



WEDNESDAY SLIDE CONFERENCE 2009-2010

# Conference 8

19 November 2009

*Conference Moderator:*

Gary D. Coleman, DVM, PhD, Diplomate ACVP, Diplomate ACVPM

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**CASE I: A08-352 (AFIP 3133678).**

**Signalment:** 5-year-old, male, intact Rhesus macaque (*Macaca mulatta*).

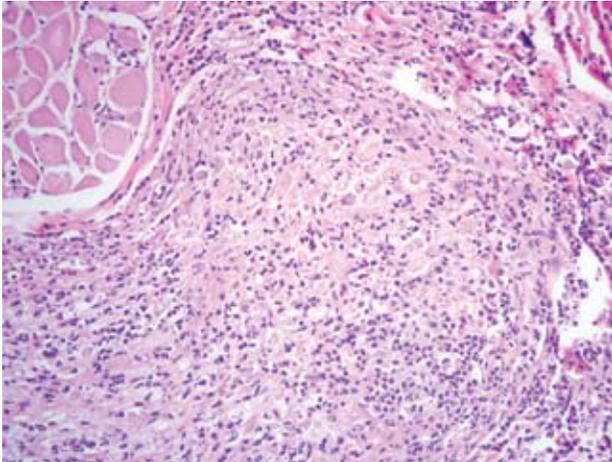
**History:** This macaque presented for necropsy at the end of an experiment investigating vaccine candidates for Simian Immunodeficiency Virus (SIV). The animal had been losing weight for the previous four months but was clinically unremarkable. Experimental manipulation included biweekly phlebotomy. Two years prior to necropsy, the animal had been vaccinated with an experimental vaccine and seven months prior to necropsy had been inoculated with SIVmac251.

**Gross Pathology:** The animal was thin with few fat reserves and the right atrium was moderately dilated. There was a moderate amount of serous pericardial effusion. The lungs were firm and failed to deflate upon opening the chest cavity.

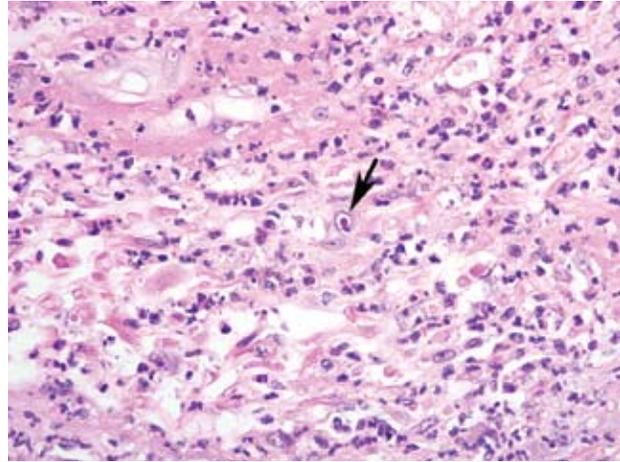
**Laboratory Results:** Serial chemistry profiles were unremarkable.

**Histopathologic Description:** Labial mucosa: Multifocally submucosal nerves and hair follicles are partially to completely effaced by large aggregates of degenerate and non-degenerate neutrophils admixed with fewer histiocytes, lymphocytes and necrotic cellular debris (**fig. 1-1**). Frequently, histiocytes within the inflammatory infiltrate are enlarged and contain large, eosinophilic, intranuclear, round to ovoid, 10-20  $\mu$ m diameter inclusion bodies and smaller eosinophilic intracytoplasmic inclusion bodies (**figs. 1-2 and 1-3**). Nerve fibers are completely effaced by the infiltrate and there is a dual loss of the myelin sheath and the axon. Inflammatory cells frequently infiltrate into the surrounding musculature. In these areas myofibers are variably swollen, hypereosinophilic and degenerate with sarcoplasmic vacuolation and loss of cross striations. Several large hair follicles are surrounded by a moderate amount of fibrosis and a similar neutrophilic and histiocytic infiltrate with frequent intracytoplasmic and rare intranuclear inclusion bodies. The inflammatory cells are especially abundant in the perifollicular tissue, but also are present through all layers of the hair follicle and are associated with moderate perifollicular hemorrhage and congestion.

**Contributor's Morphologic Diagnosis:** 1. Labial



1-1. Lip, peripheral nerve, rhesus macaque. Separating and surrounding axons, expanding the perineurium, and infiltrating adjacent collagen and myocytes are high numbers of neutrophils and fewer histiocytes admixed with necrotic debris. Multifocally axon sheaths are swollen and degenerate. (HE 200X)



1-2. Lip, peripheral nerve, rhesus macaque. Multifocally, cells contain large lightly basophilic intranuclear inclusions that marginalize the chromatin (arrow). Multifocally there is axonal degeneration and abundant necrotic debris admixed with many neutrophils and histiocytes. (HE 400X)

mucosa and haired skin: Severe, multifocal, subacute neutrophilic neuritis with cytomegaly and intrahistiocytic, intranuclear and intracytoplasmic herpetic inclusions (Cowdry type A).

2. Labial mucosa and haired skin: Severe, multifocal, subacute to chronic neutrophilic folliculitis with perifollicular fibrosis, cytomegaly, and intrahistiocytic, intranuclear and intracytoplasmic herpetic inclusions (Cowdry type A).

**Contributor's Comment:** Simian immunodeficiency virus (SIV) was first isolated in rhesus macaques at the New England Primate Research Center in 1985 and since then numerous strains of SIV which are endemic to different species of African monkeys have been identified. When these viruses are transmitted to Asian species they induce a disease similar to human AIDS, making the rhesus macaque model of SIV infection uniquely suited to studying HIV/AIDS pathogenesis.<sup>2</sup>

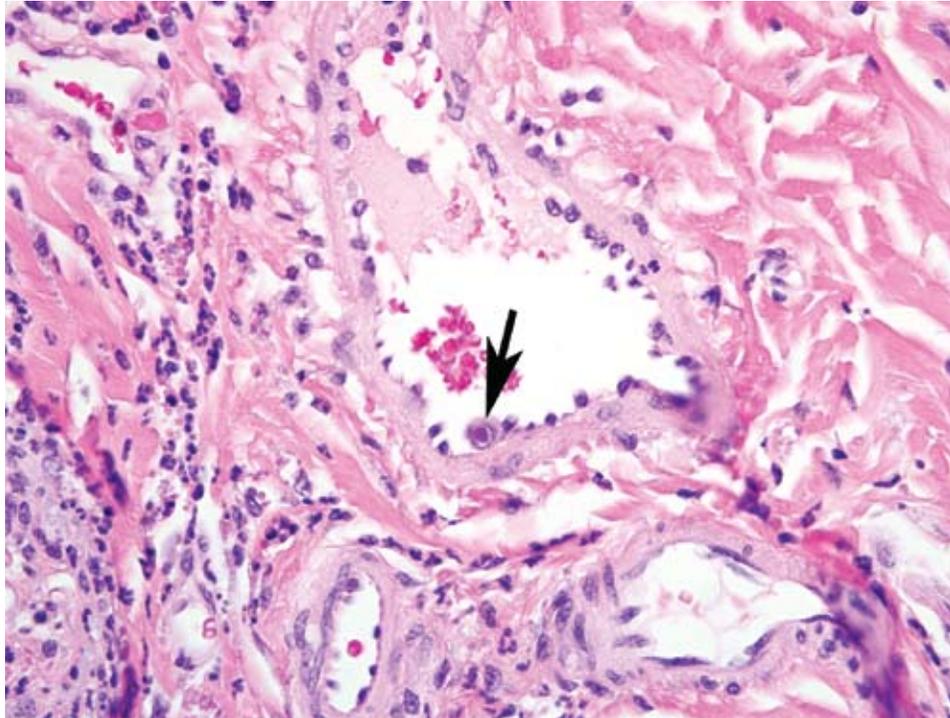
Common clinical and pathologic findings in SIV infected rhesus macaques include lymphadenopathy/lymphoma, chronic diarrhea and wasting, giant cell disease, pulmonary arteriopathy, viral associated dermatitis, and a wide spectrum of opportunistic infections. The most common opportunistic infectious agents in rhesus macaques progressing to AIDS include *Pneumocystis carinii*, *Mycobacterium avium* complex, *Trichomonas* sp., cytomegalovirus, adenovirus, *Cryptosporidium*, SV-40, *Candida* sp., and rhesus lymphocryptovirus.<sup>10</sup> Additionally, infections with *Enterocytozoon bienusi*, *Entamoeba*, *Giardia* and various alphaherpes viruses are

also seen regularly.

Cytomegaloviruses (CMV) are host-specific beta-herpesviruses that frequently establish latent infections in both rhesus macaques and humans. In rhesus macaques, CMV seroprevalence approaches 100% by one year of age but there are generally no clinical or pathologic findings associated with infection in otherwise healthy individuals.<sup>3,11</sup> Transmission is thought to be through contact with virus shed in urine, saliva, and genital secretions which is similar to findings in humans. Unlike in humans, vertical transmission has not been documented, most likely due to near 100% seroprevalence with resultant high maternal antibodies and fetal protection.

With immune suppression, as occurs with AIDS, latent CMV becomes reactivated. It is the most common viral opportunistic infection identified and among the most common opportunistic infections overall in both rhesus macaques and man with isolation from the retina, gastrointestinal tract, lungs, and adrenal gland common in humans and gastrointestinal tract, lungs, central nervous system, liver and lymph nodes in macaques.<sup>4,5</sup>

Common gross pathologic features of cytomegalovirus infection depend on the organ involved. A common finding at the NEPRC is gastrointestinal pseudotumors which present as raised, red lesions multifocally throughout the small intestine.<sup>7</sup> Less common gross findings include multifocal interstitial pneumonia, necrotizing orchitis, and gastrointestinal ulceration. Histologically, lesions are frequently characterized by intense neutrophilic infiltrates



*1-3. Lip, rhesus macaque. Rarely endothelial cells contain large lightly basophilic intranuclear inclusions which are surrounded by a clear halo and peripheralize the chromatin (arrow). (HE 400X)*

admixed with fewer lymphocytes and plasma cells. Scattered throughout these areas there are frequently large intranuclear inclusion bodies, so-called “owl’s eye cells” and also smaller intracytoplasmic inclusion bodies.

Unfortunately, these cells are not always present and as many as one half of infected animals may show varying degrees of pathology without characteristic inclusion bodies making immunohistochemistry important for definitive diagnosis of suspected cases. In fact, immunohistochemistry has shown that some animals with no underlying pathology have viral reactivation in multiple organs.<sup>5</sup>

In this case, the CMV-associated neuritis and folliculitis of the labial mucosa is quite striking. While not a common finding with CMV reactivation, neuritis is seen occasionally in rhesus macaques at the NEPRC and is most commonly seen in the labial and buccal mucosa. In humans, CMV optic neuritis and retinitis is a common finding. We have not previously identified folliculitis and this is an uncommon finding in humans as well.

**AFIP Diagnosis:** 1. Haired skin and mucocutaneous junction, lip: Polyneuritis, suppurative, acute, moderate, with axonal degeneration and intranuclear and intracytoplasmic eosinophilic and amphophilic inclusion bodies.

2. Haired skin and mucocutaneous junction, lip: Epidermal hyperplasia, focally extensive, moderate, with hyperkeratosis.

**Conference Comment:** The contributor provides an excellent synopsis of a unique presentation of CMV infection in this case, and conference participants used the discussion as a starting point to review the concepts of latency and recrudescence in herpesviral infection. Immunocompetent, asymptotically-infected rhesus macaques expend substantial immunological resources to maintain a stable virus-host relationship, and possess high numbers of circulating CD4+ and CD8+ rhesus CMV-specific T cells in peripheral blood.<sup>11</sup> Like other herpesviruses, human CMV evades the immune system by downregulating MHC class I and II molecules, and producing homologues of MHC class I molecules, IL-10, and tumor necrosis factor (TNF) receptor.<sup>6</sup> Rhesus CMV has also been shown to encode inhibitors of natural killer cell function and class I MHC assembly and transportation, and homologues of cellular IL-10, a viral inhibitor of caspase activation, and a viral inhibitor of apoptosis.<sup>11</sup>

Participants briefly reviewed other cytomegaloviruses of importance in veterinary medicine, which are summarized in the table below:<sup>1,8,9</sup>

Cytomegaloviruses in Veterinary Species			
Name	Virus Subfamily	Species Affected	Summary
Simian CMV (includes Rhesus CMV)	Betaherpesvirus	Rhesus macaque; other NHP have host-specific CMV	Seroprevalence nearly 100% in rhesus macaques; clinical disease occurs only with immunosuppression (SIV) or experimental manipulation
Caviid herpesvirus	Betaherpesvirus	Guinea pig	Subclinical infection is common; useful model of human CMV; typical "owl's eye" inclusions; targets are salivary gland, kidney, and liver
Murine CMV (Murid herpesvirus 1)	Betaherpesvirus	Mouse	BALB/c and A strain mice are susceptible; B6, B10, CBA, and C3H mice are resistant; disease occurs in immunocompromised mice; typical lesions are in salivary glands; persistent infections may cause immune complex glomerulitis
Rat CMV	Betaherpesvirus	Rat	Common in wild rats; nonexistent in laboratory rats; typical lesions in salivary and lacrimal glands with intranuclear and intracytoplasmic inclusions in ductal epithelium
Bovine herpesvirus 4	Gammaherpesvirus	Ox	Associated with "epivag" (i.e. vaginitis, salpingitis, oophoritis, or epididymitis), pneumonia, enteritis, metritis, mamillitis, and abortion
Suid herpesvirus 2	Betaherpesvirus	Pig	Causes inclusion body rhinitis in neonates; infection in pregnant sows results in small litters, mummification, stillbirth, or weak, premature offspring; typical cytomegaloviral inclusions in nasal mucosa, lung, or kidney, but not seen in the placenta
Equid herpesvirus 2	Gammaherpesvirus	Horse	Clinical significance unknown; has been identified in few cases of bronchointerstitial pneumonia in foals

The contributor's observation of inflammation involving the hair follicles was also discussed. Most conference participants interpreted the folliculitis as a secondary extension of the neurocentric inflammation into the sinus hairs, rather than primary folliculitis due to viral infection of hair follicles. When present, inflammation of the hair follicles was generally confined to well-innervated sinus hairs, and in less severely affected follicles, the inflammatory cells appeared to extend from the peripheral nerve into the stroma surrounding the adnexa.

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<http://www.hms.harvard.edu/nerprc/>

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## CASE II: T292/09 (AFIP 3134307).

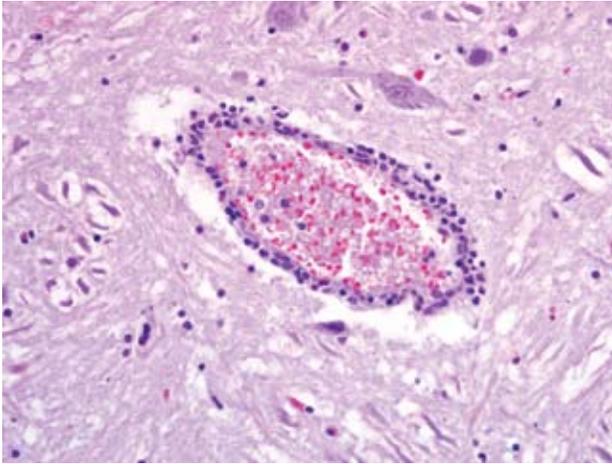
**Signalment:** 4-year-old female German shorthaired pointer dog (*Canis familiaris*).

**History:** The dog was regularly used for hunting purposes. In January 2009 the owner noticed single coughing attacks after trailing a wounded animal. The next morning the dog showed apathy, inappetence and recurrent coughing attacks. Clinical observations revealed no other symptoms. Body temperature was normal. There were no abnormal auscultation findings of thorax and abdomen. Antibiotic therapy was initiated and the owner gave a cough syrup. In the evening the animal showed regurgitation of reddish-brown fluid, salivation and progressive deterioration with dyspnea. The dog was referred to a veterinary clinic immediately. There were no specific radiographic findings and bronchoscopy was inconclusive. The animal suddenly died 30 hours after the first attack of coughing. The clinicians suspected intoxication. Pseudorabies was discussed differentially but ruled out due to the lack of itch and other central nervous signs.

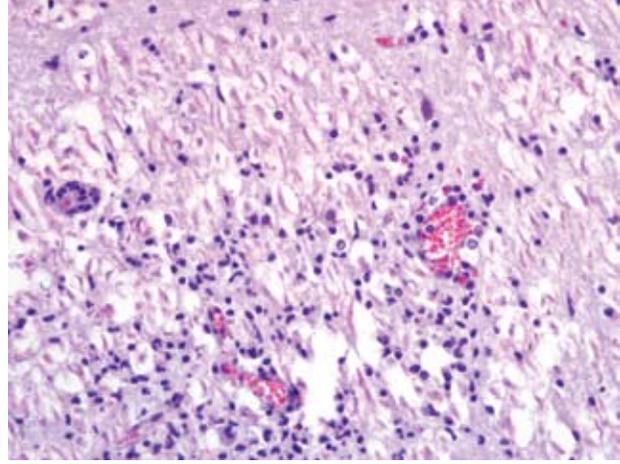
**Gross Pathology:** Unfortunately, the owner did not permit a full necropsy. However, the clinician collected samples of stomach and intestine and removed the brain for histology and/or toxicology 48 hours post mortem.

**Histopathologic Description:** Due to the suboptimal brain sampling, slides contain an angular section of cerebellum and brainstem. In the brain stem there is a moderate perivascular infiltration and cuffing composed of mononuclear cells (lymphocytes and macrophages) (**fig. 2-1**). Focally there are acute perivascular hemorrhages. Multifocally in the brainstem there are single degenerating neurons (dark neurons) and within the neuropil there are multifocal accumulations of activated microglial cells (rod cells, microgliosis) admixed with few infiltrating neutrophils (**fig. 2-2**). Single neurons and glial cells show intranuclear round eosinophilic inclusion bodies with chromatin margination. The cerebellum is unaltered. Immunohistochemically, porcine herpesvirus 1 antigen was detected within neurons and glial cells using the PAP method (**fig. 2-3**). Initially, using fresh material from intestine and brain, herpesvirus DNA was not detectable with PCR or in cell culture. In paraffin embedded material, using the gB-region in a nested PCR, porcine herpesvirus DNA was detectable.

**Contributor's Morphologic Diagnosis:** Cerebellum and brain stem: encephalitis, nonsuppurative, multifocal, moderate with perivascular lymphohistiocytic cuffing,



2-1. Brainstem, dog. Multifocally within the gray matter, Virchow-Robbins spaces contain moderate numbers of lymphocytes and fewer macrophages. (HE 400X)



2-2. Brainstem, dog. Multifocally there are glial nodules, composed of reactive astrocytes and microglia and fewer lymphocytes. (HE 400X)

neuronal degeneration, microgliosis and perivascular hemorrhages; Aujeszky's disease, pseudorabies; German shorthaired pointer dog (*Canis familiaris*).

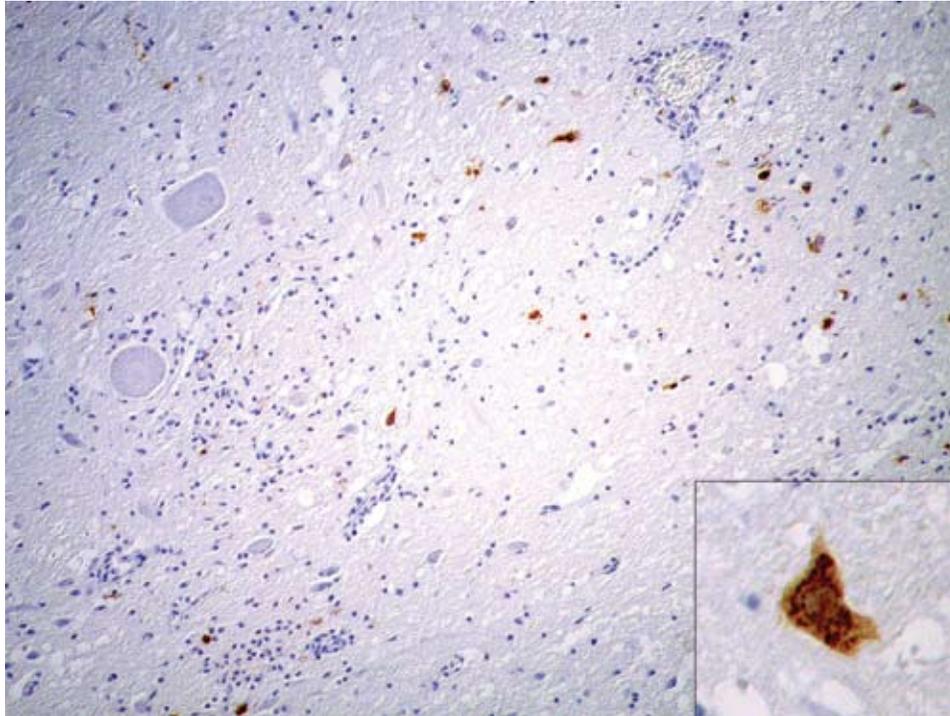
**Contributor's Comment:** Pseudorabies, also known as Aujeszky's disease, infectious bulbar paralysis or mad itch, is caused by suid herpesvirus 1, an alphaherpesvirus of the genus varicellovirus.<sup>4</sup> The disease was first described and its infectious cause identified by Aladár Aujeszky (1896-1933), a Hungarian veterinary pathologist, in 1902. An interesting historical essay was published in 2003 by Köhler and Köhler.<sup>3</sup> Farm animals in many countries in Europe are free of the disease. But the increasing population of wild pigs in some areas (especially in Germany) serves as a reservoir and there is a general risk of transmission to livestock.<sup>8</sup> Suid herpesvirus was the object of intensive virological research in the past and the virus serves as model for other alphaherpesvirus infections and for developing marker vaccines and DNA vaccines.<sup>2,5,7,9</sup> The typical clinical symptom in animals other than pigs is intense pruritus at the locus of inoculation and the outcome is lethal in nonnative hosts. Dogs can be infected by ingestion of raw meat from infected pigs (farm or wild pigs) or percutaneously (bites, trauma). The virus spreads centripetally after inoculation along nerves to the spinal ganglia and the central nervous system.<sup>4</sup> Symptoms in dogs are similar to rabies but the clinical course is much shorter. Gross lesions are limited to self trauma due to severe itching.<sup>4</sup> Histologically, nonsuppurative encephalitis with gliosis and ganglioneuritis (e.g. trigeminal or spinal ganglia) can be found. Typical herpesvirus inclusion bodies can be found in neurons and glial cells. Virus antigen can be

detected immunohistochemically on paraffin-embedded formalin-fixed material.<sup>4</sup>

The clinical course in the present case was very rapid (only 30 hours) with early lethal outcome. Contact of the dog with a wild pig or blood and/or carcasses of hunted wild pigs can be presumed. Due to the coughing attacks as the predominant symptoms, inhalation as possible route of infection can be discussed. Unfortunately, lung material was not submitted for examination. Stomach and intestine were unaltered. Virus antigen could not be detected immunohistochemically within tissues other than the brain.

**AFIP Diagnosis:** Brainstem: Encephalitis, non-suppurative, multifocal, mild to moderate, with gliosis.

**Conference Comment:** Participants readily attributed the striking nonsuppurative encephalitis in this case to a viral etiology; however, nonsuppurative inflammation in the central nervous system (CNS) is not entirely specific for viral infections, with salmonellosis in pigs and neorickettsial infection (i.e. "salmon poisoning") in dogs being notable examples of non-viral causes of nonsuppurative CNS inflammation.<sup>4</sup> There was variation with respect to the presence of intranuclear inclusion bodies and scattered neuronophagia, and most participants developed a differential diagnosis that included several viral etiologies. Many conference participants suspected canine morbillivirus as the etiology; however, this case lacks the characteristic morbilliviral intracytoplasmic and intranuclear viral inclusions, which are generally detectable in astrocytes, and occasionally ependymal



2-3. Brainstem, dog. Neurons and glial cells are immunohistochemically positive for porcine herpesvirus 1 antigen. Photomicrograph courtesy of Institute for Veterinary Pathology, University of Giessen, Frankfurter Str. 96, 35392 Giessen, Germany, kernt.koehler@vetmed.uni-giessen.de

cells and neurons, in dogs with canine distemper virus infection. In early canine distemper, lesions include demyelination, status spongiosus, astrocytic hypertrophy and hyperplasia, and variable syncytial cell formation, all of which are lacking in this case. In late-stage canine distemper, lesions include nonsuppurative perivascular cuffing, leptomeningitis, and choroiditis, with occasional gitter cells, and most participants who favored a diagnosis of distemper specifically favored “old dog encephalitis” associated with distemper.<sup>4</sup> Other viral etiologies considered by conference participants included rabies and the arboviruses.

Pseudorabies is an unusual alphaherpesvirus in that it is proficient in interspecies transmission. Conference participants briefly discussed two other closely-related alphaherpesviruses that share this capability: human herpesvirus-1 (HHV-1, herpes simplex virus) and cercopithecine herpesvirus-1 (herpes B virus, BV). Humans are the primary hosts of HHV-1, with a seroprevalence of approximately 80%. Infection in humans is generally mild, characterized by labial, oral, and ocular lesions, with encephalitis being a rare consequence of centripetal infection through the olfactory, optical, or trigeminal nerves. However, rabbits are exquisitely sensitive to HHV-1, where the virus is exclusively neurotropic and almost invariably fatal. Less susceptible species include rats, mice, and chinchillas.<sup>6</sup> An analogous

situation exists with BV, which is endemic in macaques and a seroprevalence of 80-100% in most populations. Following initial replication in mucosal epithelial cells, the virus is transmitted by axonal transport to dorsal root ganglia, where it establishes latency characterized by a lack of viral replication and a limited pattern of transcription. Episodes of recrudescence are generally asymptomatic, or less frequently cause oral herpetic lesions. Humans, by contrast, are exquisitely sensitive to BV, with infection being almost uniformly fatal in the absence of early treatment with antiviral therapy.<sup>1</sup> Other alphaherpesviruses of veterinary importance were briefly reviewed in WSC 2009-2010, Conference 5, case II.

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[http://www.uni-giessen.de/cms/fbz/fb10/institute\\_klinikum/institute/pathologie](http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie)

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### CASE III: 08N592 (AFIP 3134541).

**Signalment:** Four 5-week-old, cross-bred pigs (*Sus scrofa*).

**History:** The pigs were weaned two weeks previously, and treated with ivermectin at weaning. About 20 pigs died in a herd of 200 animals.

**Gross Pathology:** All four pigs had a generalized, greasy, moist appearance on the skin with multifocal crusting (**fig. 3-1**). Multifocally, there were multiple, raised, circular, crusty lesions (0.5-2.0 cm in diameter) around the eyes, and on the snout, chin, thorax, abdomen and legs (**figs. 3-2 and 3-3**). Exudate matted the eyes of one pig closed. There was mild separation of the hooves and erosions were present on the coronary bands.

**Laboratory Results: Bacteriology:** *Staphylococcus*

*hyicus* was cultured from multiple skin samples. **Virology:** Swinepox virus was identified with electron microscopy from the circular, raised lesions.

**Histopathologic Description:** Multifocally, the epidermis is ulcerated with an overlying serocellular crust composed of neutrophils, fibrin, and serous proteinaceous material admixed with numerous colonies of bacterial cocci; additionally, there are frequent intracorneal pustules composed of degenerate neutrophils that expand the stratum corneum (**figs. 3-4, 3-5, and 3-6**). The epidermis in intact areas is multifocally, moderately hyperplastic with prominent rete pegs, and there are frequent neutrophils noted in exocytosis across the epithelial surface. Multiple follicular lumina are also filled with numerous degenerate neutrophils with occasional eosinophils. Multifocally, epithelial cells exhibit ballooning degeneration or hydropic change and contain occasional 5-7 µm diameter, eosinophilic, intracytoplasmic inclusion bodies (poxviral inclusions) (**fig. 3-7**). There are occasional apoptotic cells scattered throughout the epidermis and follicular epithelium characterized by shrunken, hypereosinophilic cells with pyknotic or karyorrhectic nuclei. The dermis subjacent to the ulcerated areas is heavily infiltrated by lymphocytes, plasma cells, histiocytes, and scattered neutrophils with frequent fibroblasts and small capillaries (granulation tissue).

**Contributor's Morphologic Diagnosis:** 1. Skin: dermatitis, proliferative and ulcerative, multifocal severe, with ballooning degeneration, granulation tissue, and intracytoplasmic eosinophilic inclusion bodies, porcine (*Sus scrofa*).

2. Skin: epidermitis and folliculitis, suppurative (exudative), multifocal to coalescing, severe, with numerous bacterial cocci colonies, porcine (*Sus scrofa*).

**Contributor's Comment:** Greasy pig disease, or exudative epidermitis, is caused by *Staphylococcus hyicus*, a gram positive bacterium. The disease occurs most commonly following introduction of carrier animals into a naïve herd.<sup>12</sup> The pathogenesis of exudative epidermitis is not completely known; however, trauma from fighting, unclipped teeth, rough bedding, or other factors leading to exposure of the dermis may allow the bacteria to establish the infection.<sup>6,12</sup> Initially, there is reddening of the skin with multiplication of bacteria on skin surface and growth between keratinocytes. In infected skin, there is generally marked inflammation with hyperplasia of the stratum corneum and neutrophilic infiltration followed by epidermal erosion.<sup>12</sup> The most important factor in pathogenesis of infection is the production of exfoliative toxins. The exfoliative toxins are known as: ExhA, ExhB, ExhC, ExhD, SHETA and SHETB.<sup>3,10</sup> Exudative epidermitis



3-1. Haired skin, pig. There is generalized, greasy, moist skin with multifocal crusting. Photograph courtesy of Louisiana State University, School of Veterinary Medicine, Dept. of Pathobiological Sciences, Baton Rouge, LA 70803, dcho@vetmed.lsu.edu

is regarded as a porcine homologue of Staphylococcal Scalded Skin Syndrome (SSSS) or bullous impetigo in humans. SSSS results in loss of keratinocyte cell-to-cell adhesion and leads to blister formation.<sup>9</sup> The virulent strains of *S. aureus* produce exfoliative toxins, namely ETA, ETB and ETD.(1) It has been reported that these toxins are glutamate specific serine proteases that cleave a single peptide bond in the extracellular region of human and mouse desmoglein (Dsg) 1.<sup>2</sup> In human epidermis, Dsg1 and Dsg3 are present predominantly in stratified squamous epithelium.<sup>11</sup> Recent studies have suggested that pathophysiological mechanisms of intraepidermal splitting in exudative epidermitis are similar to human SSSS by cleavage of Dsg1.<sup>5,8,9</sup> The detailed differential list for exudative epidermitis has already been described in WSC 2008-2009, Conference 9, case I.

Swine pox is a typical poxvirus primarily affecting young pigs and lesions are usually confined to the ventrolateral abdomen.<sup>3</sup> Transmission is typically through direct contact, although lice (*Haematopinus suis*) and other blood-sucking insects can be an important means of transmission in swine herds as well.<sup>3</sup> Lesions follow the typical pox progression: erythematous macules becoming papules, then vesicles progressing to pustules, leading to rupture and crust formation.<sup>3</sup> Differentials for swine pox

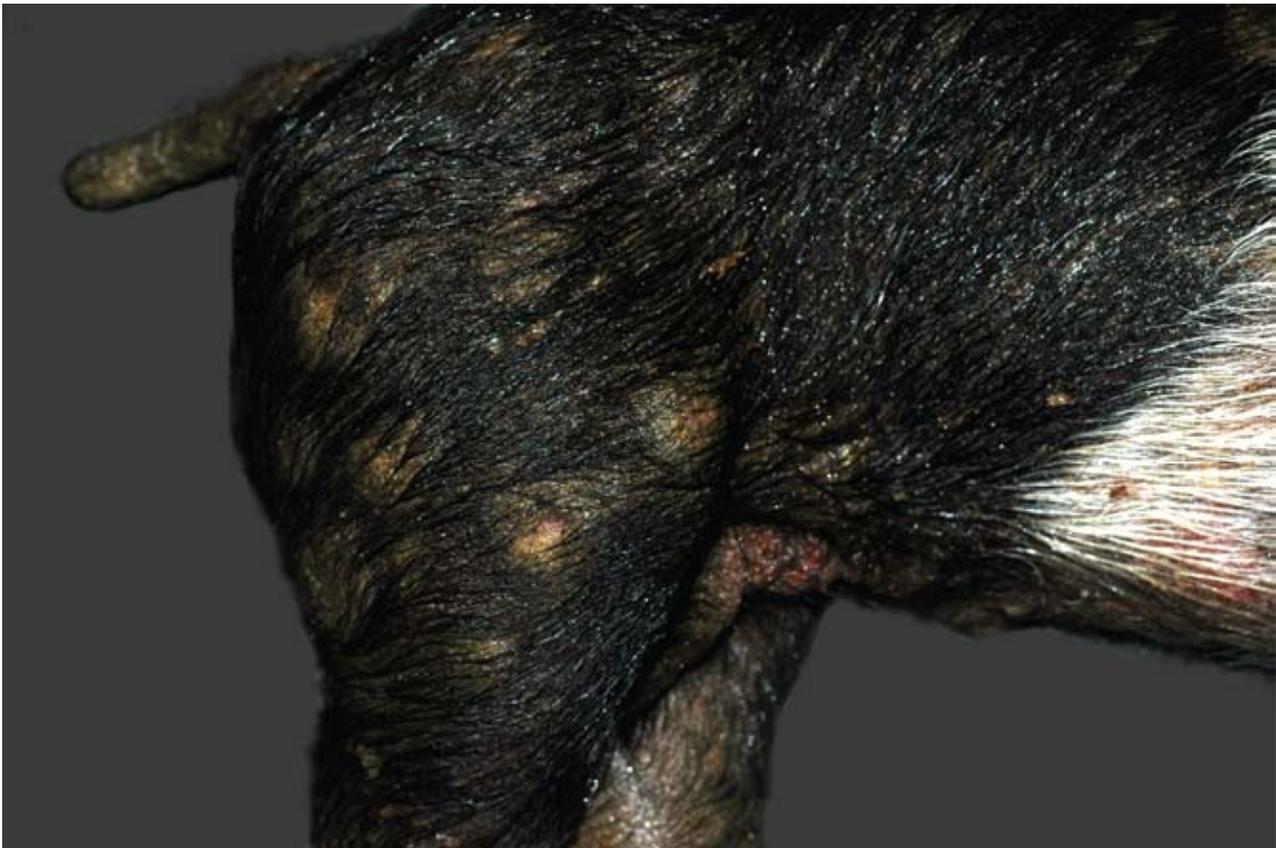
must include other vesicular diseases, sarcoptic mange, and erysipelas.<sup>3</sup> Swinepox virus is the sole member of the *suipoxvirus* genus and is morphologically similar to vaccinia virus; it has a double-stranded DNA genome of 146 kilobase pairs and 150 predicted genes.<sup>4</sup> Following an abrasion to the skin, swinepox may enter the host and preferentially replicates in epidermal keratinocytes of the stratum spinosum; replication occurs exclusively in the cytoplasm, as indicated by the intracytoplasmic inclusion bodies.<sup>4</sup>

In this case, the swine pox infection was likely the predisposing factor leading to the staphylococcal infection. In addition to the skin lesions in these cases, there was evidence of embolic showering of bacteria within the lungs and lymph nodes with suppurative inflammation in all pigs examined (evidence for systemic infection). Therefore, the cause of death in these pigs appears to be sepsis secondary to epidermal infection with *Staphylococcus hyicus*, made possible by the ulcerative lesions caused by the poxviral infection.

**AFIP Diagnosis:** Haired skin: Dermatitis, proliferative and necrosuppurative, chronic, diffuse, marked, with epidermitis, folliculitis, and many cocci.

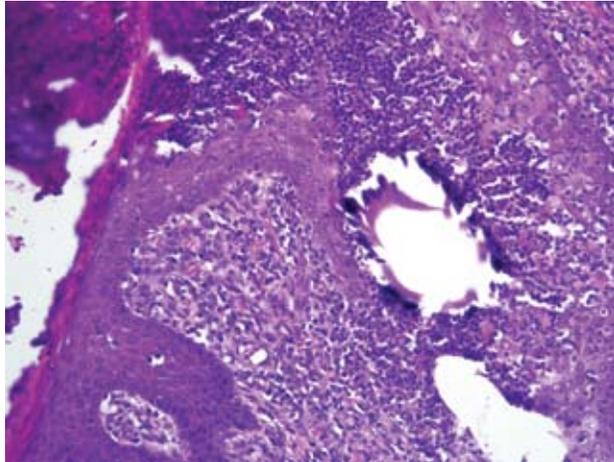


3-2, 3-3. Haired skin, pig. Multifocally there are multiple raised, circular, crusty lesions on the snout, chin, thorax, abdomen, and legs. Photograph courtesy of Louisiana State University, School of Veterinary Medicine, Dept. of Pathobiological Sciences, Baton Rouge, LA 70803, dcho@vetmed.lsu.edu

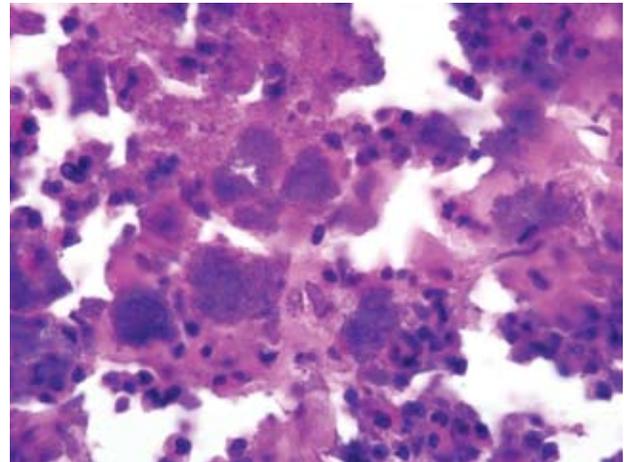


**Conference Comment:** During the conference, the moderator highlighted that the presence of many acantholytic keratinocytes in this case is reminiscent of the autoimmune condition of pemphigus foliaceus. This observation is meaningful in light of the contributor's comments concerning the pathogenesis of staphylococcal cutaneous infections, and this case provides an exquisite

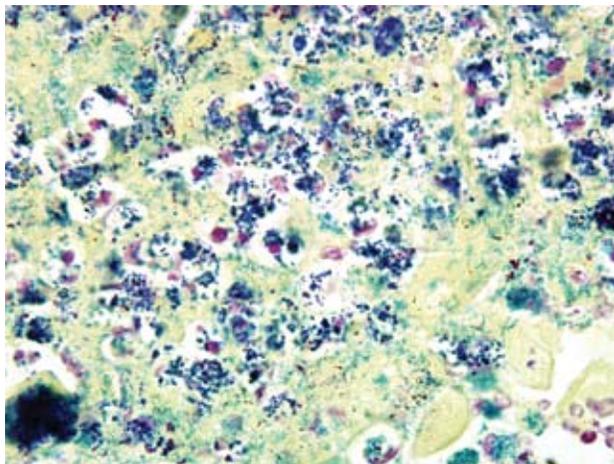
example of two diseases sharing a common molecular link, i.e. desmogleins as the target of bacterial toxins and autoantibodies, resulting in histomorphological similarities. As noted by the contributor, Dsg1 and Dsg3 are both components of desmosomes, but differ with respect to their histologic distributions within the epidermis and anatomic locations. The Dsg1 protein, the target of



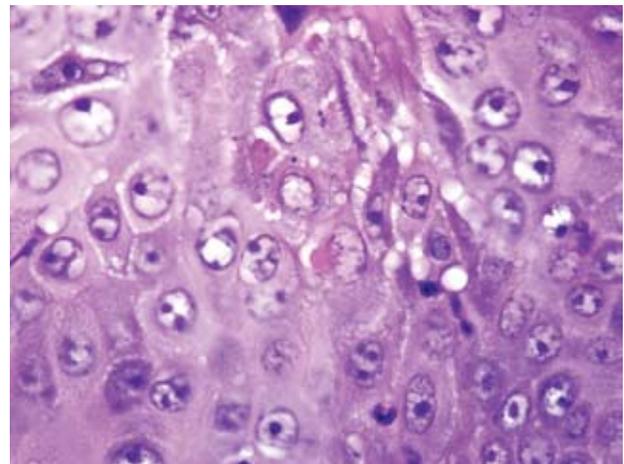
3-4. Haired skin, pig. Within the epidermis, dermis, and hair follicle epithelium are numerous pustules containing many degenerate neutrophils, with overlying parakeratotic and orthokeratotic hyperkeratosis. (HE 400X)



3-5. Haired skin, pig. Within the degenerate hair follicle are numerous aggregates of neutrophilic inflammation and necrotic cellular debris which center upon large colonies of 2µm diameter basophilic cocci. (HE 1000X)



3-6. Haired skin, pig. Large colonies of cocci are gram positive. (B&B 1000X)



3-7. Haired skin, pig. Rarely epithelial cells contain large eosinophilic cytoplasmic viral inclusions and nuclear chromatin of affected cells is peripheralized. (HE 1000X)

autoantibodies in pemphigus foliaceus, is most abundant in the superficial layers of the epidermis and is scarce in oral mucosa, which accounts for the characteristic histologic pattern of acantholytic subcorneal and intragranular pustular dermatitis and an absence of oral lesions in the disease. The Dsg3 protein is more abundant in the basal layers of the epidermis and oral mucosa and, along with Dsg1, is targeted by autoantibodies in pemphigus vulgaris thus explaining the characteristic suprabasilar clefting in the epidermis and lesions found in the oral mucosa.<sup>7</sup>

There is substantial slide variation with respect to the ballooning degeneration and poxviral inclusions described by the contributor. Most conference participants' slides

lacked this feature or had only rare examples. This variation is not surprising given that the submission consisted of samples from multiple animals. The contributor provided a succinct review of swinepox, and readers are referred to WSC 2009-2010, Conference 5, case II for additional discussion regarding poxviruses.

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#### CASE IV: 07-684-5 (AFIP 3136053).

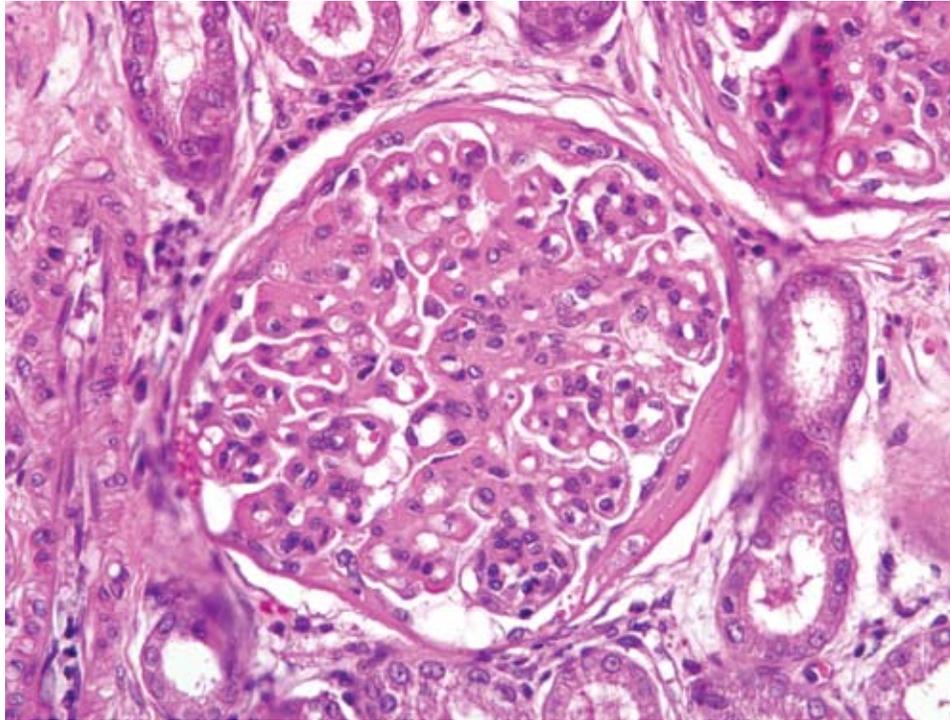
**Signalment:** 5-year-old spayed female golden retriever dog (*Canis familiaris*) weighing 30.9 kg.

**History:** The dog, who lived in the northeastern United States, initially presented to the referring veterinarian for a two-week history of vomiting, decreased appetite, polydipsia, and lethargy.

**Gross Pathology:** Both kidneys were reniform in shape with smooth capsular surfaces and diffusely pale tan cortices; medullae were diffusely bulging on cut section. There was marked perirenal edema and subcutaneous tissues were severely edematous. Hematomas were present in the right retroperitoneal space and ventral abdominal midline. Parathyroid glands were enlarged bilaterally (5 x 3 x 0.5 mm and 6 x 3 x 1 mm).

**Laboratory Results:** **Hematocrit:** 17.4 (37-55%). **Reticulocyte count:** 15,750 (>60,000 indicates regeneration). **Serum biochemistry:** BUN 70 (7-27 mg/dl), creatinine 4.9 (0.4-1.8 mg/dl), phosphorous 16.1 (2.1-6.3), total protein 4.1 (5.1-7.8 g/dl), albumin 1.0 (2.3-4.0 g/dl). **Urinalysis:** urine protein 1,253 (10-50 mg/dl), creatinine 76.8 (100-500 mg/dl), protein/creatinine ratio 16.3 (<0.5). Urine culture: no growth. **Coagulation profile:** normal PT 7.5 (6.8-10.2 s), slightly prolonged PTT 16.9 (10.7-16.4 s), increased d-dimers 0.28 (<0.2 ug/ml), and decreased platelets 49 (177-398 x 10<sup>3</sup>/ul), consistent with disseminated intravascular coagulation. **Serology:** negative for *Rickettsia rickettsii*, *Bartonella* spp., *Ehrlichia canis*, *Anaplasma* spp., *Dirofilaria immitis*, and *Leptospira* spp.; positive for *Borrelia burgdorferi* (Idexx SNAP® 4Dx® test).

**Histopathologic Description:** Kidney: Diffusely throughout the cortex, glomerular tufts and capillary walls are thickened by hyaline, eosinophilic material, with increased cells in the glomeruli. The parietal epithelium is hypertrophied; often there are synechiae between glomerular tufts and Bowman's capsule (**fig. 4-1**). Occasional glomerular capillaries contain fibrin thrombi. The urinary space occasionally contains cellular and rarely mineralized debris and hyaline to globular proteinaceous material. Bowman's capsule is moderately thickened by hyaline to fibrillar eosinophilic material and surrounded by fibrosis (**fig. 4-2**). Tubules throughout the cortex and medulla are moderately ectatic and contain eosinophilic stippled proteinaceous material, protein casts, occasional sloughed epithelial cells, and rare crystals.



4-1. Kidney, dog. diffusely and globally, glomeruli have increased cellularity, increased thickness of mesangium, thickened capillary loops, periglomerular fibrosis, hyperplasia of the parietal epithelium with multifocal synechia, and occasional necrotic cells within the glomerular tuft. (HE 400X)

Tubules multifocally contain individual cells with brightly eosinophilic cytoplasm and pyknotic nuclei (necrosis), and are lined by thin, attenuated epithelium, cuboidal epithelial cells with swollen, vacuolated cytoplasm, or by epithelial cells with basophilic cytoplasm, increased nuclear to cytoplasmic ratio and rare mitoses (regeneration) (fig. 4-3). Occasional tubules are mineralized. Multifocally, tubular epithelial cells contain small amounts of brown globular cytoplasmic pigment. The interstitium is multifocally infiltrated by small numbers of plasma cells, lymphocytes, and fewer macrophages and neutrophils. Occasional hilar arterioles have walls expanded by acellular hyaline eosinophilic material with narrowing of the arteriole lumen. Occasionally there is proliferation of small arterioles within the cortical interstitium.

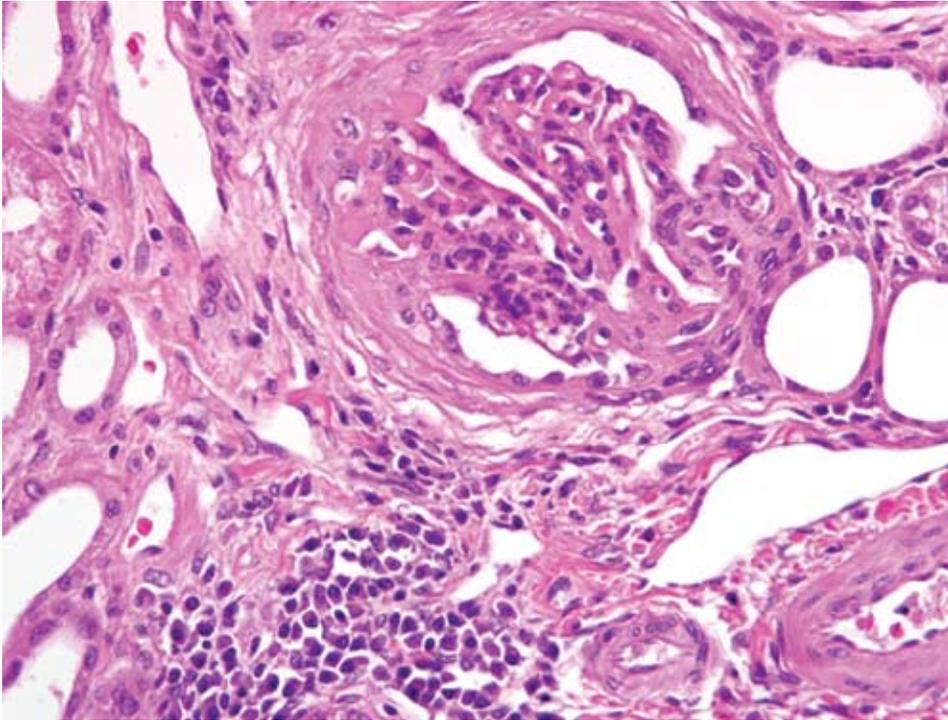
**Contributor's Morphologic Diagnosis:** Kidneys: Moderate diffuse global membranoproliferative glomerulonephritis with tubular necrosis, regeneration, luminal protein casts, tubular mineralization, multifocal chronic lymphoplasmacytic tubulointerstitial nephritis, and glomerular fibrin thrombi.

**Contributor's Comment:** The glomerular, tubular, and interstitial changes are consistent with a histologically and clinically unique renal syndrome in dogs associated with infection by the spirochete, *Borrelia burgdorferi*. Golden and Labrador retriever dogs are more likely to develop Lyme nephritis than the general canine population.<sup>2</sup>

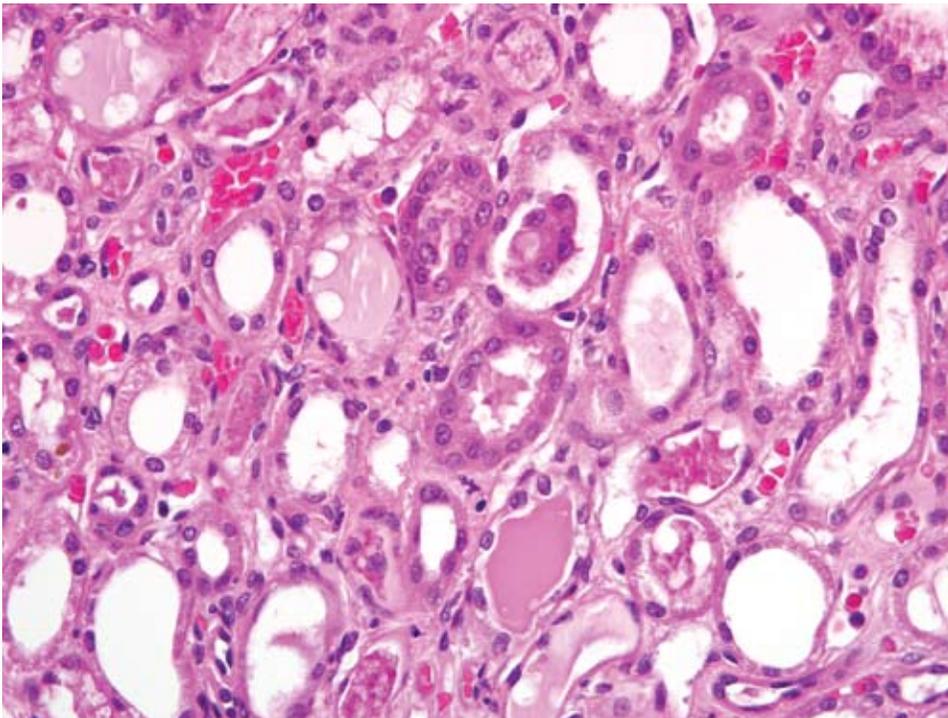
This syndrome is characterized clinically by a protein-losing nephropathy, the presence of serum antibodies to *B. burgdorferi*, and rapidly progressive fatal renal failure in dogs in Lyme endemic areas.

Diagnosis of infection can be determined by a commercially available enzyme-linked immunosorbent assay (ELISA) that does not react to commercially available Lyme borreliosis vaccines.<sup>8</sup> This ELISA detects the C<sub>6</sub> peptide derived from a conserved immunodominant region (IR<sub>6</sub>) of a segment of a *B. burgdorferi* surface protein named VlsE (Vmp-like sequence, expressed).<sup>8</sup> Alternatively, Western blot may be used to distinguish between natural and vaccine exposure in dogs by detecting antibodies to OspA or OspB.<sup>3</sup> Minimal evidence exists to support the presence of *B. burgdorferi* or other bacterial organisms in the kidneys of affected dogs; rare spirochetes have been seen in silver stains.<sup>4</sup> *B. burgdorferi* DNA is rarely amplified by PCR in renal tissue from affected dogs, and often *B. burgdorferi* antigen is not detected by immunohistochemical staining.<sup>1</sup> It is thought that the lesions seen in Lyme nephritis are due to a sterile immune complex disease.

The most common glomerular lesion in Lyme nephritis is membranoproliferative glomerulonephritis, with adhesions of glomerular tufts to Bowman's capsule, and often periglomerular fibrosis of Bowman's capsule. The membranous component is the result of immune mediated glomerulonephritis with subendothelial deposits, IgG, IgM,



4-2. Kidney, dog.  
Multifocally the cortical interstitium is mildly expanded by low numbers of lymphocytes and plasma cells. Occasional glomeruli are sclerotic. (HE 400X)



4-3. Kidney, dog.  
Multifocally tubular epithelium is degenerate, necrotic, or regenerative, and there is often an eosinophilic proteinaceous material within tubular lumina. (HE 400X)

and C3 present along glomerular basement membranes. The proliferative component is due to increased mesangial cell number and influx of inflammatory cells (macrophages and neutrophils). The severe, diffuse glomerular lesions

are likely the primary lesion and responsible for subsequent tubular changes including multifocal dilation with necrosis and regeneration. Tubular damage most likely results from cellular hypoxia and/or nephrotoxin exposure due to

profound proteinuria; damaged tubules express vimentin. Interstitial fibrosis is often present to some degree, and mild to moderate lymphoplasmacytic inflammation is present throughout the cortex. Arteriolar changes are occasionally noted and include fibrinoid necrosis of small to medium arterioles or deposition of PAS-positive hyaline material in hilar arteriolar walls, lesions commonly associated with renal disease-induced hypertension.<sup>2</sup>

*B. burgdorferi* is transmitted by *Ixodes* spp. ticks, which may feed on and cause disease in several species including dogs, horses, cattle, cats and people. The most common manifestation of disease in dogs is polyarthritis, with fewer cases of nephritis, and one case report of myocarditis.<sup>5,9</sup> In addition to arthritis, horses may also develop ocular disease and probably encephalitis, and abortions may be seen in cattle. The tick vector for *B. burgdorferi* has a two-year life cycle. Larvae and nymphs primarily feed on birds and small mammals; the main mammalian host is the white footed mouse and deer mouse (*Peromyscus* spp.). Adults primarily feed on white-tailed deer (*Odocoileus virginianus*), which are asymptomatic carriers. Adult ticks must attach to the host for a minimum of 24 hours in order to transmit the bacterium. Deer flies and horse flies may act as mechanical vectors in areas where infection is well maintained in the wildlife population by tick vectors.<sup>9</sup>

**AFIP Diagnosis:** Kidney: Glomerulonephritis, membranoproliferative and fibrinous, global, diffuse, marked, with synechiae, tubular degeneration, necrosis, regeneration, and proteinosis, and mild multifocal lymphoplasmacytic cortical interstitial nephritis.

**Conference Comment:** The clinical history of relatively acute onset of renal failure, laboratory evidence of *Borrelia burgdorferi* infection, and the distinctive histologic feature of extensive tubular necrosis with concurrent membranoproliferative glomerulonephritis and interstitial nephritis are consistent with canine Lyme nephritis in this dog. The contributor provides an outstanding description of this renal syndrome of suspected sterile immune complex disease. A partial list of the numerous other diseases associated with immune-complex glomerulonephritis in the dog follows:<sup>6,7</sup>

- Adenoviral hepatitis (infectious canine hepatitis caused by canine adenovirus-1)
- Borreliosis (Lyme nephritis caused by *B. burgdorferi*)
- Chronic disease (pancreatitis, hepatitis, pyoderma, neoplasia)
- Dirofilariosis (heartworm disease caused by *Dirofilaria immitis*)
- Hereditary C3 deficiency
- Immune-mediated disease (hemolytic anemia,

polyarthritis, systemic lupus erythematosis)

- Polyarteritis
- Prostatitis
- Pyometra

This case also stimulated conference participants to review the proposed pathogenesis of immune-complex glomerulonephritis. Classically, immune-complex glomerulonephritis is thought to result from prolonged antigenemia, with circulating antigen equivalent to or in slight excess of circulating antibody; this results in the formation of soluble antigen-antibody complexes that deposit in glomerular capillaries within or on either side of the glomerular basement membrane (GBM), where they initiate complement fixation and cause elaboration of neutrophil chemotaxins (e.g. C3a, C5a, and C567). Neutrophils release proteinases, arachidonic acid metabolites, oxygen-derived free radicals, and hydrogen peroxide, damaging the GBM and inciting monocyte infiltration.<sup>7</sup> There is some controversy concerning the significance and importance of soluble immune complexes in the pathogenesis of glomerulonephritis; several investigators suggest the presence of immune complexes within and around the GBM may be secondary to the damage on the glomerulus rather than the inciting stimulus for the glomerular lesions.<sup>6</sup>

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