.<sup>2,4</sup> Plasmacytomas with amyloid are invariably composed of well-differentiated tumor cells.<sup>1</sup> Occasional tumors are overwhelmed by amyloid and contain only scattered islands of residual tumor cells.<sup>1</sup> Amyloid usually is accompanied by a granulomatous inflammatory reaction, which includes foreign body-type multinucleated giant cells.<sup>1,3</sup> Similar giant cells have been reported in association with amyloid in both respiratory plasmacytomas and solitary plasmacytomas of bone in human beings as well as cutaneous amyloidosis of horses.<sup>1</sup> No correlation has been described for the presence of amyloid in plasmacytomas and the cell type, location, recurrence or biological behaviour.<sup>3,4</sup> Dense cytoplasmic staining with methyl green pyronine stain (MGP) lends support to the diagnosis of a cutaneous plasmacytoma but it is not specific for plasma cells and must be interpreted with caution. Canine plasmacytomas consistently express the common leukocyte marker CD45 but are often negative for CD18.<sup>1</sup> About 80% of canine plasmacytomas express CD79a [MB-1].<sup>1</sup>

Most canine cutaneous plasmacytomas are benign and surgical excision appears curative.<sup>1-4</sup> However, some authors suspect that digital plasmacytomas as well as plasmacytomas of the oral cavity or subcutis may be more behaviorally aggressive.<sup>1</sup> Occasionally solitary and multiple cutaneous plasmacytomas exhibit malignant behavior and may metastasize to internal organs.<sup>1</sup>

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**AFIP Diagnosis:** Haired skin and subcutis: Plasmacytoma with amyloid, Cocker Spaniel (*Canis familiaris*), canine.

**Conference Comment:** The contributor provides a concise summary of cutaneous plasmacytomas in the dog. Canine cutaneous plasmacytomas are usually circumscribed, unencapsulated, uni- or multilobular neoplasms primarily confined to the dermis. There is often a narrow uninvolved zone of the superficial dermis (Grenz zone). The neoplasms are composed of round to polygonal cells arranged in sheets, packets, and cords separated by a fine fibrovascular stroma. The plasmacytic character of the neoplasm is typically more apparent at the periphery. Marked variation in tumor cell differentiation is common, anisocytosis and anisokaryosis can be moderate to marked, and there is often variation in chromatin pattern. Previous studies have presented subclassifications of canine plasmacytomas based on the variable morphologic features, and while the features may be useful as a diagnostic tool, they are of no prognostic significance.<sup>1,3,4</sup>

The differential diagnosis for cutaneous plasmacytomas includes other cutaneous round cell tumors including histiocytoma, lymphoma, transmissible venereal tumor, and mast cell tumor. Conference participants reviewed common tumors of the canine digit including melanoma, squamous cell carcinoma, and subungual keratoacanthoma. Participants also reviewed the ultrastructural features of plasma cells and amyloid as well as types of amyloid (readers are encouraged to review Conference 8, Case 4 – a case of amyloidosis in a macaque - for a more in-depth discussion about amyloid).

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**CASE III – 46349 9A (AFIP 3027711)**

**Signalment:** 3-year-old, male, canine, Shih Tzu.

**History:** The animal was presented to The Animal Medical Center on 11/05/05 with a history of 3 days of decreased appetite, vomiting and diarrhea with melena. On physical examination, the dog had hyphema and abdominal pain. While hospitalized, the dog collapsed, was resuscitated once, but died after 3 hours on a ventilator. The clinical suspicion for the cause of death was acute internal hemorrhage.

**Gross Pathology:** On necropsy, the gross findings were as follow:

1. Severe, multifocal gastric ulceration with moderate amount of dark brown hemorrhagic content in gastric lumen
2. Severe fibrinous pericarditis with multifocal myocardial hemorrhage
3. Mild hydrothorax and ascites
4. Moderate lymphadenomegaly with hemorrhages (retropharyngeal and mesenteric lymph nodes)
5. Severe, diffuse pulmonary congestion and edema
6. Severe, diffuse small intestinal mucosal hyperemia

**Laboratory Results:** There was significant increase of creatinine (4.2 mg/dl [normal: 0.4 - 1.8]), blood urea nitrogen (135 mg/dl [7 - 27]) and phosphorus (>25 mg/dl [2.1 - 6.3]) values. The serologic tick-borne disease panel was positive for exposure to *Borrelia burgdorferi* (1:256), and negative for *Ehrlichia canis* and *Rickettsia rickettsii*.

**Histopathologic Description:** Two sections of kidney reveal diffuse glomerular, interstitial and tubular changes. The glomerular lesion is characterized by increased overall glomerular size and severe thickening of the glomerular capillaries by a deep acidophilic and glassy homogeneous material, evenly deposited along the capillary walls, with occasional nodular areas. The glomerular tufts also have increased cellularity, with moderate amounts of pyknotic nuclei, and synechiae are multifocally seen. The Bowman's capsules reveal mild to moderate hypertrophy and hyperplasia of the visceral epithelium, with thickening of the basement membrane, and lamellar peri-glomerular fibrosis. A deposition of an irregularly floccular and deep acidophilic fibrinous material is occasionally seen in the interstitium surrounding the glomerular capsules. The interstitium has multiple scattered aggregates of moderate numbers of plasma cells and fewer lymphocytes. Minimal amount of loose fibrous tissue is multifocally seen between tubules. Small

numbers of tubules reveal intra-luminal sloughed epithelial cells with pyknotic nuclei. A few tubules exhibit cells with enhanced cytoplasmic acidophilia and loss of nuclear detail, associated or not with an attenuated epithelial lining. Moderate numbers of tubules are dilated and contain acidophilic (proteinaceous) casts. There is mild and multifocal mineralization of cortical tubular basement membranes, glomeruli and Henle loops. A finely granular dark brown pigment is rarely seen within the cytoplasm of the tubular epithelium.

**Contributor's Morphologic Diagnoses:** 1. Kidneys: Marked, chronic, diffuse, global membrano-proliferative glomerulonephritis  
2. Kidneys: Moderate, multifocal lymphoplasmacytic interstitial nephritis with minimal interstitial fibrosis  
3. Kidneys: Mild, acute, multifocal tubular necrosis with moderate tubular regeneration

**Contributor's Comment:** In this case, the most significant gross changes, such as gastric ulcerations with hemorrhage, fibrinous pericarditis, hydrothorax, hydroperitoneum and hyphema, were secondary to severe multi-systemic necrotizing fibrinoid vasculitis caused by uremia. Although not grossly visible, microscopic evaluation of the kidney revealed a severe nephropathy, which had two major components: a marked membranoproliferative glomerulonephritis, and an interstitial nephritis, in which plasma cells were the predominant cell type, without significant interstitial fibrosis. A third component was mild acute tubular necrosis with mild tubular regeneration. The histologic features of the nephropathy observed in this dog is compatible with the distinctive renal lesion that has been putatively associated with *Borrelia burgdorferi* infection in dogs, and that has been referred to as "Lyme nephropathy" in recent literature.<sup>1,4,5</sup> Exposure to *Borrelia burgdorferi* was documented in this dog by a positive serologic titer.

Lyme borreliosis is caused by *Borrelia burgdorferi*, of the order *Spirochetes*, which also contains other genera of pathogenic bacteria such as *Leptospira* spp. and *Treponema* spp. *Borrelia* spp. are spiral-shaped, 10-30 x 0.18-0.25  $\mu\text{m}$ , Gram-negative and have an outer sheath encasing endofibrils.<sup>2,4</sup> They are not free-living organisms (quickly die outside the body) and have special culture requirements.<sup>4</sup> Unique features are a linear chromosome and life cycles that require arthropod vectors and mammalian hosts.<sup>4</sup> Mechanisms for survival in the hosts include change in surface protein expression, a lack of requirement for iron, and the fact that they can change their shape and hide in folds of cellular membranes, which might contribute to protection against the host's immune response and antibiotics.<sup>4</sup> Lyme disease occurs in humans and multiple nonhuman animal species, and is transmitted by ticks of the *Ixodes* genus.<sup>2,3,4</sup> *B. burgdorferi sensu lato* is divided into multiple strains (genospecies) that include *B. burgdorferi sensu stricto* (the

cause of Lyme disease in North America), and others such as *B. garinii* and *B. afzelii* which occur in Europe and Asia.<sup>3,4</sup>

The commonly recognized clinical signs and lesions associated with spontaneous canine Lyme disease are fever and polyarthritis with reactive lymphadenopathy. Neurologic and cardiac disease (common disorders in human infection) has been rarely reported in naturally infected dogs, and cutaneous rash does not occur.<sup>2,3,4</sup> Histological studies of experimental canine Lyme disease have also shown dermatitis at the site of tick bite, and a mild subclinical meningitis and/or encephalitis.<sup>6</sup> A protein losing nephropathy has been described in a relatively small number of natural cases, but the exact prevalence of this manifestation has not been determined.<sup>5</sup> Lyme nephropathy has been described as morphologically and clinically unique, and as the only fatal form of Lyme disease in dogs.<sup>1</sup> It affects mostly dogs under 5 years of age, and has a rapid progression. Usually the clinical course is of 6 to 8 weeks, but sudden onsets of lethargy, anorexia and vomits can occur, and the clinical course can be as acute as 24 hours.<sup>1</sup> A predilection for Labrador and Golden Retrievers and Shetland sheepdogs has been suggested.<sup>1,5,6</sup> In canine spontaneous glomerulonephritis of other etiologies, the dogs are usually older, the disease is slowly progressive, and there is no breed predilection. The presence of tubular necrosis and regeneration is an unusual feature not seen in other canine glomerular diseases, in which tubular changes are usually restricted to dilation with luminal protein casts. In Lyme nephropathy, the glomerular lesion is membranoproliferative in most cases, and associated with C3, IgG and IgM deposits, which supports an immune-mediated pathogenesis.

Ultrastructurally, the lesion is characterized by the presence of electron-dense subendothelial deposits, multifocally along the glomerular basement membrane. Although these deposits are not seen within the mesangium, mesangial matrix is increased. Glomerular endothelial cells present with cytoplasmic vacuolation and separation from the basement membrane. Also parietal cells will present swollen and with cytoplasmic vacuolation.<sup>1</sup> Another microscopic feature described on Lyme nephritis is a lamellar periglomerular fibrosis of the Bowman's capsule, which was also seen in our case.<sup>6</sup> The exact cause for the tubular necrosis is unknown. Deposition of complement or immunoglobulins has not been detected along tubular basement membranes. The suggested mechanisms for tubular necrosis in these dogs include hypoxia (possibly related to a perfusion disorder caused by the glomerular lesions) or nephrotoxicity. In spite of the glomerular and tubular changes, and the interstitial infiltration by lymphocytes and plasma cells, interstitial fibrosis is not a significant feature of Lyme nephropathy. Other necropsy findings of dogs with Lyme nephropathy are usually related to uremia.<sup>6</sup>

In cases of Lyme nephropathy, the glomerular deposits are PAS positive and Masson's trichrome and Congo Red negative, although amyloidosis is an occasional

component of the lesion, and then be positive for Congo Red. Silver stains can help for the visualization of spirochetes within the lesions, although spirochetes are rarely found and can also be seen in dogs with Lyme disease but without renal disease.<sup>1</sup> Experimental infection of dogs and other species have failed to reproduce the renal syndrome putatively associated with *B. burgdorferi* in dogs, therefore Koch's postulates has not been satisfied.<sup>1,5,6</sup> It is important to note that no studies of experimental Lyme disease have been conducted in dogs of the breeds that are presumably susceptible to Lyme nephropathy.<sup>5</sup> Why the Lyme nephropathy occurs in some dogs and not in others, and why the disease cannot be reproduced experimentally is unknown at this point.<sup>5</sup> Factors that might be involved in the specificity of the development of canine Lyme nephropathy include age and breed of the dogs, genetic susceptibility, different strains of *B. burgdorferi*, likelihood of multiple exposures and the presence of coinfections.<sup>1,5</sup>

It has been suggested that *B. burgdorferi* may not be the causative agent of Lyme nephropathy, but just a marker for tick exposure. Since mixed infections with *Ehrlichia* spp., *Babesia* spp. and *Bartonella* spp. can occur in dogs exposed to ticks, and thrombocytopenia, glomerulonephritis and neurologic signs (also seen in Lyme disease) have been seen in dogs infected by *Ehrlichia* spp. and *Babesia* spp., it has been suggested that another agent, rather than *B. burgdorferi* may cause the nephropathy.<sup>4</sup> It is also unknown if vaccination for Lyme disease in dogs can have a role in the development of the lesion.<sup>1,3</sup> This specific renal lesion has been seen in vaccinated and non-vaccinated animals, and titers for *B. burgdorferi* are higher for dogs with Lyme nephropathy than for healthy dogs exposed to the agent.<sup>1,5</sup>

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**AFIP Diagnosis:** Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, marked, with tubular degeneration, necrosis, regeneration, proteinosis, and moderate chronic lymphoplasmacytic interstitial nephritis, Shih Tzu (*Canis familiaris*), canine.

**Conference Comment:** The contributor provides an excellent overview of Lyme nephropathy in the dog caused by *Borrelia burgdorferi*.

Conference participants reviewed causes of immune complex glomerulonephritis in different species. Readers are encouraged to review WSC 17 / Case IV, 2006-2007 – membranoproliferative glomerulonephritis in a macaque – which includes a chart summarizing the cause of immune complex glomerulonephritis in various species.

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**CASE IV** – Abbott 2006 (AFIP 3030458)

**Signalment:** 10-week-old Sprague-Dawley, *Rattus norvegicus*, rat.

**History:** The rat was part of a high dose group in a one month oral toxicity study using a proprietary compound. Necropsy was conducted at the end of the treatment period, followed by routine ultrastructural evaluation of the kidneys.

**Gross Pathology:** Kidneys were unremarkable at necropsy.

**Histopathologic Description:** Kidney: The image on the right is that of a renal glomerulus, characterized by capillary loops, recognizable by its content of a tangential section of an erythrocyte in one loop. The capillaries are lined by a thin layer of fenestrated endothelial cytoplasm and an endothelial cell body can be seen bulging into one of the capillary lumens. At least three podocytes can be seen giving rise to several primary and many secondary foot processes that rest on the glomerular basement membrane. A mesangial cell, grazing sections of mesangial matrix and the tortuous course of the urinary space can also be recognized. The cytoplasm of podocytes, endothelial cells and mesangial cells contain four to eighteen predominantly uncentric, electron dense, round to oval bodies of varying size that are composed of multi lamellar membranous whorls (lamellar bodies).

The image at the left is that of a renal proximal tubular epithelial cell, characterized by simple cuboidal epithelium and a prominent brush border of tall microvilli. The epithelial cell contains at least twelve predominantly multicentric lamellar bodies of variable size, randomly distributed in the cytoplasm. A few lamellar bodies are also present in the lumen.

**Contributor's Morphologic Diagnosis:** Kidney: Lamellar bodies, intracytoplasmic, multiple, glomerulus, proximal tubular epithelium, consistent with renal phospholipidosis.

**Contributor's Comment:** Similar ultrastructural changes were also observed in the liver (hepatocytes, Kupffer cells, biliary epithelial cells and endothelial cells), lungs (alveolar macrophages, type I pneumocytes, type II pneumocytes, Clara cells, ciliated bronchial epithelial cells and endothelial cells, and circulating lymphocytes. The changes observed are consistent with phospholipidosis consequent to administration of cationic amphiphilic drugs (CADs). Many drugs belonging to this family of compounds are known to induce this lesion. Aminoglycosides (Gentamycin) are good examples, and cause renal toxicity in humans and animals.

There are three mechanisms by which this group of compounds causes phospholipidosis:

1. By direct interaction with cellular phospholipids. CADs bind to phospholipids and the complex resists degradation by lysosomal enzymes.
2. By inhibition of the enzymes (phospholipases) that degrade phospholipids.
3. By upregulating the synthesis of phospholipids in the cell.

Excessive intracellular accumulation of poorly degraded phospholipids occurs in the lysosomes. They often acquire a multilamellar morphology that is recognizable ultrastructurally. Although such lamellar bodies can potentially occur in any tissue, they are commonly present in lung, liver, kidney, eye, brain, and occasionally in circulating lymphocytes. There is wide variation in species, breed and strain susceptibility to phospholipidosis. Because the distribution of specific phospholipids varies by tissue, target tissues for specific compounds vary, and because composition of specific phospholipids within a tissue varies by age, susceptibility may also vary by age. In the context of most of the compounds studied, phospholipidosis is thought to be largely an adaptive response and its functional consequences are not completely known, although accumulation of lamellar bodies in large numbers in a cell is known to induce apoptosis.<sup>1</sup>

**AFIP Diagnosis:** Kidney, glomerulus and proximal convoluted tubular epithelium: Lamellar bodies, intracytoplasmic and extracellular, consistent with renal phospholipidosis, Sprague Dawley rat (*Rattus norvegicus*), rodent.

**Conference Comment:** The contributor provides a concise summary of phospholipidosis and the mechanisms by which cationic amphiphilic drugs (CADs) can cause phospholipidosis.

Conference participants reviewed the ultrastructural components of a glomerulus to include capillary loops, fenestrated endothelial cells, podocytes and mesangial cells. The ultrastructure of renal proximal tubular epithelial cells was also reviewed to include cuboidal to columnar epithelium, a prominent brush border with tall microvilli, and mitochondria arranged perpendicularly to the basement membrane. Additionally, ultrastructural abnormalities in various organelles and reversible and irreversible ultrastructural changes were discussed.

Reversible cell injury manifests as morphologic and functional changes that are reversible if the damaging stimulus is removed. The hallmarks of reversible injury are reduced oxidative phosphorylation, depletion of ATP, and cellular swelling. If the damage continues, the injury becomes irreversible. Certain structural changes such as amorphous densities in mitochondria, indicative of severe mitochondrial damage and loss of membrane permeability, indicate that cells have undergone irreversible injury.<sup>3</sup>

Included below are two charts summarizing basic ultrastructural abnormalities in various organelles as well as reversible and irreversible ultrastructural changes.<sup>2,3</sup>

## **Basic Ultrastructural Abnormalities in Various Organelles**

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### **Plasma membrane**

- Loss of surface specialization
- Breakdown of attachments
- Cytoplasmic blebs

### **Cytosol**

- Rarefaction (swelling)
- Depletion of glycogen
- Presence of myelin figures

### **ER**

- Swelling/dilation
- Detachment of ribosomes

### **Lysosome**

- Swelling
- Rupture

**Mitochondria**

Cristolysis  
 Condensation  
 Swelling  
 Rupture  
 Matrix flocculent densities  
 Vacuolation

**Nucleus**

Clumped chromatin  
 Pyknosis, karyorrhexis, karyolysis

**Other**

Inclusions  
 Parasites, bacteria, fungi, algae

**Reversible and Irreversible Ultrastructural Changes**

<b>Reversible</b>	<b>Irreversible</b>
Plasma membrane alterations: Blebbing, blunting, distortion, creation of myelin figures, loosening of intercellular attachments	Overt discontinuities in plasma membrane Disruption of lysosomes
Mitochondria: Swelling, rarefaction, small amorphous densities	Large amorphous densities in mitochondria
Dilation of ER with detachment of ribosomes	Pronounced myelin figures Amorphous osmophilic debris
Clumping of nuclear chromatin	Profound nuclear changes

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