Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 15

19 January 2022

CASE I: 20N-0086 (JPC 4153158)

Signalment:
9-year-old, spayed female, Otterhound (*Canis familiaris*), canine

History:
The dog had an approximately 3-week history of right forelimb lameness with a swollen, warm, painful elbow joint that was nonresponsive to nonsteroidal anti-inflammatory drugs. Bilateral elbow radiographs showed a right fragmented medial coronoid process with mild degenerative joint disease. A complete blood count was performed and revealed a mild thrombocytosis (651 x $10^3/\mu L$), leukocytosis (15.6 x $10^3/\mu L$), and neutrophilia (13.3 x $10^3/\mu L$) and a presumptive diagnosis of septic arthritis was made. The night before presentation to necropsy, the owner reported an increased respiratory rate and effort. The following morning, the dog was found deceased.

Gross Pathology:
Gross examination revealed diffuse generalized muscle wasting. The lungs were diffusely heavy. Scattered throughout all lung lobes were pinpoint to 2 mm in diameter tan to red to dark red foci that extended into the parenchyma on cut surface. On cut surface, the lungs exuded a moderate amount of dark red watery to foamy fluid. The trachea contained a moderate amount of tan to red foamy fluid.

Lateral to the right elbow joint, there was a multicavitated structure that exuded a mild amount of tan watery fluid and extended caudally and proximally encircling the olecranon and dissecting through the surrounding musculature. This structure did not directly communicate with the elbow

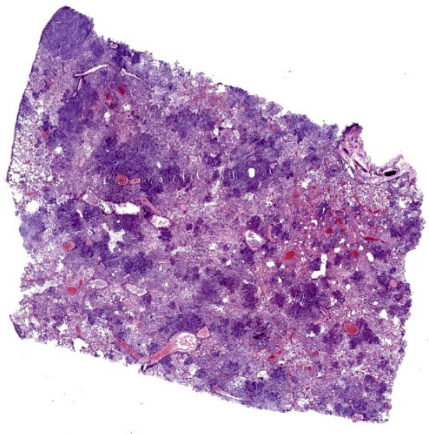

Figure 1-1. Lung, dog. At subgross magnification, fully half of the alveoli are filled with a cellular infiltrate, and another 30% are filled with edema fluid. (HE, 4X)

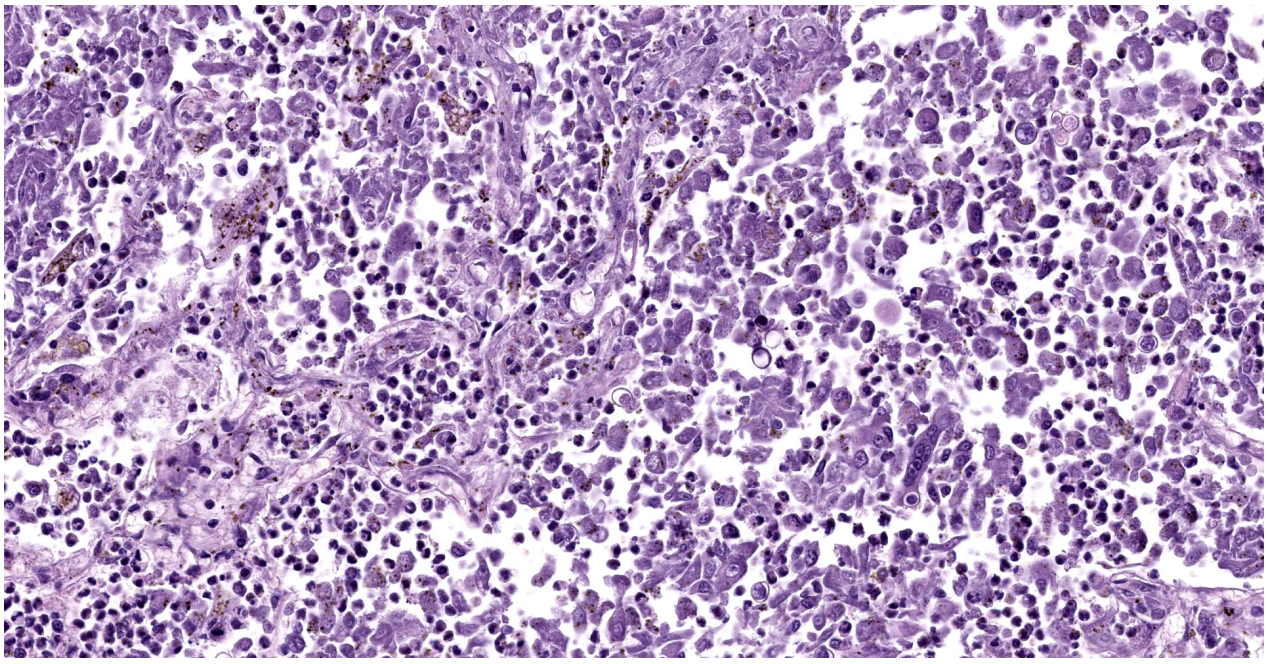

Figure 1-2. Lung, dog. Alveoli are filled with varying combinations of spindled epithelioid macrophages, foamy alveolar macrophages, neutrophils, and lymphocytes. Yeasts consistent with *B. dermatitidis* are scattered throughout the section. (black arrows) There are rare siderophages (upper left). (HE, 4X)

joint but did infiltrate the synovium externally. The olecranon also had multifocal areas of boney lysis and the proximal tip was markedly friable. There was a fragment on the proximal-medial surface of the ulna that was similar in color and texture to the adjacent bone (fragmented medial coronoid process). There was also mild roughening and thinning of the articular cartilage in the right elbow joint (degenerative joint disease). The synovial fluid in the joint was clear with the viscosity of saliva.

Laboratory Results:
4DX SNAP: Negative x 4

Bilateral elbow radiographs revealed a right fragmented medial coronoid process with mild degenerative joint disease. The left elbow was within normal limits.

Synovial fluid analysis revealed a markedly increased protein concentration; however, interpretation was inconclusive due to hemodiluted sample.

Aerobic and anaerobic culture of the right joint demonstrated no growth after 7 days.

CBC: mild thrombocytosis (651 x 10^3/ µL), leukocytosis (15.6 x 10^3/ µL), and neutrophilia (13.3 x 10^3/ µL)

Serum chemistry: Within normal limits

Microscopic Description:
Lung: Affecting approximately 50 to 90% of the examined sections, there is marked infiltration, expansion, and replacement of alveoli, terminal bronchioles, and less often alveolar septa by a densely cellular inflammatory infiltrate. Inflammatory cells are composed predominately of foamy and epithelioid macrophages and viable and degenerative neutrophils with fewer multinucleated giant cells (foreign body and

Langhans types), lymphocytes and plasma cells. Inflammatory cells form disorganized loose aggregates that expand nearly all alveoli and bronchioles to varying degrees but also occasionally form poorly organized pyogranulomas that are variably surrounded by a thin wall of fibrous connective tissue. In the most severely inflamed regions, there is multifocal to coalescing coagulative necrosis with retention of cellular architecture but loss of differential staining, which affects approximately 20%-60% of the tissue.

Within areas of inflammation and necrosis, there are numerous extracellular and intrahistiocytic (including multinucleate giant cells), 5-30 µm in diameter, round yeast with a 1-2 µm distinct double-contoured refractile wall. They typically lack nuclear material / protoplasm and rarely exhibit broad-based budding. In less affected areas, alveoli contain fibrillar material (fibrin), hemorrhage, eosinophilic proteinaceous edema fluid and mildly increased numbers of foamy macrophages and neutrophils. Vessels are diffusely congested and variably lined by hypertrophied endothelial cells. Large caliber bronchioles and bronchi are largely unaffected but do contain a small amount of sloughed epithelial cells (postmortem autolysis) and erythrocytes, and the submucosal connective tissue is infiltrated by individual to loosely arranged small clusters of lymphocytes and plasma cells with fewer macrophages and neutrophils.

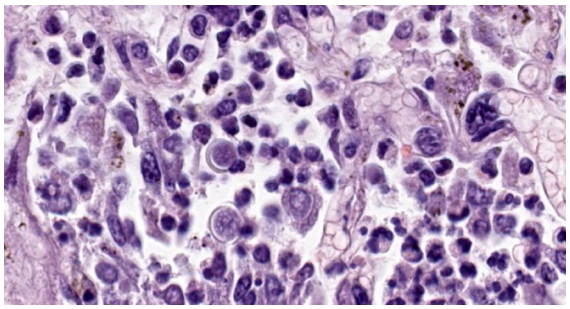

Figure 1-3. Lung, dog. High magnification of extracellular *B. dermatitidis*. (HE, 1100X)

Tissue from around the right elbow (not submitted) is affected by pyogranulomatous inflammation containing numerous yeasts similar to those described in the lung.

Contributor's Morphologic Diagnoses:
Lungs: marked, multifocal to coalescing, pyogranulomatous interstitial pneumonia with myriad intrahistiocytic and extracellular broad-based budding yeasts consistent with *Blastomyces dermatitidis* (pulmonary blastomycosis)

Contributor's Comment:
The histologic changes in this case represent a classic presentation of pulmonary blastomysosis caused by *Blastomyces dermatitidis*. Blastomycosis is a fungal disease that primarily affects dogs and humans and most commonly presents as pulmonary disease. Infection is typically acquired through inhalation of the environmental saprophytic mycelial form of the fungus (*Ajellomyces dermatitidis*) that, once within airways and at body temperature, propagates into yeast forms and establishes a primary infection in the lungs.[3,8] After a primary infection is established, it can spread hematogenously throughout the body, with the eyes, skin, subcutis, lymph nodes, bones, and joints among the most common sites of dissemination.[3,8] Although there have been rare reports of focal cutaneous infections from direct inoculation, and, within humans, rare reports of extrapulmonary infection, notably bone, without a known route of inoculation, in cases where the lungs are

affected, the extrapulmonary lesions are generally regarded as sites of dissemination from the lungs rather than sites of primary inoculation.[3, 5-9] In the dog of this case, along with the lungs, the connective tissue and skeletal muscle surrounding the right elbow were also affected. Although the dog initially presented for clinical signs referring to the elbow, based on the severity of pulmonary lesions, known pathogenesis of Blastomyces, and absence of a clinical history of penetrating trauma to the elbow, we favor primary pulmonary infection with dissemination to the joint. The lack of nuclear material / protoplasm in the yeasts in this case was interpreted as autolytic change.

Within the United States, *Blastomyces dermatitidis* is endemic to the Ohio River valleys, Mississippi, Missouri, and the mid-Atlantic states and is considered highly endemic along waterways in North Central Wisconsin, specifically near Eagle River.[1,3] Defining the environmental niche of *Blastomyces* has been challenging due to difficulty isolating the organism from soil; however, a study of the endemic area of North Central Wisconsin revealed close proximity to waterways to be a risk factor for blastomycosis, which is part of what makes this region ideal for growth of the organism.[1,11] Other environmental conditions that have been demonstrated to support its growth include acidic sandy soil, animal waste products, and wood by-products.[3,8] Immunologic response to the *Blastomyces* infection is primarily mediated though a T-helper-1 (TH-1) immune response, which targets the primary virulence factor for *Blastomyces*, *Blastomyces* adhesion 1 (*BAD-1*) antigen. *BAD-1* antigen has been demonstrated to promote cellular adhesion and invasion of mononuclear phagocytes and non-phagocytic cells and evades immune defenses by increasing production of transforming growth factor β and suppressing the production of tumor necrosis factor-α.[3,11] Clinical signs of infection include cough, dyspnea, anorexia, weight loss, uveitis, and skin ulceration with drainage. Infection typically presents with purulent to pyogranulomatous lesions, with the most consistent finding being generalized coalescing, grey-white granulomatous nodules in the lungs.[3,8] Histologically, infection is characterized by the presence of coalescing granulomas which surround round yeasts that measure 5-30 µm in diameter, have 1-µm thick double-contoured refractile walls, and central granular protoplasm. Yeasts occasionally exhibit broad-based budding. Infrequently, filamentous and pseudohyphal forms and conidia are found within tissues. Histologic diagnosis can be aided by the use of special stains, including histochemical staining by Gomori's methenamine silver

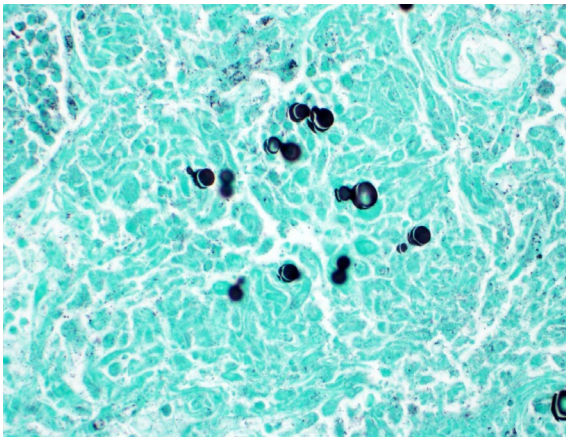

Figure 1-4. Lung, dog. A Gomori methenamine silver demonstrates the yeasts cell wall and internal contents but yields little detail (GMS, 400X)

(GMS) and periodic acid-Schiff (PAS) reaction.[3,8]

Contributing Institution:
University of Wisconsin
School of Veterinary Medicine
Department of Pathobiological Sciences
https://www.vetmed.wisc.edu/departments/pathobiological-sciences/

JPC Diagnosis:
Lung: Pneumonia, interstitial, granulomatous, diffuse, severe, with intrahistiocytic and extracellular yeasts, etiology consistent with *Blastomyces dermatitidis.*

JPC Comment:
Although the pulmonary form is the most common manifestation of blastomycosis, this entity was first described in humans with cutaneous lesions. In 1894, Thomas Casper Gilchrist described "peculiar parasitic bodies" he initially believed to be protozoans in sections of cutaneous tissue submitted from a patient in Philadelphia. However, he later realized the organism was a fungus after its isolation and named the agent *Blastomyces dermatitidis*; blastomycosis was subsequently known for some time as Gilchrist's disease.[2,4]

In 1898 Gilchrist published a report detailing a second case in a human patient, including detailed characteristics of the organism's characteristic budding and also noting yeasts were often enclosed within giants cells "…in various stages of germination". Gilchrist then injected a suspension of the cultured *Blastomyces dermatitidis* into the jugular vein of a dog, which over the next two months became emaciated and had a nasal discharge. Both lungs "presented a striking picture" upon opening the thoracic cavity, with numerous pea sized or larger, discrete, firm, light yellow nodules that upon histologic examination contained the characteristic yeast. The fungus was then cultured from the samples, essentially fulfilling Koch's postulates. Furthermore, Gilchrist intravenously inoculated two additional dogs, a sheep, and a horse with suspensions composed of lung nodules containing the organism. Pulmonary lesions containing the *B. dermatitidis* were identified in both dogs, the sheep, and the horse.[4]

Blastomycosis was initially thought to be restricted to North America and caused by a single species, *Blastomyces dermatitidis*. However, in 1952 a case was reported in Tunisia, with additional cases since reported in Africa and less commonly in India, the Middle East, and occasionally in Europe. In addition to *B. dermatitidis*, additional species have since been added to the genus, including

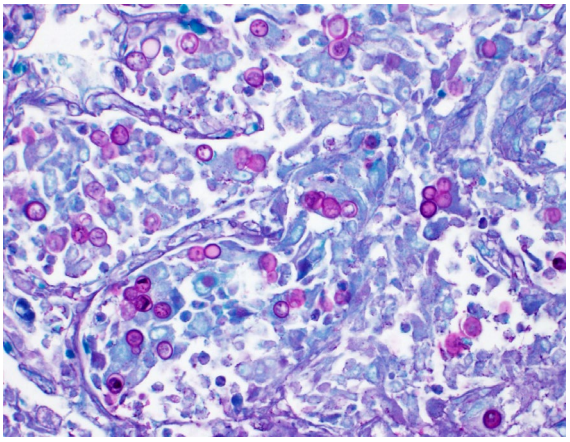

Figure 1-5. Lung dog. A periodic acid-Schiff stain demonstrates yeasts with better illumination of internal detail, but may miss non-viable fungal structures, unlike GMS. (PAS, 400X)

B. gilchristii, *B. percursus,* and *B. silverae.* In addition, both *Emmonsia parva* and *E. helica* were reclassified as *B. parva and B. helicus* following a taxonomic revision in 2017 based on DNA sequence analysis. Interestingly, human cases of blastomycosis in Africa typically present as cutaneous lesions or osteomyelitis compared to pulmonary disease in North America. A recent retrospective study evaluating 20 human cases of blastomycosis in South Africa between 1967 and 2014 that had been attributed to *B. dermatitidis* were found to actually *B. percursus* and *B. emzantsi* based in *ITS* sequences. In addition, *BAD-1,* the important adhesion-promoting protein and virulence factor associated with *B. dermatitidis* could not be identified in these strains. Although *BAD-1* has clearly been demonstrated to be an important virulence factor in North American strains, the significance of its absence in these African strains is unclear. However, it is possible the difference in virulence genes associated with the different strains may contribute to the variations of clinical manifestations between North American and African blastomycosis.[10]

Clinically, most canine patients present with normal complete blood count although mild anemia and mild neutrophila +/- mild left shift may be present. Hypoproteinemia is the most common abnormality noted on blood chemistry panels and mild hypercalcemia is present in approximately 10% of cases; most chemistry panels are normal. Radiographs reveal an interstitial pattern in approximately 70% of canine cases although nodular, bronchointerstital, and alveolar patterns have also been described. Osteolytic lesions may also be identified radiologically, particularly those affecting the long bones of the distal limbs, with the forelimbs affected more often than the pelvic limbs. An antigen enzyme immunoassay (EIA) for galactomannan, a sugar present in the cell wall of *B. dermatitidis*, can detect the substance in both urine and serum samples. However, galactomannan is only useful for determining the presence for a fungal infection in general as the test shows cross reactivity for blastomycosis, histoplasmosis, coccidioidomycosis, penicilliosis, and paracoccidioidomycosis in humans and therefore most likely canines as well. Serology for antibodies is generally considered to be insensitive and non-specific, with one study reporting only 50% of dogs with confirmed blastomycosis to be positive. Agar-gel immunodiffusion (AGID) is the most commonly utilized serologic test although additional types include complement fixation, enzyme linked immunosorbent assay (ELISA), counterimmunoelectrophoresis, and agar-gel precipitation.[12]

Similar to as noted in WSC21-22 Conf. 14 Case 2 with coccidiomycosis, the microbiology lab should be informed when samples are submitted for culture that are consistent with blastomycosis. This dimorphic fungus presents a serious risk to laboratory personnel as they may be exposed to the infective mycelial form if plates are mishandled.[12]

Conference participants discussed the variable patterns of pneumonia, including bronchopneumonia, interstitial, bronchointerstitial, and embolic. Bronchopneumonia

is characterized by exudative lesions that originate at the bronchiolar-alveolar junction due causative agents entering via the airborne route and typically has a cranioventral distribution. Depending on individual and institutional preference, interstitial pneumonia may be used to describe a broad range of diseases that damage the interstitium (alveolar or interlobular septa) or be restricted to simply indicate increased numbers of leukocytes within alveolar septa. Diffuse alveolar damage is the most commonly recognized form of interstitial lung disease. Interstitial pneumonia typically has a diffuse distribution due to the causative agent being hematogenously distributed, such as in cases of endotoxemia and 3-metylindole toxicity in cattle. Bronchointerstitial pneumonia is also associated with two forms of use. In most cases, bronchointerstitial pneumonia indicates the presence of both bronchiolar necrosis and diffuse alveolar damage. However, the term has also been used to describe lesions in which airways are encircled by mononuclear cells that also infiltrate the alveolar septa. Embolic pneumonia occurs as the result of hematogenous distribution of infectious agents or inflammatory processes within the lung, such as with disseminated aspergillosis.[3]

The moderator also discussed additional infectious causes of granulomatous interstitial pneumonia in veterinary species, including other fungal agents such as *Coccidioides, Histoplasma,* and *Cryptococcus,* as well as bacterial agents such as *Mycobacterium, Rhodococcus, Actinobacillus, Actinomyces,* and *Nocardia.* Additional viral etiologies to be considered in canines with interstitial pneumonia include canine influenza, canine adenovirus-2, and canine distemper virus.

References:

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CASE II: C-23638-17 (JPC 4119604)

Signalment:
5 mont- old, intact, female, Nova Scotia duck tolling retriever, *Canis lupus familiaris*

History:
The owners report that until the morning of presentation the dog had been an apparently normal active puppy, although they thought she always appeared to drink and urinate excessively. On the morning in question, she refused her breakfast, went outside to urinate and had a semi-solid bowel movement, then came back in the house and laid down on her bed. The owners looked in on her 30 minutes later and she was not moving and did not appear to be breathing. The owners rushed her to their vet where she was pronounced dead on arrival. The owners consented to a postmortem.

Gross Pathology:
The puppy was thin, having small visceral and subcutaneous fat stores. A few streaks of hemorrhage were noted on the abdominal surface of the diaphragm. The urinary bladder was small and empty. The caudodorsal lung lobes were red, heavy and wet, while cranioventral areas were pale pink and pliable.

Otherwise, significant postmortem findings were restricted to the kidneys. Both were mildly enlarged, slightly pale and the capsules were mildly, diffusely adhered to the cortices. When sectioned, much of the cortical parenchyma is effaced by poorly defined, multifocal to coalescing, pale, white-tan, streaks which extend into the peripheral medulla.

Laboratory Results:
A scant growth of *E. coli* (2 colonies) was isolated from the kidney.

Immunohistochemistry performed on sections of kidney revealed moderate, multifocal, staining for Leptospiral antigens within areas of inflammation. The staining was cytoplasmic in inflammatory cells

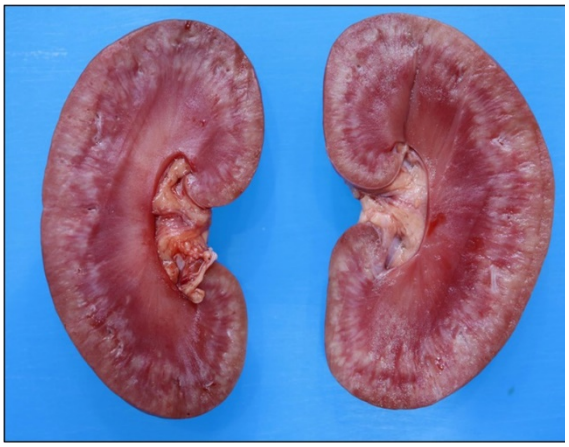

Figure 2-1. Kidney, dog. Sagittal sections through each kidney. In both, the cortices and peripheral medullary areas contain poorly defined, coalescing, white-tan areas which partially obscure the normal architecture. (*Photo courtesy of:* Atlantic Veterinary College, University of Prince Edward Island www.upei.ca/avc)

(largely macrophages) and was also occasionally noted at the luminal surface of tubular epithelial cells.

Microscopic Description:
The white-tan areas noted grossly in the renal cortices and peripheral medullary areas represents numerous, multifocal to coalescing, areas where the interstitium is expanded and the parenchyma is replaced by dense infiltrates of lymphocytes admixed with fewer plasma cells, and sometimes central aggregates of pale epithelioid macrophages. Sparse cell debris is scattered amongst these infiltrates which are associated with a mild to moderate increase in collagenous stroma that often extends into the adjacent medulla. Scattered cortical and medullary tubules within the intervening parenchyma contain small amounts of proteinaceous fluid and, rarely, sparse cell debris. These tubules are often lined by epithelial cells which appear slightly floccular with unapparent nucleoli and/or the epithelium has partially sloughed. Gram, PAS and acid-fast staining does not reveal infectious agents. Warthin-Starry staining reveals finely stippled black material (putative bacteria or possibly cell debris) and occasional fine, thin bacilli-like structures within macrophages and loose in areas of inflammation, and rarely within epithelial cells lining adjacent intact tubules.

Contributor's Morphologic Diagnoses:
Kidney: Severe, bilateral, chronic, multifocal to coalescing, lymphohistiocytic, interstitial nephritis

Contributor's Comment:
The sudden death of this puppy was attributed to renal failure caused by chronic interstitial nephritis due to leptospiral infection. The diagnosis was confirmed by immunohistochemistry; leptospiral antigens were demonstrated within macrophages in areas of inflammation, as well as within the lumen of occasional adjacent renal tubules. Unfortunately, further serotyping/genotyping of the bacteria was not performed.

Leptospirosis is a zoonotic spirochetal bacterial infection with worldwide distribution that affects many animal species. The taxonomy of leptospires is complicated, confusing, and evolving. Leptospires were originally subdivided via culture and immunologic reactivity into 2 species; *L. interrogans sensu lato* (pathogenic strains) and *L. biflexa sensu lato* (all saprophytic strains from the environment). Within these 2 species Leptospires were then classified

into approximately 25 serogroups which have been further subdivided to more than 250 serovars of Leptospira.[3] Serogroups include serovars that share common antigens resulting in cross-reactions with antibody detection methods, while serovars are identified by distinct antibody reactivity to a variety of carbohydrate moieties in the outer lipopolysaccharide membrane.[3] Unfortunately, due to the nature of this type of antibody testing, it has limited value in trying to definitively identify these organisms by species because different species and serovars often share common antigenic determinants and cross-reactions are extremely common. That is, antigenically similar serovars may belong to different Leptospira species. Serogrouping and serovar typing, although not perfect, has had practical uses in epidemiologic studies and it has been thought that the protection induced by current vaccines is restricted to the serogroup used for their production.[7] Serotyping is increasingly being replaced by restriction enzyme analysis of chromosomal DNA which has resulted in the identification of approximately 20 genomospecies of Leptospira.[3]

In general, serovars of leptospires are carried and spread in the environment by specific maintenance hosts to which that serovar is well adapted. The maintenance host does not typically develop clinical disease but carries the bacteria in renal proximal tubules, or, in some hosts (cattle, sheep, horses), the genital tract. These animals shed bacteria in the urine for several months or throughout life. Rodents, raccoons, skunks, dogs, cattle, sheep, and horses have all been identified as

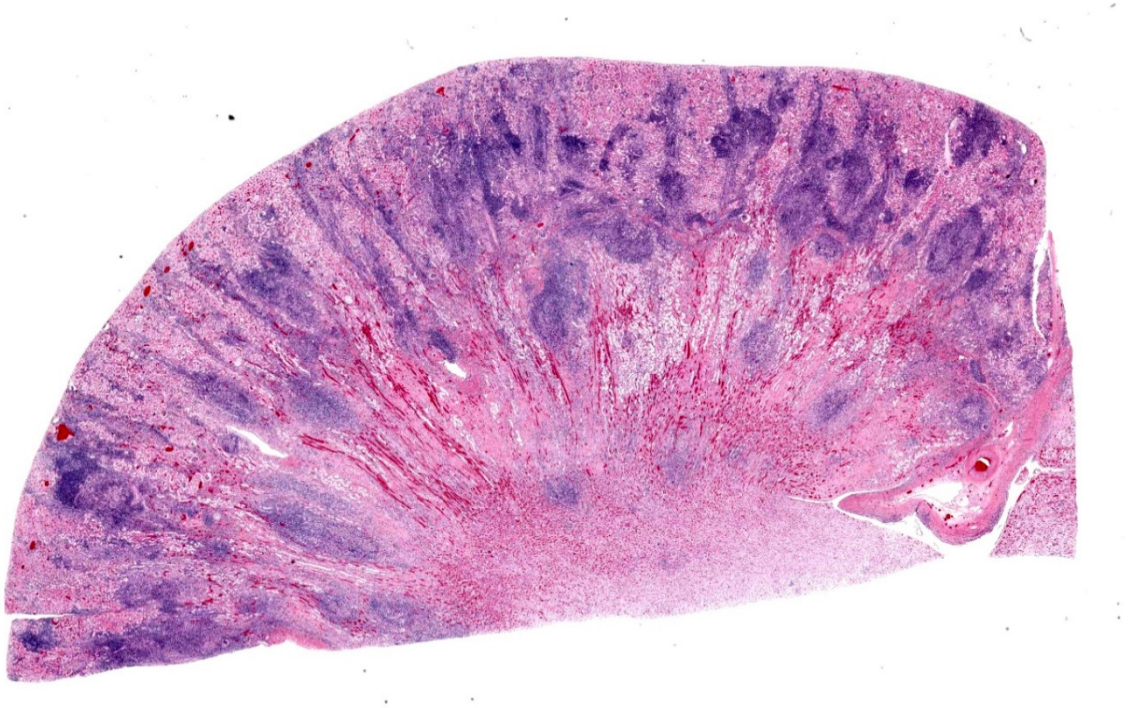

Figure 2-2. Kidney, dog. Large often coalescing linear inflammatory infiltrates effaced the renal parenchyma from the cortex to medulla. (HE, 5X)

maintenance hosts for one or several of the 10 serogroups/serovars of Leptospires that are associated with clinical disease in veterinary medicine. Clinical disease is generally seen when an "incidental" host becomes infected. Transmission is typically via contact with contaminated urine, or less commonly, through contact with contaminated placental/ fetal tissues or by venereal transmission.[1] Leptospires do not replicate outside of the maintenance host but they can survive in warm, moist, environmental conditions (such as stagnant water and water logged soil) for weeks to months. Because of this, the incidence of disease is often seasonal with clinical disease most commonly occurring in the fall in temperate climates ("fall fever"), in the winter in tropical climates, or following periods of heavy rainfall or flooding.[1]

In dogs, several serovars of Leptospira are associated with clinical disease. Historically, *canicola* and *icterohaemorrhagiae* are best recognized as a cause of disease and mortality. In the 1970's, a bivalent vaccine for dogs targeting these serovars became available, and now, disease in dogs due to these serovars is rare. However, in recent years, northeastern North America, including

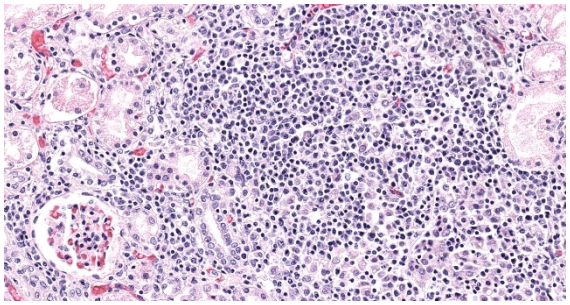

Figure 2-3. Kidney, dog. Large numbers of lymphocytes and fewer macrophages and plasma cells entrap and efface cortical tubules and glomeruli. (HE, 328X)

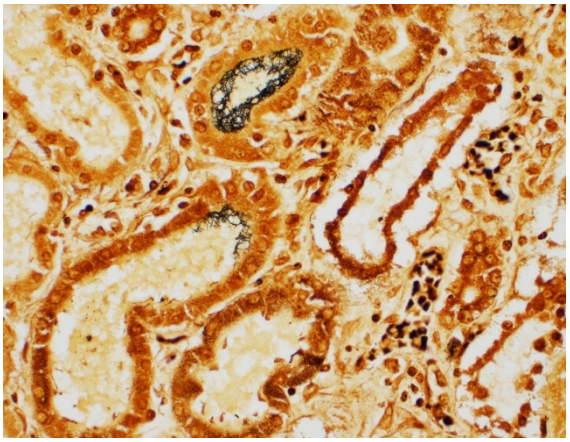

Figure 2-4. Kidney, dog. A silver stain demonstrates numerous spirochetes in proximity to the apical surface of proximal convoluted tubular epithelium. (Warthin Starry 4.0, 400X)

areas within the maritime provinces of Canada[6], has seen an apparent resurgence of leptospirosis in urban dogs. Serovars identified in these dogs include *grippotyphosa*, *pomona*, *autumnalis*, *bratislava* and other serovars typically seen in wildlife. This resurgence may be due to a combination of factors including, vaccination (current vaccines do not offer protection against these serovars), changes in climate (increased periods of heavy rain and flooding, etc) and increased contact with wild maintenance hosts in urban environments (skunks, rodents, raccoons, etc).[7]

The severity of clinical signs induced by leptospiral infection varies widely from subclinical disease to peracute or chronic manifestations of disease and depends on factors such as age, immune status or the host's ability to contain infection, infective dose, serovar type and virulence. Leptospires can penetrate intact mucous membranes of the mouth, nose, eyes and abraded, water-softened skin. The organisms then enter

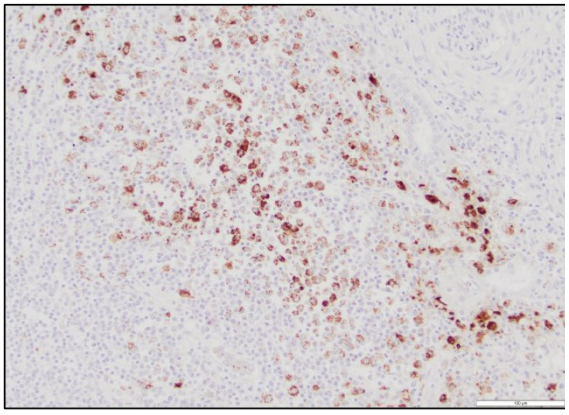

Figure 2-5. Kidney, dog. Immunohistochemical staining demonstrates leptospiral antigen within areas of inflammation. (anti- *Leptospira,* 200X) (*Photo courtesy of:* Atlantic Veterinary College, University of Prince Edward Island www.upei.ca/avc).

systemic circulation and quickly begin to replicate. Severe peracute infection resulting in septicemia has been reported in puppies (usually infected with *icterohemorrhagiae*) and death may occur in a few hours to 2-3 days.[1,7] Endothelial damage and hemorrhage are commonly seen in these animals. In most infected dogs this stage of infection is subclinical or associated with vague clinical signs. Organisms then disperse and replicate in many tissues, most commonly the kidney and liver. Acute death has been reported in dogs in this stage of infection. At postmortem, these dogs may have areas of hepatic necrosis and microscopic lesions consisting of dissociation of hepatic cords, canaliculi plugged with bile, acute hepatocellular degeneration and early regeneration, and organisms may be noted with silver stains in sinusoids and within hepatocytes.

Subacute to chronic leptospirosis is the form of disease most often seen in dogs with leptospirosis. This typically manifests as renal insufficiency resulting from subacute to chronic interstitial nephritis as the bacteria localize and replicate in the kidney. The severity and progression of disease may be quite variable. In acute renal lesions, tubular degeneration and regeneration predominates. As lesions progress, tubular lesions resolve, interstitial infiltrates of lymphocytes and plasma cells increase and then often generally decrease, as interstitial fibrosis becomes gradually more prominent. The organisms may be noted in areas of inflammation and within cortical tubules with Warthin-Starry stains in subacute lesions but generally become less numerous with chronicity.[1]

Leptospirosis is of zoonotic concern. Humans (usually pet owners, veterinary staff, etc.), may be infected via contaminated urine, or rarely via bite wounds, so special care and hygienic precautions should be taken when handling infected dogs.[3]

Contributing Institution:
Atlantic Veterinary College, University of Prince Edward Island
www.upei.ca/avc

JPC Diagnosis: Kidney: Nephritis, tubulointerstitial, lymphoplasmacytic and histiocytic, chronic, multifocal to coalescing, marked, with tubular degeneration, necrosis, and loss, and interstitial fibrosis.

JPC Comment:
The contributor provides an excellent review of leptospirosis, the most common zoonotic infection in the world.[7] German physician Adolf Weil first described leptospirosis in 1886 after recognizing a "new" infectious disease characterized by splenomegaly,

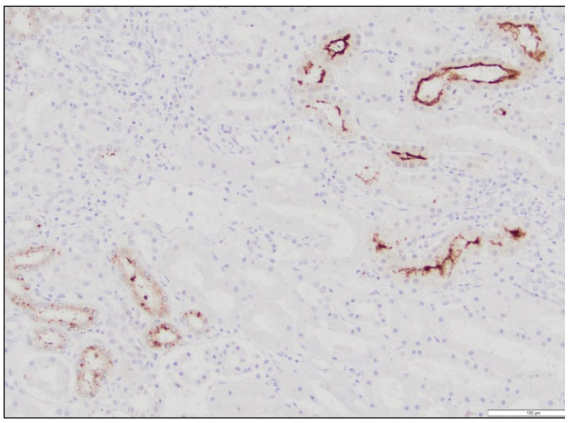

Figure 2-6. Kidney, dog. Immunohistochemical staining demonstrates leptospiral antigen lining proximal convoluted tubules. (anti- *Leptospira,* 200X) (*Photo courtesy of:* Atlantic Veterinary College, University of Prince Edward Island www.upei.ca/avc)

jaundice, and nephritis which was subsequently known as "Weil's disease" in humans. Inada et al. at Kyushu University first successfully isolated *Leptospira* spp. nearly three decades later during a 1914-1915 outbreak of Weil's disease in Japanese coal miners. Although Weil typically receives credit for first recognizing the distinct clinical disease, historical records indicate knowledge of this condition since ancient times based on its seasonality, occupational exposure, and duration of symptoms with names such as "rice-harvest jaundice" in ancient China as and "7-day fever" and "autumn fever" in Japan.[4] As noted by the contributor, certain leptospiral serovars are associated with specific maintenance hosts that are often asymptomatic, such as *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Hardjo in cattle, *Leptospira interrogans* serovar Canicola in dogs, *Leptospira interrogans* serovar Pomona in pigs, and *Leptospira interrogans* serovar Copenhageni in rats. In contrast, "incidental" hosts typically develop clinical disease.[4]

The pathogenesis of subclinical chronic tubulointerstital nephritis in maintenance hosts has been the subject of interest. Based on experimental *L. interrogans* serovar Copenhageni infection in rats, the pathogen disseminates hematogenously to nearly all tissues shortly after infection, followed shortly afterward by its elimination from nearly all tissues, with the exception of the kidneys, which is likely facilitated by circulating anti-leptospiral immunoglobulin (IgM and IgG). However, the kidneys present a permissive environment for the organism's survival and proliferation, and leptospiruria develops within a week of infection. Given the clearance of leptospirosis from nearly every organ with the exception of the kidneys, it is likely that kidneys are not a specific target of *Leptospira* spp. but rather their survival in this organ, specifically in the renal tubules, is facilitated by an immunologically permissive environment that is exploited by the pathogen.[5]

A possible explanation for this survival despite the presence of anti-leptospiral IgG in the renal tubules is the absence of complement in the renal tubules. In addition, pathogenic *Leptospira* genomes encode a group of proteins known as Len (leptospiral endostatin-like) proteins that bind complement regulatory proteins such as plasma factor H, which promote deactivation of the compliment cascade, essentially preventing destruction of the cell. Interestingly Len proteins are not present in non-pathogenic *Leptospira*, further supporting the hypothesis of their role in immune invasion. In addition, human complement regulator C4BP is also

bound by pathogenic *Leptospira* spp., which provides additional host complement resistance.[5]

In addition to affecting the kidney and liver, leptospirosis may also cause meningitis, uveitis (suspected etiologic agent of equine recurrent uveitis), abortion and infertility, and pulmonary hemorrhage secondary to previously described vasculitis.[2]

Key features noted by conference participants in this case included lympho-plasmacytic interstitial nephritis in addition to relatively minimal glomerular changes in regions with minimal inflammation and necrosis. Two additional differentials proposed by the moderator in this case include *Leishmania* spp. and *Borrelia burgdorferi.* Both of these entities are also associated with lymphoplasmacytic interstitial nephritis; however, the primary lesion associated with these etiologies in the kidney is glomerulonephritis whereas the glomerulus is not typically affected in cases of leptospirosis.

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CASE III: 525/20 (JPC 4167685)

Signalment:
21-year-old, male (gelding), Connemara, (*Equus caballus*), equine

History:
Initially presented in July with multiple suspected cutaneous melanomas mainly in the perineal region and along the tail as well as a suspected squamous cell carcinoma of the penis. The pony was initially treated with penile amputation and sheath ablation. Enlarged inguinal lymph nodes were re-moved. A number of the melanomas were removed at a second surgery. Presented again in November with neurological signs include-ing head pressing and ataxia. Given the rapidly deteriorating clinical condition, the pony was euthanized.

Gross Pathology:
Carcass preservation and body condition were good. Multiple, firm, well-demarcated darkly pigmented dermal masses were observed around the anus and along the tail base. Black pigmentation was observed throughout these masses on sectioning. Further masses of similar appearance and up 6 cm in diameter were found over the ribs, in the region of the brachial plexus, in the dorsal lumbar and gluteal fascia and in the tensor fascia latae muscle. Masses were focally embedded on the pleural aspect of the ribs: approximately 0.5 cm thick and extending to the width of the affected rib. The deep inguinal lymph node was markedly enlarged and subtotally effaced by yellow firm irregular masses. A firm pale yellow lesion infiltrated the psoas muscles and iliac lymph

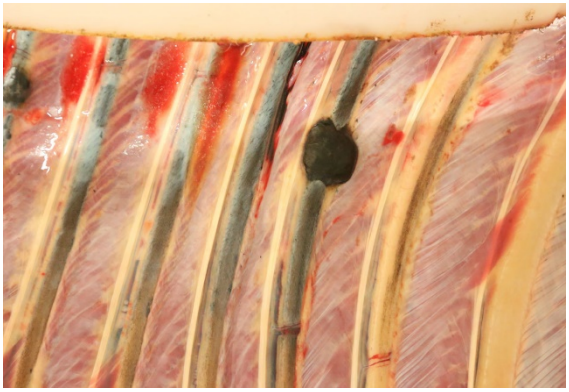

Figure 3-1. Pleura, horse. Pigmented masses infiltrate and expand the pleura. (*Photo courtesy of:* Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland http://www.ucd.ie/vetmed/)

nodes and surrounded the aorta, the ureter and the renal hilus over a distance of some 50cm. This mass was 30cm wide and 15cm deep and abutted the lumbar vertebrae. On sagittal sectioning the lumbar spinal column multiple small pigmented masses (melan-omas) were noted embedded within the pale yellow mass. Similar pigmented masses infiltrated numerous lumbar vertebral bodies. On sagittal sectioning of the skull a 5 cm diameter pigmented mass infiltrated the sphenoid bone midline at the level of the pituitary fossa and protruded approximately 1 cm into the cerebral cavity.

Laboratory Results:
None submitted.

Microscopic Description:
Inguinal lymph node: sub-totally effaced by a poorly demarcated densely cellular mass that infiltrates the surrounding capsule and adipose tissue. The mass is composed of nests and islands of large polygonal cells in a fibrovascular stroma. The majority of the cells have distinct borders and moderate to

abundant eosinophilic cytoplasm. The nucleus is either relatively large and ovoid with coarse stippled chromatin or vesicular with a prominent nucleolus. There is marked anisocytosis and anisokaryosis. More than 40 mitotic figures are counted in 10 high power fields. Multifocally the neoplastic islands show areas of central necrosis. In addition to this neoplastic lesion there is a second neoplastic cell population within the node. This consists of more loosely arranged large polygonal cells with distinct cell borders and moderate to abundant cytoplasm featuring myriad large brown granules (melanin). Nuclear details are obliterated by the dense cytoplasmic pigment. Pigmented polygonal cells are occasionally admixed with more spindled cells that contain lesser amounts of cytoplasmic granulation and have a more elongated nucleus. Clustered neoplastic pigmented cells noted in subcapsular sinus.

Histopathology confirmed that the penile squamous cell carcinoma had also metastasized to the iliac lymph nodes and had severely infiltrated the psoas muscles and retroperitoneal region. The melanoma had metastasized widely: skeletal muscles, lymph nodes, fascia, peripheral nerves and bones of the vertebral column and the skull

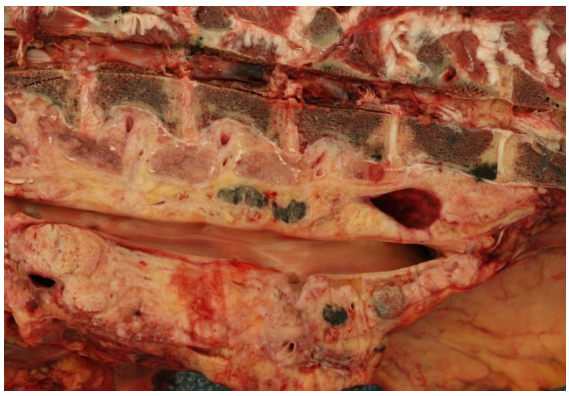

Figure 3-2. Lumbar spine and posterior aorta: A 50m non-pigmented mass infiltrates the psoas muscles and iliac lymph nodes and surrounded the aorta, the ureter and the renal hilus .Pigmented masses are scattered throughout. (*Photo courtesy of:* Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland http://www.ucd.ie/vetmed/)

Contributor's Morphologic Diagnoses:
Inguinal lymph node:

(1) Squamous cell carcinoma
(2) Melanoma

Contributor's Comment:
These 'two for the price of one' diagnoses showcase two 'classic' equine tumors occurring contemporaneously in the same lymph node as a result of metastasis from different anatomical sites.

Between 4 and 15% of all equine skin tumors are melanocytic.[6] Melanoma is a result of uncontrolled proliferation of melanocytes that can arise within the epidermis but can also originate from mucosal surfaces and the eye. Although the vast majority of cutaneous melanomas in the horse are benign at initial presentation, over 60% can progress to malignancy with attendant widespread metastasis.[6] As in this case, the majority of melanocytic tumors occur in grey or white horses, usually at or before the age of 5 years: the time at which they undergo changes in coat colour.[9] A retrospective study of the pathological features of 53 cases of equine melanoma proposed four categories of tumor: melanocytic naevus; discrete dermal melanoma (benign and malignant forms); dermal melanomatosis; and anaplastic malignant melanoma.[13] Equine melanomas result from the activation of oncogenes (e.g. *NRAS*) or

from the loss of tumor suppressor genes (e.g. *TP53*).[18] In contrast to melanomas in other/non-grey horses, which are highly malignant, those in grey horses have a longer benign growth phase prior to malignant transformation and metastasis.[6,10] Up to 80% of grey horses over 15 years old have melanocytic tumors at some anatomical location.[16]

The STX17 (syntaxin 17) gene is central to the inheritance of skin pigmentation in grey horses. Both this and the adjacent NR4A3 gene are overexpressed in melanomas from grey horses: elevated STX17 expression is associated with activation of the extracellular signal-regulated kinase (ERK) pathway in melanocytes.[5] Increased signaling of the melanocortin-1 receptor (MC1R) gene, a gene that regulates skin pigmentation, also promotes melanoma development in grey horses, as in human patients.[16]

As in this case, equine melanomas occur most frequently on the perineum/ventral tail region, as well as on the vulva, lips, and eyelids.[3,9] One unifying feature of these locations is their adjacency to mucosal epithelium. Equine melanomas are typically positive for S100, PCNA, Ki-67, and CD44 on immunohistochemistry.[13,14] In addition, RACK1, has also been found to be effective in distinguishing benign melanocytic tumors from melanomas in horses.[1]

After sarcoids, squamous cell carcinomas (SCC) are the most common equine neoplasm.[14] Around 10% of all equine neoplasms are found on the penis, vulva and ocular adnexa, of which penile SCC is the most common[15]: between 50–80% of all tumors of the external genitalia. While there is no breed predisposition, a higher incidence of penile SCC is reported in ponies relative to horses.[7] Although SCC can develop at any location on the skin surface, like melanoma, it is over-represented on regions contiguous to mucocutaneous junctions. Horses are typically diagnosed with SCC between the ages of 9 and 15 years.[7] Risk factors associated with the development of SCC in horses include lightly pigmented skin and chronic exposure to excessive sunlight. It is suspected that SCC frequently develops from pre-existing lesions such as actinic keratosis, carcinoma-in-situ, and the chronic keratosis associated with exposure to accumulated smegma.[7] As in human patients, evidence suggests cutaneous SCC in horses result from mutations in the p53 tumor suppressor

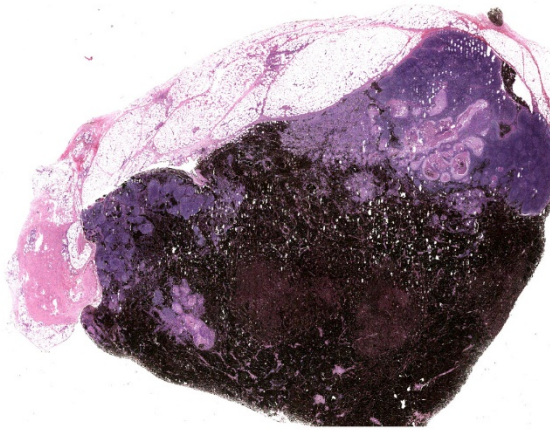

Figure 3-3. Inguinal lymph node, horse. The lymph node is expanded by two separate neoplasms, one of which is deeply pigmented. (HE, 4X)

gene.[11] Other research points to oncogenesis being initiated, in part, by equine papillomavirus type 2-infection, similar to the sexually-transmitted infection model proposed for cervical cancer in human patients.[2,17,19]

Most SCC in horses arise as solitary masses but the adjacent skin may be at similar risk of tumor development, particularly the prepuce.[6] Cutaneous SCC are locally invasive, metastasizing to local lymph nodes in some 19% of cases (as described here). These tumors can ulcerate or become traumatized leading to secondary bacterial infections and purulent inflammation.

Contributing Institution:
Room 012, Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
http://www.ucd.ie/vetmed/

JPC Diagnosis:
1. Lymph node: Metastatic squamous cell carcinoma.
2. Lymph node: Metastatic melanoma.

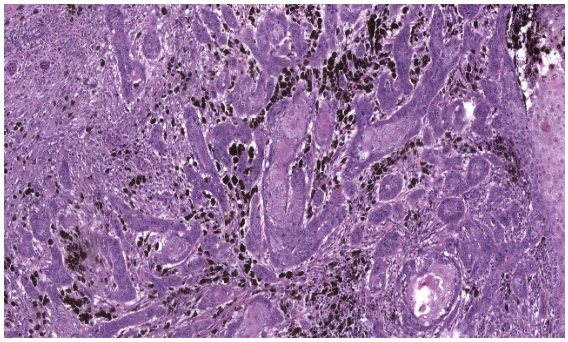

Figure 3-4. Inguinal lymph node, horse. Trabeculae and keratinizing nests of squamous epithelium efface the capsular sinus and infiltrate the underlying cortex. (HE, 108X)

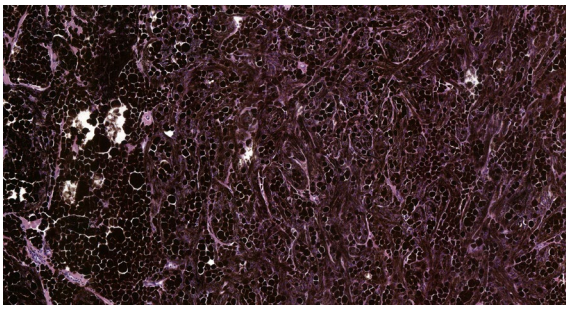

Figure 3-5. Inguinal lymph node, horse. Nests of polygonal to spindles and deeply pigmented neoplastic melanocytes infiltrate the lymph node (HE, 106X)

JPC Comment:
We would like to thank the contributor for this unique submission of two classic equine neoplastic entities in one tissue section. In addition, the contributor concisely highlights key features of each entity.

As noted by the contributor, both graying and melanoma in horses have been linked to a duplication of the *syntaxin-17* (*STX17*) gene. The significance of this duplication is the *STX17* gene plays a regulatory role in the regulatory binding sites for microphthalmia-associated transcription factor (MITF) and *NR4A3*, both of which play key roles regulating melanocyte gene expression and cellular function. Horses homozygous for the *STX17* duplication have two additional copies of this regulatory element and have a higher incidence of melanoma as well as a higher mean melanoma grade in comparison with heterozygous grey horses.[12]

An additional mutation associated with increased melanoma severity is the deletion

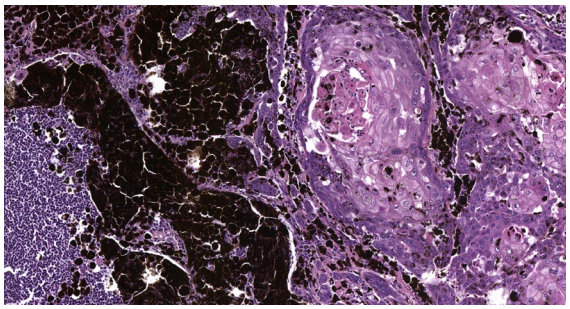

Figure 3-6. Inguinal lymph node, horse. Nests of neoplastic melanocytes infiltrate the tumor stroma of the squamous cell carcinoma and impart pigment to neoplastic squamous cells. (HE, 163X)

of exon 2 in the agouti-signaling protein (*ASIP*) gene, which regulates the melano-cortin-1-receptor (MC1R) pathway. *NR4A3* and *MITF* transcription is dependent on the MC1R pathway and cause an increase in the cellular concentration of cAMP. Under normal conditions, the MC1R signaling pathway is inhibited (i.e. regulated) by agouti-signaling protein, which in turn decreases cAMP, *MITF*, and *NR4A3* expression. Horses homozygous for the deletion of the exon 2 of the *ASIP* gene are unable to antagonize the MC1R pathway, resulting in increased signaling and has been associated with increased melanoma severity in horses.[12]

In contrast, "chestnut" horses occur as the result of a loss-of-function mutation of the *MC1R* gene, resulting in reduced melanocyte cAMP levels and decreased *MITF* and *NR4A3* transcription. In humans, similar loss of function mutations in the *MC1R* gene do not decrease the risk of developing melanoma but are associated with better survival rates. Although the *MC1R* loss of function mutation may be associated with similar outcomes in horses, this phenomenon has yet to be definitively validated in this species.[12]

Horses are affected by nine types of *Equus caballus* papillomavirus (EcPV), which are non-enveloped double stranded DNA viruses. As noted by the contributor, EcPV-2 has been associated with genital lesions and that in some cases undergo neoplastic transformation to squamous cell carcinoma. Potential ongogenic mechanisms have not yet been definitively determined. However, genomic sequencing has shown the presence of both E6 and E7 genes.[4] In contrast to high risk-human paplillomaviruses (hr-HPVs) which are known to cause cervical cancer, EcPV-2 E7 lacks the RB-binding motif. Therefore, the primary oncogene of interest EcPV-2's is E6, which in hr-HPVs inactivates p53 by signaling for its proteosomal degradation.[4] Also known as "the guardian of the genome", p53 is a tumor suppressor gene that regulates the cell cycle, DNA repair, cellular senescence, and apoptosis. Loss of p53 function results in the accumulation of damaged (i.e. unrepaired) DNA resulting in the accumulation of driver mutations in oncogenes that ultimately predispose the cell to malignant transformation.[8]

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CASE IV: 487/0821 (JPC 4165420)

Signalment:
45-day-old, female, Canary Black Pig (*Sus scrofa domesticus*)

History:
One female piglet, from a farm with 28 breeders (25 sows and 3 boards), had been found outside the farm wandering near rubbish dumps. Two days later, it exhibited lethargy, dehydration, hypothermia, and cachexia, along with vomit and muco-purulent nasal discharge. The animal died within a day after the signs were noticed. No specific treatment was administered other than fluids and electrolytes. No other pigs from the farm showed any clinical signs.
The animal was submitted to our diagnostic laboratory for a complete necropsy.

Gross Pathology:
The most remarkable macroscopic lesions were observed in the caecum, colon, liver, and palatine tonsils. Multiple well-demarcated white nodules were observed on the serosa of the spiral colon. The mucosa of both caecum and colon show numerous multifocal to coalescent well-demarcated

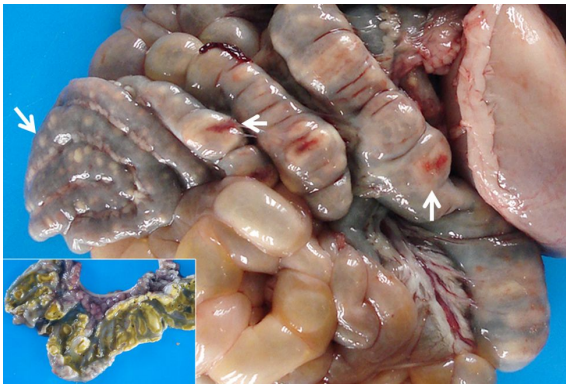

Figure 4-1. Spiral colon, pig. Multiple well-demarcated white nodules are scattered through the serosa of the spiral colon (arrows). The nodules correspond to areas of ulceration visible in the inset. (*Photo courtesy of* : Unit of Veterinary Histology and Pathology. University Institute of Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria, c/ Trasmontaña, s/n, 35413, Arucas, Canary Islands, Spain.)

ulcers, covered with fibrinosuppurative (diphteroid) membranes. In the liver, on the visceral surface of the caudate lobe, a locally extensive 5 cm-wide area of necrosis was observed. On cut surface, the necrotic lesion extends deep into the parenchyma. The palatine tonsils have focal and bilateral areas of necrosis and congestion. Other gross findings included serous abdominal effusion, and enlarged and congested mesenteric lymph nodes.

Laboratory Results:
Bacterial analyses including typing (Reference Laboratory, Algete-Madrid, Spain) identified Salmonella enterica subspecies enterica Serotype: Enteritidis 9,12: g.m. from samples of large intestine, liver and mesenteric lymph nodes.

Microscopic Description:
Colon: affecting the full thickness of the mucosa and extending into the submucosa

there are large, segmental, well-demarcated areas of necrosis covered with fibrin (button ulcers). These areas are characterized by loss of differential staining, loss of tissue architecture and replacement by abundant eosinophilic amorphous material, cellular debris, fibrin and degenerated neutrophils, admixed with abundant short rod bacteria. The underlying submucosa is severely infiltrated by viable neutrophils, macrophages and cellular debris, and expanded by edema. Few blood vessels are occluded by fibrin thrombi, together with occasional small number of short rod bacteria. Vascular walls show karyorexis/karyolisis and loss of cellular detail and are replaced by eosinophilic amorphous material and debris (fibrinoid necrosis). Moderate numbers of neutrophils and macrophages, with rare lymphocytes, infiltrate vascular walls and occasionally fully occlude the lumen (vasculitis). There is marked hypertrophy and hyperplasia of submucosal fibroblasts with plump nuclei (reactive). Multifocally, the same inflammatory infiltrate extends into the muscular layer, dissecting the myofibers.

Contributor's Morphologic Diagnoses: Colitis, necrotizing, fibrinosuppurative (diphtheroid), multifocal, severe, subacute, with vasculitis, thrombosis and intralesional colonies of short rod bacteria.

Contributor's Comment:

In addition to the lesions observed in the colon and caecum, other significant histological findings were hepatitis, necrotizing and lymphohistiocytic, tonsilitis necrotizing and neutrophilic, pneumonia, bronchointersticial, lymphohistiocytic, and microthrombosis in the spleen, lymph nodes, brain and leptomeninges. Gram stain highlighted intralesional positive and, to a lesser extent, negative rod-shaped bacteria in palatine tonsils, and small to moderate numbers of negative bacteria in the liver.

The Canary Black Pig (Cochino Negro Canario) is a breed exclusive to the territory of the Canary Islands archipelago. It seems to come from North African pigs, mixed with breeds introduced during the Spanish and British occupation of the islands in the 15th century. The breed was officially recognized once included in the Official Register of Spanish Livestock Breeds published in the Real Decreto (Royal Decree) 1682/19974 and it is currently under a Special Protection Status due to the reduced number of individuals and high risk of extinction. However, since the 80s, the population has been increasing, thanks to the numerous initiatives taken by both local authorities and a group of farmers who decided to introduce the breed in their facilities.[6]

Salmonellosis is known to be one of the main causes of food-borne enteric disease in humans worldwide. *Salmonella* Enteritidis and *S.* Typhimurium are the most commonly reported serovars associated with human

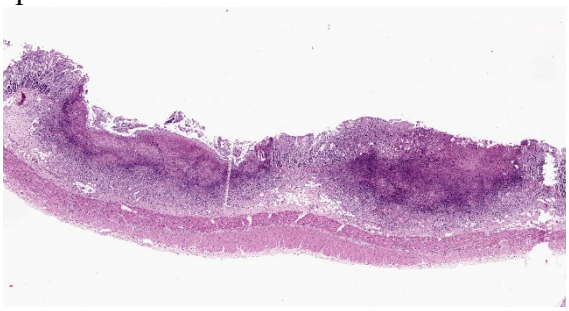

Figure 4-2. Spiral colon, pig. Multiple well-demarcated areas of full-thickness mucosal necrosis extend into the underlying submucosa. (HE, 5X)

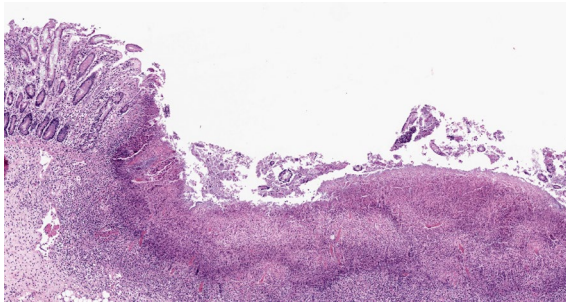

Figure 4-3. Spiral colon, pig. Ulcers are well-demarcated with a dense band of cellular debris at their margins. (HE, 108X)

cases. These zoonotic strains are carried by both livestock and wild animals, with avian species and pigs being the main reservoirs. In general, infection in birds is caused by serovar Enteritidis, while porcine infections are usually due to serovar Typhimurium or serovar Derby. However, in several European countries, *S.* Enteritidis has also been reported, in both domestic and wild suids.[2,5,8,9] Thus, in swine, salmonella infections are of concern for two main reasons: clinical disease in animals (salmonellosis) and the threat to human health by means of contaminate pork products with a broad range of serotypes.[3]

Fecal–oral transmission is the most common route of transmission of virulent salmonellae. Transmission can occur from pig to pig, contaminated environment to pig, or dam to offspring. Tonsils, and thus oropharyngeal secretions, become rapidly contaminated, favoring nose-to nose transmission. Aerosolized secretions, faeces, and contaminated dust particles are potential sources for short-distance aerosol transmission as well.[3] Over 200 virulence factors have been associated with salmonellae but only few have been completely characterized. Generally, these factors are involved in adhesion, invasion, cytotoxicity, and resistance to intracellular killing. The ability to invade is a requirement for pathogenesis and depends on a serotype-specific plasmid coding for virulence factors.[3] Many epithelial cell types as enterocytes, goblet cells or M cells in the jejunum and ileum may be invaded. Invasion of M cells seems to be the preferred route due to the shorter glycocalyx that coats these cells.[3] Attachment of the bacteria to epithelial receptors triggers microfilament-controlled uptake, vacuole formation, vacuole transport through the cell cytoplasm, and entry into the lamina propria via exocytosis through the basement membrane.

During the invasion process, there is selective induction of synthesis of new proteins that enhance intracellular survival within macrophages and neutrophils in the lamina propria.[3] *Salmonella* uses mucosal macrophages and dendritic cells to spread systemically, reaching, for instance, liver and lymph nodes, which are common sites of secondary infection (septicemic form). *Salmonellae* produce disease via enterotoxins, cytotoxins (verotoxins), and endotoxins causing microvascular thrombosis and endothelial necrosis in the submucosa and lamina propria. Mucosal ischemia is most likely the result of microvascular thrombosis and the action of enzymes and mediators released during the inflammatory process.[10] The systemic signs and lesions of septicemic salmonellosis, are commonly attributed to the endotoxemia that follows bacterial dissemination.

The number of potential sources of *Salmonella* infection in a swine population is

virtually endless, [3] and many stressors can be involved (e.g. overcrowding, transportation, concurrent diseases, feed changes, parturition and antibiotic treatments).[7,10] Although none of those were identified in this specific case, individual susceptibility and the age of the animal could be considered predisposing factors, as disease is usually more common and severe in young individuals.[10] In the present case, it is possible that contaminated material at the reported small rubbish dump was the source of infection. The fact that all the other animals at the farm tested negative for *Salmonella* supports this hypothesis. Housing of pigs in facilities other than total confinement is a factor associated with infection.[3]

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JPC Diagnosis:
Colon: Colitis, ulcerative, multifocal, severe, with vasculitis and thrombosis.

JPC Comment:
With the notable exception of the host adapted *Salmonella* Cholerasuis, the majority of porcine *Salmonella* infections result in self-limiting diarrhea or are asymptomatic. As mentioned by the contributor, the majority of porcine infections are caused by *Salmonella* Typhimurium, which has been linked to numerous outbreaks of human salmonellosis directly related to the consumption of pork products.[1]

As noted by the contributor, the jejunum and ileum are believed to be the primary sites of *Salmonella* invasion following ingestion. Utilizing previously described mechanisms, the organism breaches the mucosal epithelium, enters the lamina propria, undergoes leukocyte trafficking to the subepithelial dome of Peyer's patches and then quickly spreads to mesenteric lymph nodes where in most cases its dissemination is halted by cellular and inflammatory responses. However, it is believed high numbers of infected pigs become carriers with latent organisms remaining in the tonsils and mesenteric lymph nodes, which presents a challenge for control of the disease within a herd.[1]

A recent study assessed the persistence of *Salmonella* Typhimurium infection of weaned pigs using using Taqman qPCR to quantify *Salmonella* in the contents of the ileum, cecum, colon, and feces and while also performing histologic examination of tissues. Mucosal epithelium was severely damaged 1 day post infection (dpi) and 2 dpi with large quantities of *Salmonella* detected in the ileum, cecum, and colon early in the

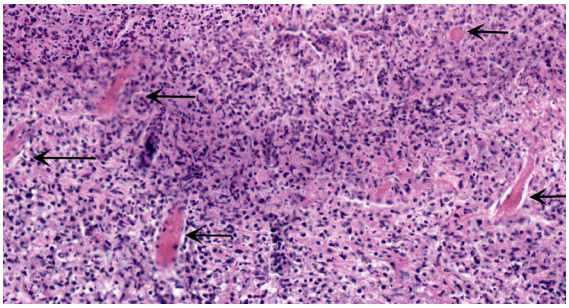

Figure 4-4. Spiral colon, pig. At the base of each ulcer, vessels are occluded byfibrin thrombi. (HE, 380X)

infection, likely bearing resemblance to lesions seen in this case. After 6dpi, signs of recovery with progressive epithelial restoration, reduction of inflammatory infiltrate, and elimination of *Salmonella* from the mucosa were noted. Although clinical recovery correlated with decreased shedding, *Salmonella* continued to be passed four weeks post infection. In addition, the bacteria were identified within the mesenteric lymph nodes up to 30dpi, the end point of the study. This further supports the latent risk of reinfection of pigs as well as potential food chain contamination.[1]

Interestingly, recent studies have demonstrated *Salmonella* infects and survives not only within macrophages but also fibroblasts, which both may serve as Trojan horses, shielding the organism from immune surveillance until conditions are favorable for reinfection.[1]

Although *Salmonella* Enteritidis was isolated in this case, the lesions observed are essentially identical to those caused by classical swine fever virus (hog cholera), an endotheliotropic *Pestivirus*. This virus predominantly affects the immune system, vascular endothelium, and epitheial cells. CSFV replicates within lymphoid tissue macrophages and causes lymphocytolysis via expression of cytokines such as TNF-α. Lesions consistent with this phenomenon in the submitted tissue sections include GALT abscessation and lymphoid depletion, although these findings in this case are likely secondary to circulation endotoxin. Epithelial damage due to CSFV is also as the result of macrophage derived cytokines. Damage to the endothelial cells can ultimately result in disseminated intravascular coagulopathy and hemorrhage.[7]

The moderator discussed additional etiologies that may cause similar lesions of the colonic mucosa in swine, including classical swine fever virus, *Brachyspira hyodysenteriae* and *B. hampsonii*, *Lawsonia intracellularis,* and in very young piglets, *Clostridium difficile.*

Additional sections stained with Warthin-Starry revealed numerous argyrophilic spirochetes associated with the superficial aspect of mucosal epithelial cells. It is possible these represent a coinfection with *Brachyspira* spp. in addition to *S.* Enteritidis.

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