Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #22 12 April 2024

CASE I:

Signalment:
18-month-old Angus heifer, *Bos taurus*, bovine.

History:
Two out of a herd of one hundred heifers had a history of progressive wasting and diarrhea. One of the heifers had a body condition score of 1/5 and was euthanised.

Gross Pathology:
A field post-mortem was performed and over 10 adult liver flukes were observed in the liver parenchyma, as well as occasional multifocal areas of chronic pulmonary consolidation and suspected ileum thickening.

Laboratory Results:
Serum liver fluke ELISA S/P % - 158 (strong positive).

Serum pestivirus antigen capture ELISA - negative.

Bovine Johne's disease liquid culture and PCR - negative.

Microscopic Description:
Portal areas are markedly expanded by proliferative fibroblasts, mature connective tissue, markedly increased numbers of small caliber bile ducts (biliary hyperplasia) replacing periportal hepatocytes and frequently joining between adjacent portal areas and isolating hepatic lobules (bridging fibrosis), and numerous eosinophils and lymphocytes. Macrophages scattered in portal areas and sinusoids often contain yellow-brown pigment (likely bile). Multifocally replacing parenchyma, there are several irregular, well demarcated areas of homogenous eosinophilic material (proteinaceous oedema) with extravasated erythrocytes (haemorrhage) and frequent intact and degenerate eosinophils and macrophages, surrounded by fibrillar eosinophilic material (fibrin) (liver fluke migration tract). Focally replacing the parenchyma, there is a longitudinal

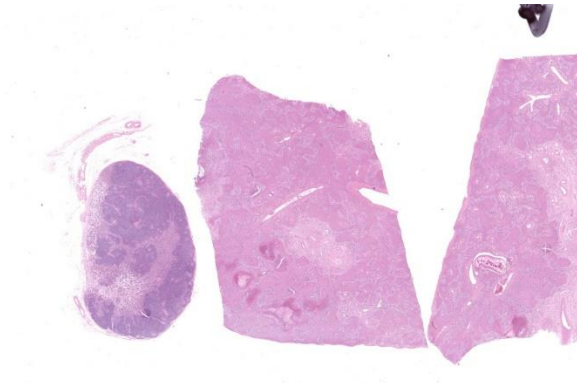

Figure 1-1. Liver, ox. One section of reactive lymph node (left) and two section liver are submitted for examination . At subgross magnification, there is bridging fibrosis between portal areas, and on the section at right, a cross section of a fluke is present in a bile duct. (HE, 4X)

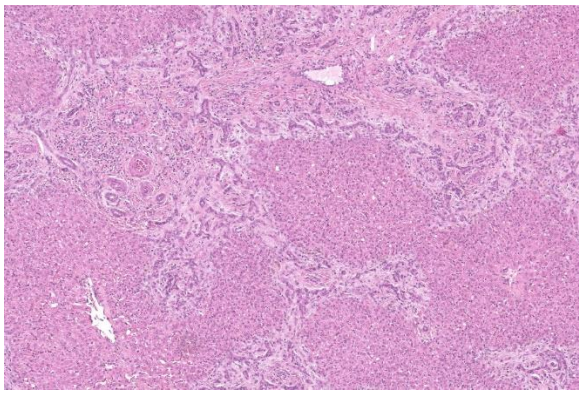

Figure 1-2. Liver, ox. There is bridging fibrosis of portal areas and marked biliary hyperplasia. (HE, 82X)

section of a 3.1 x 0.9 mm metazoan parasite which features a prominent outer tegument with spines on the anterior two thirds, muscular anterior (oral) and ventral suckers, with a digestive tract which includes a caecum containing yellow-brown pigment, surrounded by parenchyma, and no evidence of a uterus, vitellarian glands, or eggs (morphology consistent with juvenile *Fasciola hepatica*).

Contributor's Morphologic Diagnosis:
Liver: Fibrosis, periportal and bridging, marked, diffuse, chronic, with marked biliary hyperplasia, eosinophilic and lymphocytic portal hepatitis, parasitic migration tracts and intralesional juvenile trematode, morphology consistent with *Fasciola hepatica, Bos taurus.*

Contributor's Comment:
Liver fluke (*Fasciola hepatica*) is a common endoparasite of cattle and sheep which is endemic to Eastern Australia and poses a major economic threat to producers.[1] The life cycle of the liver fluke in the animal begins with excystment of metacercariae in the small intestine where newly emerged juvenile flukes migrate to the liver and then over 8-10 weeks migrate through the hepatic parenchyma to the bile ducts. Here, they mature into adult flukes and produce eggs in the bile and feces 8-10 weeks post infection.[2] In the acute phase, the migration through the liver causes extensive inflammation and hepatocellular damage, which induces an antibody response.[2]

There are several different methods used to diagnose liver fluke, including fecal sedimentation examination, necropsy, histopathology, and immunological based tests which include an antibody ELISA, or a commercial fecal copro-antigen ELISA (cELISA; Bio X diagnostics, Belgium). Each method has advantages and disadvantages, and test results should be interpreted in light of the tests' shortcomings.

Fecal sedimentation results in cattle have variable sensitivity (30-60%) due to the intermittent shedding of the fluke eggs, volume of the feces produced, and the relatively long prepatent period.[14] The specificity is high at 99%.[7] This test will only detect the presence of female adult fluke.

Liver fluke antibody ELISA may be performed on serum and milk and will detect IgG antibodies for *F. hepatica* excretory- secretory (E/S) antigen from 2-4 weeks post infection.[13] The sensitivity and specificity for the serum antibody ELISA varies depending on the season and ranges from 72-94% and 76-89%, respectively.[7] Another advantage of the antibody ELISA is that it can be used for surveillance and herd level diagnosis in bulk milk samples with ranges of sensitivity and specificity being 81.3-95% and 98.2-100%, respectively.[11] The antibody ELISA can only detect exposure and may remain positive for at least 4 weeks post flukicide treatment; therefore, veterinarians should take caution interpreting these results as cattle may not be currently infected.[2]

Test	Sensitivity (%)	Specificity (%)
Fecal sedimentation	30-60[12]	99[12]
Liver fluke serum antibody ELISA	72-94[6]	76-89[6]
Liver fluke milk antibody ELISA	81-95[9]	98-100[9]
Commercial fecal copro-antigen ELISA	80-87[8]	100[8]
Liver necropsy	99[6]	98[6]
Abattoir liver inspections	68[6]	88[6]

Table 1: Summary of sensitivity and specificity for different diagnostic tests for *F. hepatica* detection.[7]

The cELISA detects *F. hepatica* E/S antigen in the feces 6-8 weeks post-infection and can detect low liver fluke burdens.[6] The cELISA was designed for and is commercialized for the use of detecting liver fluke in sheep. Many studies have reviewed the use of the fecal copro-antigen ELISA in cattle. Palmer et al. (2014) illustrated that the specificity of the cELISA was 100% in cattle.[9]

When following the recommended cut off for the commercial kit the sensitivity was 80% in comparison to 87% when using a lower custom cut off for liver fluke detection in Australian cattle.[9] Multiple studies have used a modified cut off when using the commercial cELISA in cattle and therefore there is no consensus on the method and hence sensitivity of the commercial kit.[2,3,9] Furthermore, the sensitivity of this test varies over time as the antigens are released intermittently.[5,7] An advantage of the method over the antibody ELISA is that worm burden can be differentiated pre- and post- anthelmintic treatment.[2] Unfortunately, a fecal sample was not submitted for testing in this case.

Finding *F. hepatica* on liver necropsy is considered gold-standard however this method is not available for living animals. Estimated sensitivity and specificity are 99% and 98%, respectively.[7] These values are much higher compared to liver inspections at abattoirs which were estimated to have a sensitivity and specificity of 68% and 88%, respectively.[7] Liver histopathology can support a diagnosis of liver fluke, although sections often do not contain trematodes themselves. In this case, the size of the fluke and lack of gonad fits with the life cycle as juvenile flukes migrate through the liver parenchyma to the bile ducts where they become sexually mature.

Typical hepatic lesions in acute infection are characterized by hemorrhagic tortuous tracts with an element of coagulative necrosis and occasional inclusion of young flukes. As the disease progresses, these lesions are infiltrated by eosinophils followed by macrophages and giant cells which clear away the debris before healing occurs by formation of granulation tissue and fibrosis. Studies have shown that immature *F. hepatica* enters the liver parenchyma at 0.2 mm long and in cattle are approximately 1.5-3 mm long 21 days post-infection.[8,12] Most juvenile flukes migrate to the bile ducts; however, some encyst in a fibrous capsule within the parenchyma.[8] Mature flukes grow to approximately 2.5 cm long and reside in the large bile ducts and cause cholangitis. Biliary hyperplasia is a result of irritation by the flukes and biliary stasis. In this case, there is evidence of both acute and chronic fluke infection, with the presence of immature *F. hepatica* and migration tracts, and biliary hyperplasia and portal fibrosis, respectively. This suggests that this animal has been previously infected, with current reinfection.

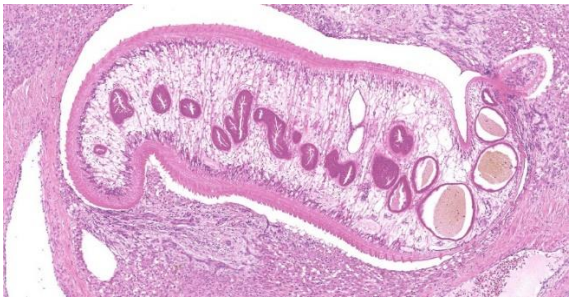

Figure 1-3. Liver, ox. Within a bile duct, there is a sagittal section of a larval trematode. The larva has a ridged tegument, a spongy body cavity with numerous subtegumental somatic cell nuclei, and numerous cross sections of intestine. (HE, 87X)

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JPC Diagnosis:
Liver: Fibrosis, portal, bridging, diffuse, moderate to severe, with biliary hyperplasia, parasite migration tracts, and larval trematode.

JPC Comment:
Fasciola hepatica has a broad geographic range and causes estimated annual losses of US $3.5 billion in the global livestock industry; beyond livestock, many species of mammals are affected with approximately 170 million humans worldwide at risk of infection.[4] Many mammals serve as definitive hosts for the fluke, including cattle, sheep, buffalo, and humans.

F. hepatica has a rather complicated life cycle which begins when non-embryonated eggs are shed in the feces of an infected definitive host.[1,10] Eggs become embryonated and hatch in water, followed by release of miracidia which then penetrate an aquatic snail which serves as the intermediate host.[1,10] Development continues within the intermediate host until the free-swimming cercarial form is released into the environment.[1,10] Cercariae encyst on plants and form metacercariae which are then ingested by a human or animal host.[1,10] As noted by the contributor, once inside the host the metacercariae migrate through the duodenal wall, the peritoneal cavity, and then through the parenchyma of the liver for six to seven weeks until they reach the bile duct, where they mature into adults.[1,10] Once a patent infection is established, flukes are prodigious egg layers, with a single adult fluke contaminating the environment with 20,000-50,000 eggs per day over a long time period.[1] In cattle, egg production eventually begins to decline as the animal develops resistance to chronic infection.

Acute fascioliasis may occur following heavy intake of metacercariae, typically when livestock are grazed on heavily contaminated, wet ground.[1] Clinical signs may be absent, or may include abdominal pain and/or jaundice. Death is usually the result of liver hemorrhage secondary to the immature fluke's migration through the hepatic parenchyma.[1] Subacute disease is heralded by jaundice, ill thrift, and anemia secondary to extensive migration-related liver damage which results in death in 8-10 weeks.[1]

Chronic fascioliasis is the most common form of the disease in cattle, sheep, goats, horses, and pigs, and develops when the flukes reach the bile ducts, begin ingesting blood, and cause anemia, chronic inflammation, and enlargement of the bile ducts.[1] The disease is insidious, with increasing severity of anemia, decreasing appetite, reluctance to move, and, in some animals, submandibular edema ("bottle jaw").[1]

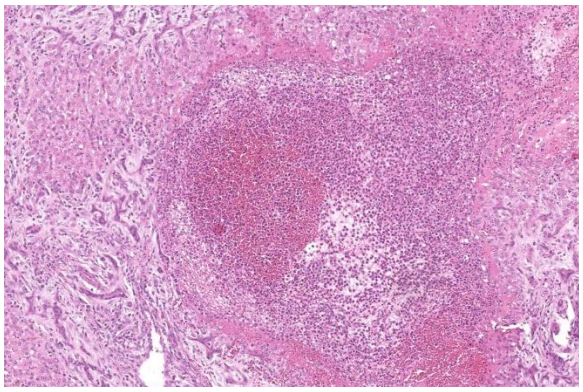

Figure 1-4. Liver, ox. Scattered randomly throughout the parenchyma, there are foci of necrosis (migration tracts) in which hepatocytes are replaced by various combinations and concentrations of neutrophils, macrophages, eosinophilis, lymphocytes, fibroblasts, and collagen. (HE, 116X)

A complication of fluke infection is Black disease, an acute and fatal liver disease that develops in fluke-infested sheep and cattle. Black disease is caused when fluke migration-induce liver damage provides a suitably anaerobic environment for the germination of *Clostridium novyi* type B spores within the liver. *C. novyi* multiplies in areas of hepatic necrosis and elaborates a necrotizing alpha toxin which causes more tissue damage, leading to a vicious cycle of bacterial proliferation and toxin elaboration. The disease, also called infectious necrotic hepatitis, causes acute death primarily in sheep, sometimes in cattle, and rarely in pigs and horses.[1] Control is largely through reduction of fluke or intermediate host (snail) numbers, though a *C. novyi* toxoid is available for use to control outbreaks.

This week's conference was a double-feature, with Dr. Paul Stromberg, Professor Emeritus at The Ohio State University College of Veterinary Medicine, and Dr. Don Meuten, Professor Emeritus at North Carolina State University College of Veterinary Medicine, sharing moderator duties. Dr. Stromberg began discussion of this case by noting the loss of tissue architecture, the "alarmed" hepatocytes, and the abundant inflammation and necrosis present within the liver. These acute changes are present against a background of severe fibrosis and chronic inflammation.

The moderator noted that the histologic features of the trematode, particularly the lack of a reproductive tract, the trematode as a juvenile and this, along with the gross findings of adult trematodes in the biliary tree, indicate that this animal is likely living in a contaminated environment and subject to recurrent bouts of infection, explaining the acute-on-chronic nature of the observed lesions. The moderator noted the dilated sinusoids and attenuated hepatocytes and speculated that these changes result from impeded hepatic circulation due to the massive hepatic fibrosis appreciated in section.

References:

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CASE II:

Signalment:
9-year-old female Tinker horse (*Equus caballus*)

History:
A mature female horse was admitted to the vet with hematuria. Bloodwork showed a hypoalbuminemia. The clinical signs improved after treatment with antibiotics and anti-helminthics. 3 weeks later, the horse developed a fever and ataxia. Treatment with doxycycline and NSAIDs was started, but one day later the horse was in lateral recumbency and had a vertical nystagmus. It was humanely euthanized.

Gross Pathology:
The horse was in a good body condition with normal musculature and normal fat reserves. Both kidneys contained multifocal, pale, firm, well-circumscribed masses varying in size from 1 to 10 cm in diameter. The mammary glands contained a moderate amount of watery, yellow fluid and were diffusely light pink on cut surface. Multifocally, the back muscles and gluteal muscles were diffusely pale compared to the other muscles.

Microscopic Description:
Mammary gland: Multifocally to coalescing, the original tissue architecture is extensively disrupted by foci of granulomatous inflammation, characterized by large numbers of epithelioid macrophages and few multinucleated giant cells (Langhans-type), admixed with large numbers of lymphocytes, plasma cells and eosinophils. The inflammation is centered around sections of nematode adults, larvae, and eggs, which are randomly spread throughout the tissue. The adult nematodes are approximately 15-20 microns in diameter and up to 300-400 microns in length, and have a thin cuticle, platymyarian-meromyarian musculature, intestinal tract, and characteristic rhabditiform esophagus with a corpus, isthmus and terminal bulb. The nematode larvae measure 10-15 microns in diameter and 150-250 microns in length and have a rhabditiform esophagus. Nematode eggs are ovoid, both embryonated and larvated, and measure approximately 10 x 50 microns. Incidentally, parasites are surrounded by karyorrhexis and karyolysis, with loss of cell detail (lytic necrosis).

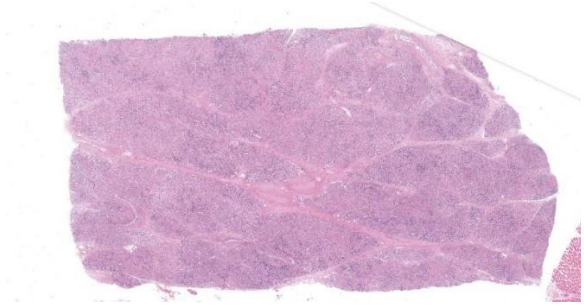

Figure 2-1. Mammary gland, horse. A section of mammary gland is submitted; the normal architecture is distorted and inflammation is evident at subgross. (HE, 4X)

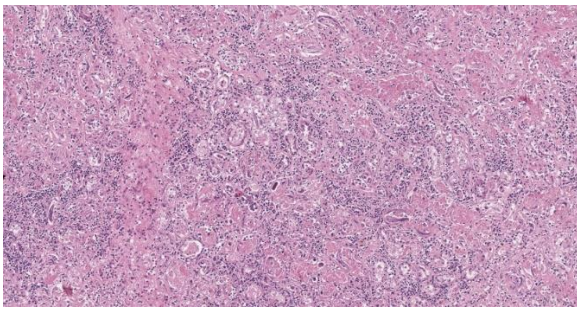

Figure 2-2. Mammary gland, horse. At higher magnification, the normal acinar architecture is effaced by fibrosis and granulatomous inflammation. Acinar epithelium is necrotic and acini are often lined by thin attenuated epithelium. Larval nematodes are scattered throughout the field. (HE, 91X)

Contributor's Morphologic Diagnosis:
Mammary gland: Severe, chronic, multifocal to coalescing, granulomatous, eosinophilic and lymphoplasmacytic mastitis with myriad intralesional rhabditoid nematode adults, larvae, and eggs, etiology presumptive *Halicephalobus gingivalis.*

Contributor's Comment:
The parasite with its accompanying inflammation was also extensively present in the cerebrum, cerebellum, kidneys and some skeletal muscles.

Halicephalobus gingivalis is a facultative, opportunistic nematode of the order *Rhabditidia*, family *Panagrolaimidae*, and causes a rare form of meningoencephalomyelitis in equids, humans, and ruminants.[4,5] The life cycle and pathogenesis of this opportunistic pathogen is still poorly understood. *Halicephalobus* nematodes are found free-living in association with water, soil, and decaying organic matter and it is thought that transmission occurs primarily via wounds in the skin or mucous membranes.[3] Rarely, other routes of infection

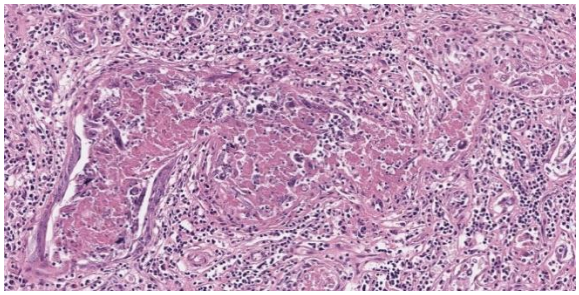

Figure 2-3. Mammary gland, horse. Lactiferous ducts are filled with cellular debris and intrepid rhabditid larvae. (HE, 209X)

have been suggested, including transmammary.

Upon infection, dissemination of the infection is then thought to occur hematogenously.[2] Sexual reproduction is believed to occur during the free-living part of the life cycle and although both males and females exist in the environment, only female nematodes have been observed within microscopic lesions. This suggests that within a host, reproduction occurs asexually via parthenogenesis.[1]

Histologic identification of consistent lesions and morphologically compatible rhabditoid nematodes found postmortem are an indication for diagnosis, although the nematodes can be extracted from fresh tissue or the parasite can be shed in the urine if there is renal involvement.

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https://www.uu.nl/onderzoek/veterinair-pathologisch-diagnostisch-centrum

JPC Diagnosis:
1. Mammary gland: Mastitis, granulomatous and eosinophilic, chronic, diffuse, marked, with numerous adult and larval rhabditid nematodes.
2. Lymph node: Reactive hyperplasia, diffuse, moderate.

JPC Comment:
Halicephalobiasis is an infection characterized by localized or disseminated granulomatous lesions that has been sporadically reported in horses and zebras and less commonly in cows and humans.[6] As the contributor notes, surprisingly little is known about the pathogenesis of the disease and the biology of the causative agent, *Halicephalobus gingivalis.* The nematode was first described in 1954 after the worms were discovered within a gingival granuloma in a horse, providing the species name for this enigmatic pathogen.[5] *H. gingivalis* has since been described in, among other tissues, the central nervous system, kidneys, oral cavity, eyes, lungs, adrenal glands, spleen, liver, heart, and, with this case, the mammary gland.[5,6]

Due to the variety of tissues that can be affected, the clinical presentations and signs of halicephalobiasis are highly variable; however

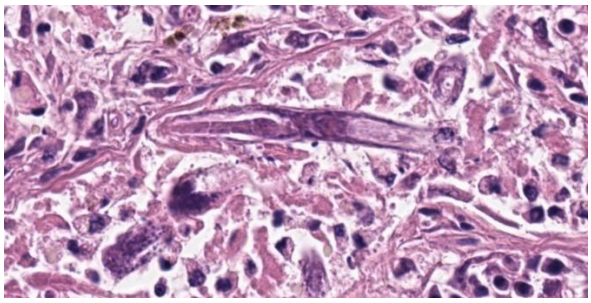

Figure 2-4. Mammary gland, horse. Larva have a rhabditoid esophagus with a corpus, isthmus, and bulb. (Obligatory picture.) (HE, 1025X)

neurologic and renal lesions, and their attendant clinical signs, appear to be overrepresented in published case reports.[6] The nematode itself has a distinctive morphology, which is nicely demonstrated in the abundant longitudinal and cross sections present in the examined slide. Adult *H. gingivalis* nematodes are cylindrical with tapered anterior ends and pointed tails, and possess a classic rhabditiform esophagus composed of the canonical corpus, isthmus, and bulb.[6]

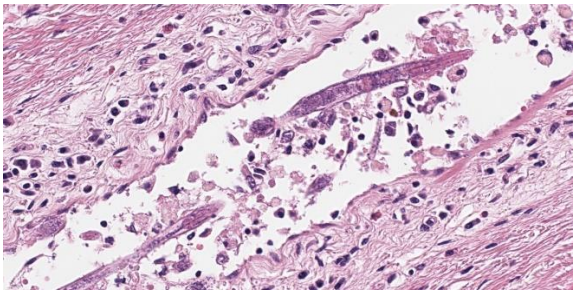

Figure 2-5. Mammary gland, horse. There goes a female *Halicephalobus* sailing away down the vessel (she's got to get away from the kids!). (HE, 500X)

The central nervous system is most commonly affected in horses, with massive intracranial invasion often causing an acute disease with a short course.[1] Gross lesions include focal arachnoid hemorrhages and multifocal thickening of the meninges. Histologically, female nematodes and larvae are found within lesions, particularly in perivascular spaces, and lesions are characterized by granulomatous and eosinophilic meningoencephalitis, myelitis, or polyradiculitis; parasitic granulomas in the kidneys and gingiva often accompany, and are perhaps antecedent to, central nervous system invasion.[1] Treatment of *H. gingivalis* infection is typically ineffective, most likely due to the inability of drugs such as ivermectin to penetrate the robust granulomatous lesions or to cross the blood-brain barrier; thus, these infections typically have a poor prognosis and a short course.[6] Other rhabditid nematodes that can infect horses include *Strongyloides westeri*, *Pelodera strongyloides* (more commonly associated with cutaneous lesions in dogs), and *Cephalobus* spp.; however, the size and distinct morphology of *H. gingivalis* allow for a presumptive diagnosis.[6]

Dr. Stromberg challenged conference participants, who all agreed with the contributor's identification of *Halicephalobus gingivalis*, to consider their level of confidence in their parasite identification. Considering the large number of rhabditid nematodes that exist in the environment, how does one know definitively that this nematode is *H. gingivalis*, particularly given the unusual anatomic location in which this nematode is found? Additionally, the extremely small size of the parasite makes evaluation of fine anatomic features, such as the type of musculature present, particularly challenging. The moderator, while agreeing with the etiology and diagnosis, urged conference participants to at least acknowledge when a diagnosis is based on probability; while, by definition, a probability-based identification is often correct, it behooves the diagnostic pathologist to realize that these diagnoses deserve extra scrutiny.

Dr. Stromberg noted that the intensity of the inflammatory response evoked by a particular parasite can give insight into the evolutionary history of the parasite/host relationship. In this case, the typically robust inflammatory response suggests that the evolutionary history of *H. gingivalis* and the horse is short and still being written. The raging chronic mastitis is evidenced by a range of fibrosis throughout the mammary gland, which conference participants appreciated by examination of a Masson trichrome stain. Dr. Stromberg noted the

correlation between the degree of damage and fibrosis and the number of adult and larval nematodes in a particular area, with fibrosis ranging from barely perceptible to obliterative in the most severely affected sections.

The JPC morphologic diagnosis omits a specific etiology, preferring, based on Dr. Stomberg's discussion of uncertainty, to describe the parasite simply as a rhaditid nematode. Participants also felt that the changes observed in the accompanying lymph node were severe enough to warrant their own mention.

References:

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CASE III:

Signalment:
10-year-old male neutered dog (*Canis familiaris*).

History:
Left hind leg/distal femur mass. Radiographs showed bone lysis and periosteal reaction. An amputated hind leg was received at the diagnostic laboratory for diagnosis.

Gross Pathology:
Left hind leg mass involving the bone and associated soft tissues (skin/muscle/subcutis).

Microscopic Description:
Multiple tissues from the amputated left hind leg received were examined. Sections of bone were examined after decalcification. The examined tissues contained a non-demarcated, non-encapsulated neoplasm composed of variably loose to solid sheets of spindle to polygonal cells supported on ample fibrovascular stroma. The cells had indistinct to variably distinct cell borders, ample eosinophilic cytoplasm, round to oval vesicular nuclei and distinct nucleoli. Six mitotic cells were observed in 2.4 mm2/equivalent to 10 N22/40x high power fields. Anisocytosis and anisokaryosis were moderate and there were multifocal multinucleated giant cells consistent with osteoclasts in the mass. The neoplastic cells multifocally surrounded eosinophilic fibrillar material (osteoid) and osseous trabeculae.

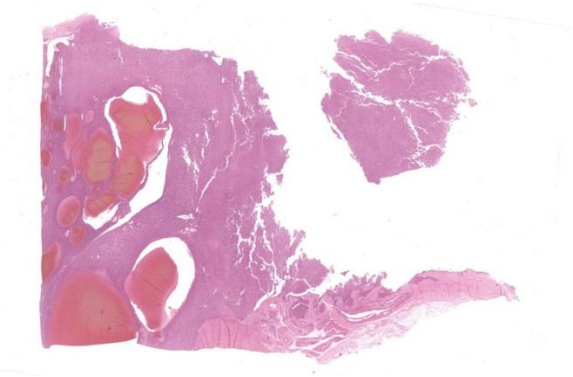

Figure 3-1. Skeletal muscle, quadriceps, dog. A neoplasm extends into and effaces the skeletal muscle. (HE, 5X)

Contributor's Morphologic Diagnosis:
Osteosarcoma, Metastatic.

Contributor's Comment:
Osteosarcoma (OSA) is the most common primary bone tumor in dogs. In general, the risk of developing osteosarcoma appears to be amplified under conditions that drive excess osteoblast activation. OSA most commonly develops at or near growth plates, where cell turnover is highest, and represents up to 85% of all primary malignant bone tumors in the dog.[4,5,7] Appendicular OSA is diagnosed most frequently in middle-aged to older, large and giant breed dogs, and affects the forelimb, particularly the humerus, more frequently than the hindlimb.

Significant differences in distribution patterns are reported between age, tumor location, and phylogenetic clusters. There are also significant variations in distribution patterns between neuter status, age, and dog sizes.[7] High risk of appendicular osteosarcoma in large and giant breed dogs may often be the result of replicative mutations caused by normal processes of cell division required to create longer bones, with only modest contributions from heritable or environmental factors.[4]

Some studies identify breed associations with osteosarcoma risk in terms of both predisposition and protection. These results can inform breed health reforms, especially in breeds such as the Rottweiler, Rhodesian Ridgeback and the Great Dane which have been shown to be highly at risk.[3] The skin or subcutaneous tissue is often the first osteosarcoma metastatic site detected as was observed in this case. After cutaneous and subcutaneous metastasis, the prognosis is grave with a median survival time of less than 2 months.[5] Treatment with surgery and chemotherapy is suggested to improve outcome and was reported to be significantly associated with survival time after the diagnosis. The median cutaneous/subcutaneous metastasis-survival time for dogs treated with surgery and chemotherapy or chemotherapy alone was significantly longer than in the untreated ones, although the prognosis for dogs with metastatic tumors much poorer than for individuals with only primary tumors.[6]

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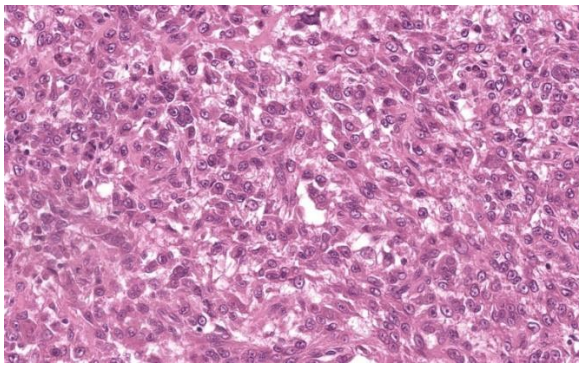

Figure 3-2. Skeletal muscle, quadriceps, dog. Neoplastic cells are arranged in short streams and bundles. (HE, 160X)

JPC Diagnosis:
Skeletal muscle and tendon: Osteosarcoma.

JPC Comment:
Osteosarcoma is well-known and well-studied in veterinary medicine; however, despite the familiarity of this tumor, it remains difficult to differentiate OSA from other primary bone neoplasms, which often present with similar clinical signs and radiographic appearances.[1] The issue is clinically urgent as OSA has a high risk of metastasis and an aggressive clinical course, yet histopathologic diagnosis of bone neoplasms was found to be only 72% accurate in one study, with only 13 of 18 malignant tumors accurately diagnosed.[1] Of the misdiagnosed tumors, 3 were initially diagnosed as less aggressive tumors, but were ultimately proved to be OSA.[1]

Histologic differentiation of bone tumors is difficult primarily because of their morphologic similarities and the small tissue samples typically provided for examination. Because of this, various immunohistochemical (IHC) markers have been evaluated for their potential to increase the sensitivity and specificity of an OSA diagnosis; however, a single sensitive and specific marker to differentiate osteoblastic cells in formalin-fixed tissue has yet to be identified.[1] A recent study in *Veterinary Pathology* characterized the expression patterns of four osteoblast-associated markers—Alkaline phosphatase (ALP), runx2, osteonectin, and osteopontin—to determine if the expression patterns of these proteins would be useful in differentiating OSA from other bone tumors.[1]

ALP is already used for this purpose in cytology, where staining for ALP enzyme activity on cytologic samples can increase the sensitity and specificity of an OSA diagnosis to 100% and 87%, respectively.[1] In contrast, ALP IHC staining identifies the protein rather than its enzymatic activity, and, on formalin-fixed tissue, has an excellent sensitivity (100%) but only a limited specificity (30%), making it unreliable for diagnosing OSA as a sole marker.[1] Runx2 is a transcription factor that is essential for osteogenesis, skeletal development, and osteoblastic differentiation. Its expression is consistently elevated in both human and canine OSA, and runx2 IHC staining was found to be 87% sensitive and 78% specific in the diagnosis of canine OSA.[1]

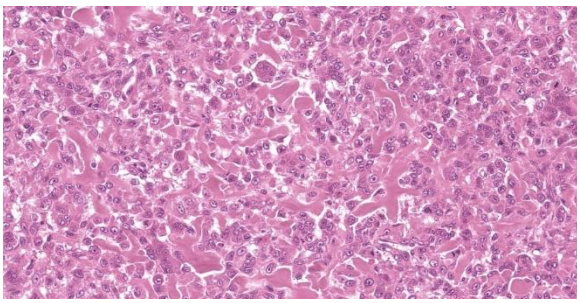

Figure 3-3. Skeletal muscle, quadriceps, dog. Neoplastic cells regionally produce and are entrapped in osteoid matrix. (HE, 350X)

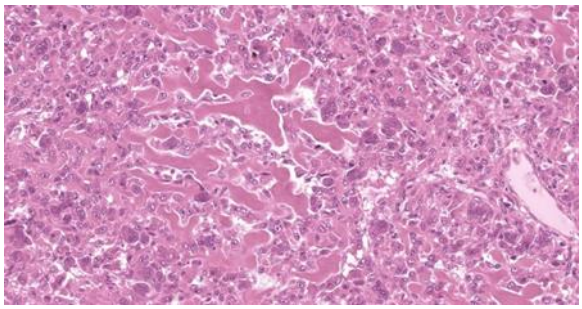

Figure 3-4. Skeletal muscle, quadriceps, dog. Neoplastic cells regionally produce and are entrapped in osteoid matrix. (HE, 350X)

Based on the sensitivity and specificity profiles of the four examined markers, the authors found the greatest diagnostic benefit from running ALP and runx2 in series.[1] The diagnostic algorithm requires running the ALP IHC first. Due to the high sensitity of ALP, if the sample is negative, the tumor is likely not an OSA and no further testing is necessary. If the tumor expressed ALP, however, runx2 should be interrogated to further characterize the tumor. When performed in series in this order, ALP and runx2 have a combined sensitivity of 87% and a specificity of 85% providing a useful set of diagnostic OSA markers.[1]

The moderator for this half of the conference, Dr. Meuten, used this classic entity to compare and contrast the traditional narrative, descriptive reports produced by most veterinary pathology diagnostic institutions, including this one, with the more streamlined, data point-driven process of synoptic reporting. After noting that synoptic reporting has become the standard in human pathology practice, Dr. Meuten led a robust discussion centered on the practical challenges associated with and the institutional and individual resistance to the transition.

Synoptic reporting dispenses with narrative descriptions and pares down diagnostic deliverables to discrete pieces of data reported in defined formats in a checklist-style report.[2] As extensively discussed in conference, the challenge with synoptic reporting is choosing what data gets collected, what data gets reported, and to whom the data is disseminated. While it is facile to state that only data which has proven diagnostic, prognostic, and predictive value should be reported, in practice, this becomes complicated by practical considerations related to the need for future data mining, the different needs of different audiences, and what is considered diagnostic or prognostic enough to merit inclusion in the report.

In the context of this particular tumor, discussion focused on whether histologic subtype, which does not currently have clear predictive value, should be included on a hypothetical synoptic report template for OSA. Some residents thought that the examined OSA might be a telangiectatic subtype and felt that this information should be collected. The moderator countered that the potential oncologist reading the report would likely treat the animal the same, regardless of histologic subtype, so should the synoptic report include only the diagnosis?

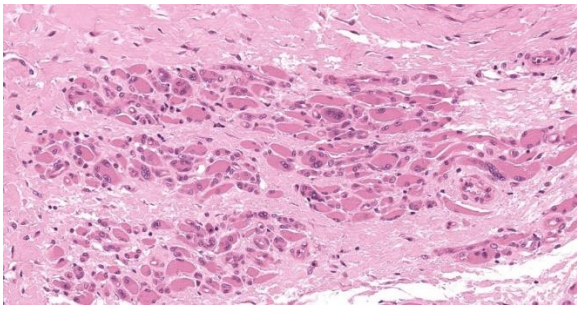

Figure 3-5. Skeletal muscle, quadriceps, dog. There is marked fibrosis for the skeletal muscle and atrophy of individual myofibers. (HE, 400X)

The interesting, rhetorical discussion raged on for some time with the moderator passionately exploring the concept of synoptic reporting to an engaged but admittedly skeptical audience. An excellent discussion of the considerations involved in synoptic reporting can be found at the Veterinary Cancer Guidelines and Protocols website.[2]

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CASE IV:

Signalment:
8-year-old female intact American Staffordshire terrier (*Canis familiaris).*

History:
The dog presented to the small animal hospital with acute onset of tachypnea, abdominal breathing, tachycardia, and bright red mucous membranes. The dog had been coughing for a month, had been inappetent, exercise intolerant, and had been losing weight for 1-2 weeks. Two weeks prior, the dog was diagnosed with a urinary tract infection. The dog was euthanized without further diagnostics and subsequently submitted for necropsy.

Gross Pathology:
The dog was in good body condition. Originating from the mucosa of the trigone area of the urinary bladder was an irregularly and indistinctly outlined mass, approximately 6 cm in diameter, with homogenous white to yellow colour, firm consistency, and a smooth, solid white cut surface. The mass extended into and infiltrated nearly the full length of the urethra. The inguinal lymph nodes were severely enlarged with a homogenous light colour, firm consistency, and a smooth, solid cut surface. Bilaterally in the adrenal medulla, 0.5 cm diameter, well-demarcated masses of similar appearance were found. Multifocally within all lobes of the lungs were abundant nodular, well demarcated masses of light colour, firm consistency, and smooth, solid cut surface, ranging from 0.3-2.0 cm in diameter. The tracheobronchial and mediastinal lymph nodes displayed a similar appearance as the inguinal lymph nodes.

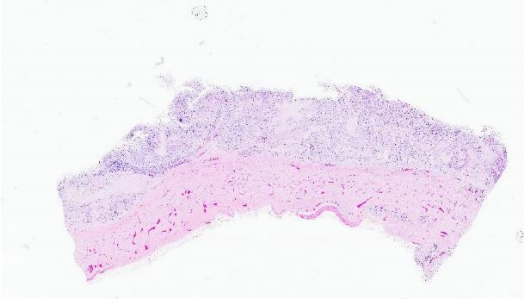

Figure 4-1. Urinary bladder, dog. One section of urinary bladder is submitted for examination. There is transmural infiltration of the bladder wall (predominantly at lower right). (HE, 5X).

Microscopic Description:
Urinary bladder: Diffusely obliterating and irregularly thickening the mucosa and extending into the submucosa is a plaque-like, poorly demarcated and unencapsulated, infiltrative, densely cellular epithelial neoplasm, extending to cut borders. A few scattered neoplastic cells are also seen within the muscular layer. Neoplastic cells are polygonal, markedly pleomorphic, and grow in cords, tubules, and poorly defined aggregates in a scant to moderate fibrovascular stroma. Nuclei are large, round to oval with vesicular chromatin and 1-2 central, prominent nucleoli. Multiple multinucleated cells and cells with bizarre, giant nuclei are seen. The cytoplasm is moderate, eosinophilic, and homogenous to vacuolated, and cell borders are mostly distinct. The N:C ratio is increased. Multifocally, neoplastic cells exhibit large intracytoplasmic vacuoles with eccentric peripherally located nuclei (signet ring formation) or large intracytoplasmic vacuoles containing homogenous or granular eosinophilic, PAS-positive material (Melamed-Wolinska bodies). The mitotic activity is high (25 mitoses per 10 HPF) and atypical mitoses are present. Multifocally, neoplastic cells display pyknotic or karryorhectic nuclei (single cell necrosis) and there are multifocal larger, confluent areas of necrosis. In the *muscularis* layer are multiple tumoral emboli within thin-walled vessels (lymphovascular invasion).

Contributor's Morphologic Diagnosis:
Urinary bladder: Urothelial carcinoma, non-papillary and infiltrative, high grade.

Contributor's Comment:
Tumors of the urinary bladder are uncommon in dogs, accounting for ≤1% of all canine neoplasms and 2% of all malignant canine neoplasms.[3,7] Lower urinary tract carcinomas have four histological types: (1) urothelial, (2) squamous, (3) adenocarcinoma, or (4) undifferentiated carcinoma.[3] Of these, the urothelial carcinoma (UC), previously called transitional cell carcinoma (TCC), is the most common bladder neoplasm in all species including dogs.[7] UC is derived from the transitional epithelium of the urinary tract (urothelium).[7] UC also occurs in cats and cattle, and may develop in cattle as a sequela to ingestion of bracken fern (enzootic hematuria).[3] Bladder neoplasms of any type are rare in other species.[7]

Urothelial carcinoma occurs primarily in older dogs (average age 9-11 years), and females seem to be more often affected than males, however a statistically significant gender difference is lacking.[7] Neutered dogs seem to be predisposed to bladder neoplasms.[7] Scottish terriers have an 18 to 20-fold higher risk for UC than other dogs. Other breeds at higher risk are Airedale terriers, Shetland sheepdogs, West Highland white terriers, fox terriers and beagles.[7]

Most of the UC neoplasms in dogs occur in the trigone area of the bladder, possibly because of a prolonged contact time of urine and potential carcinogens with the urothelium in this

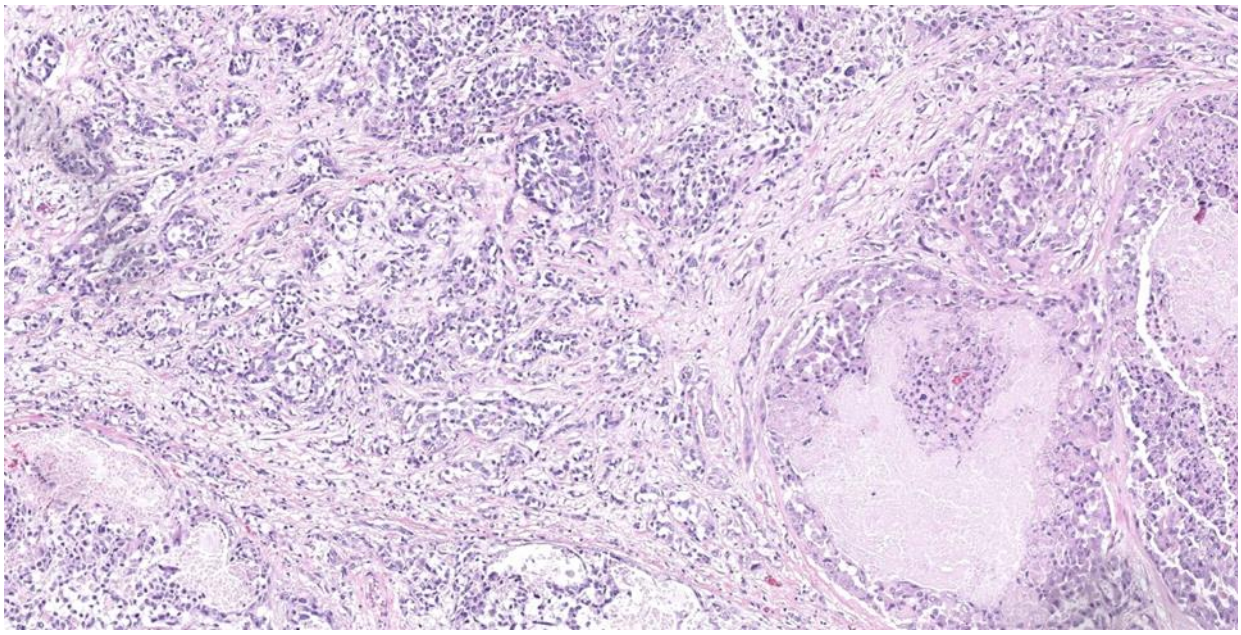

Figure 4-2. Urinary bladder, dog. Neoplastic cells form tubules, trabeculae, and occasionally solid nests on a dense fibrous stroma. There is scattered lytic necrosis within the center of large nests. (HE, 111X)

area.[7] In 30-55% of bladder UCs there will be concurrent neoplastic lesions in the prostate and/or urethra.[7] Grossly, most tumors are solitary, but they can be multiple and cover nearly the entire urinary bladder mucosa.[7] They may form either papillary growths projecting into the lumen of the bladder or non-papillary, flat plaques or masses that bulge from the mucosa into the bladder lumen.[7] Most are infiltrative into the muscle layers, producing a thickened bladder wall.[7]

A histological grading scheme is available for histological diagnosis of canine UC. The grading scheme has been based largely on the World Health Organization (WHO) histological criteria for human UCs, and was last modified in 2012.[1,7] In the classification scheme for dogs, UC is divided into low and high-grade variants.[1,7] The most aggressive region of the tumor should be used to assign a grade.[7] High-grade UCs are defined by features of malignancy such as disorganized growth and loss of cell polarity, cellular atypia, nuclear pleomorphism, mitotic activity, deep invasion and invasion into lymphatics or blood vessels.[1,7] Low-grade UCs display no invasion and are confined to the mucosa, exhibit mild pleomorphism, and contain no or only a few mitoses.[7] The great majority of UC/TCC are high-grade and invasion is present in the majority of canine UCs (>90%).[7] However, studies assessing the prognostic relevance of the grading scheme are lacking, and some studies have shown no correlation between histologic variables and prognosis.[1,11] Prospective studies determining the relationship between low- and high-grade features are needed to know if the grading scheme for UC accurately predicts clinical outcomes in dogs.[1,7]

Canine UCs can also be divided based on morphological pattern of growth, namely papillary (projecting into the lumen, around 50% of cases) or non-papillary (sessile or flat, 50%), and infiltrating (90%) or non-infiltrating (10%). This designation should be based on the dominant gross and microscopic features.[7]

In a case series of 3 canine UCs, unique additional cellular morphologies were revealed, resembling human plasmacytoid and rhabdoid variants of UC.[5] Reduced E-cadherin expression was shown by immunohistochemistry (IHC) in 2 of the cases, possibly representing increased invasiveness and epithelial-mesenchymal transition (EMT).[5]

Keys to the histological diagnosis of UC are location in the urinary bladder, presence of large epithelial cells with multiple cellular and nuclear features of atypia, invasion below the mucosa or into lymphatics or blood vessels, and the presence of so-called Melamed-Wolinska bodies.[7] Melamed-Wolinska bodies are large cytoplasmic vacuoles that may be empty or contain homogenous or stippled periodic acid Schiff (PAS) positive eosinophilic material, as was confirmed in this case. The Melamed-Wolinska bodies are also uroplakin positive and are so characteristic of UC that if seen in preparations from other locations such as lymph node, skin, or abdominal or pleural fluid, UC should be listed as the most likely differential diagnosis.[7] Urothelial tumor cells typically exhibit eosinophilic cytoplasm, large and vesicular nuclei, and numerous mitoses, some of which are bizarre. Multinucleated cells are common. Regions of squamous and/or glandular metaplasia and signet ring cell formation may occur, as well as desmoplasmic reactions.[7]

High-grade, invasive UCs are one of the most aggressive neoplasms in veterinary medicine and the prognosis is poor.[4,7] Survival times are short for almost all UCs in dogs, and most dogs (>80%) die within the first year of treatment.[4,7] Metastases occur most commonly to the lungs and regional lymph nodes, but may also occur to bones and skin.[7] Additional metastases may be found in almost any organ examined.[7] In a retrospective study, 17 of 188 canine UC cases (9%) had histologically confirmed skeletal metastasis, mainly affecting vertebrae.[2] Possible routes of metastases of canine UCs include vascular dissemination with tumor emboli, direct extension (peritoneal implantation) from transmural bladder infiltration, or iatrogenic seeding through diagnostic sampling by fine needle aspirate or surgery.[7]

Implantation of tumor along surgical excision paths is well documented with UC.[7] In one study, skin metastases were seen near the vulva or prepuce, possibly because of urine scalding of the skin and subsequent transepidermal spread.[10] Clinically, bladder carcinomas in dogs are staged with the Tumor Nodes Metastasis (TNM) system.[7] At the time of clinical diagnosis, 20% of dogs will have radiographically detectable metastases, and metastases are present in a majority of dogs (50-90%) at autopsy.[4,7]

Special genetic profiles seem to be associated with the development of canine UC.[7] Studies of canine UC biopsies have revealed aberrant chromosomal numbers (aneuploidy) of *Canis familiaris* (CFA) chromosome 13, 19, and 36, with gain of CFA13 and 36 and loss of CFA19.[13] Aberrant copy number variations in the same chromosomes were detected also by droplet digital polymerase chain reaction (ddPCR).[8] In addition, a digital PCR assay has been used to detect copy number aberrations (CNAs) in the ErbB2 (HER2) oncogene, whose product, the cell surface receptor tyrosine kinase ErbB2, is overexpressed in a variety of human malignancies.[12] In the study, ErbB2 copy number aberrations were detected in 33% of UC bladder tissue, and in 35% of UC urinary sediment, meanwhile no ErbB2 CNAs were detected in normal controls.[12] In a immunohistochemical study performed on 23 samples of canine UCs, intense membranous

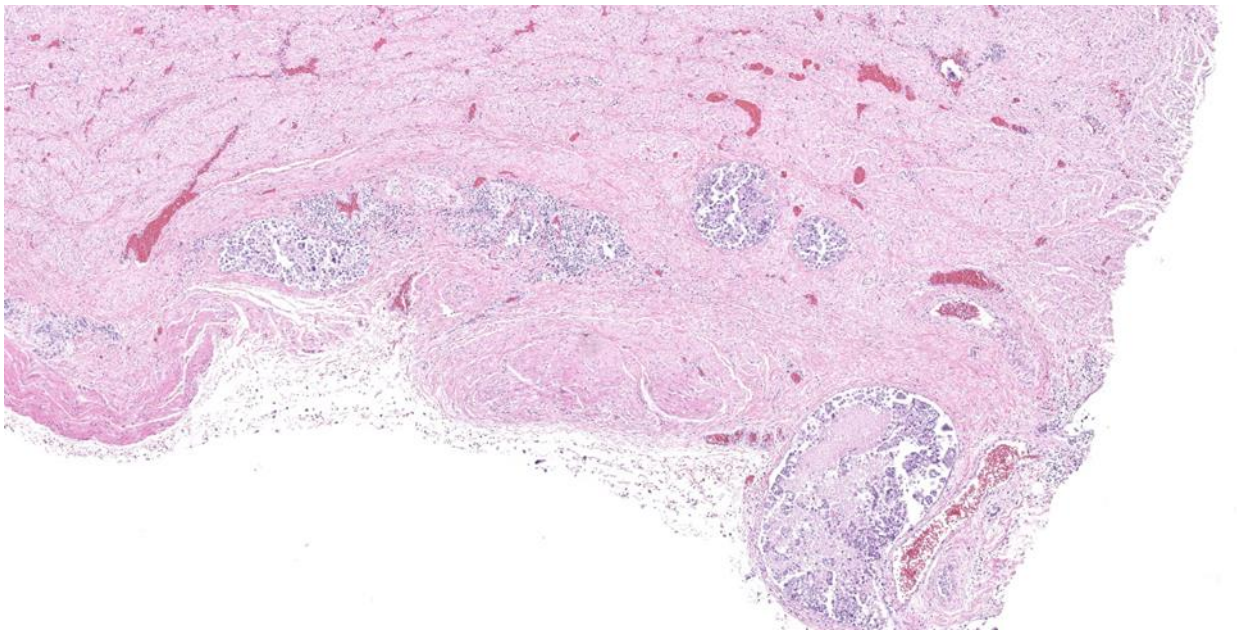

Figure 4-3. Urinary bladder, dog. Neoplastic cells are present in lymphatics within the mural smooth muscle and serosa. (HE, 46X)

ErbB2 (HER2) immunoreactivity was frequently observed in neoplastic cells.[16] In another study, 36 of 47 dogs (76.6% with UCs) had a mutation in proto-oncogene B-raf ($BRAF^{V595E}$ mutation).[6] The mutation was associated with tumor-produced chemokine CCL17 and infiltration regulatory T-cells, which may act as immune suppressors but also are thought to play a role in tumor progression.[6]

Immunohistochemistry is rarely needed to diagnose primary UC unless the tumor is anaplastic.[7] If needed, uroplakin III (UPIII) is considered the best IHC marker to recognize urothelium, and is a useful primary biomarker to identify tumors of suspected urothelial origin.[7] Uroplakin consists of four proteins located in a specialized plasma membrane (asymmetrical unit membrane, AUM) that connects the surface of urothelial cells with cytoplasmic filaments.[7] UPIII is specific to terminally differentiated superficial urothelial cells (umbrella cells) and exhibits discrete membranous immunoreactivity, primarily at the surface of cell membranes.[7] UPCIII cannot be used to differentiate neoplastic from non-neoplastic lesions, but it is highly specific for urothelial tumors, and may be helpful to rule in or out UC origin in metastases or cancer of unknown primary site.[7] Due to its high specificity in canine urothelial neoplasm, UPIII expression in less than 5% of neoplastic cells is considered diagnostic.[7] UPIII labeling may however be lost in some high-grade, anaplastic UCs, possibly due to loss of cell adhesion molecules, enhancing the ability to metastasize.[7] Lack of immunoreactivity may therefore indicate a more aggressive tumor.[7]

Antibodies to uroplakin II have been evaluated in human pathology and have been shown to outperform UPIII.[15] Urothelium (transitional epithelial cells) also contain cytokeratins associated with simple-type epithelium (CK7, CK20, CK8, CK18, CK19) and stratified-type epithelium (CK13, CK17).

CK7 and CK20 have been used in UC diagnosis in humans.[7] In a canine study, CK7 and UPIII outperformed CK20 in the diagnosis of UC.[9] CK7 staining was diffusely cytoplasmic and stained over 98% of primary UCs, whereas UPIII labeled 91% of primary UCs.[9] Another potential immunohistochemical marker for canine UC is cyclooxygenase-2 (COX-2). COX-2 and associated production of prostaglandin E_2 have been ascribed several roles in carcinogenesis, including immunosuppression, increased metastatic potential of neoplastic epithelial cells, and stimulation of angiogenesis.[14] Immunoreactivity for COX-2 has been detected in UCs of dogs, but also in non-neoplastic proliferative bladder lesions.[14]

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JPC Diagnosis:
Urinary bladder: Urothelial carcinoma, papillary and infiltrative.

JPC Comment:
The contributor provides an excellent overview of the extensively researched and described canine urothelial carcinoma. Interestingly, despite UC also being the most common bladder tumor in cats, far less has been published about feline UC. Feline UC comprises only about 0.5% of all feline malignancies, compared with around 2% of all canine malignancies, and this difference has been speculatively explained by, among other variables, the lower quantities of tryptophan metabolites excreted in feline urine, differences in the chemical composition of feline flea and tick preventatives, and potential masking by concurrent disease in aged cats.[17]

A recent descriptive study of feline UC in 38 cats found that the typical histologic presentation is of an unencapsulated, densely cellular neoplastic mass, often with ulceration or erosion of the bladder mucosa.[17] As in canine UC, the two main histologic patterns reported are papillary, exophytic growths extending into the bladder lumen (21%) or the formation of non-papillary sessile growths (79%).[17] The case series found evidence of Melamed-Wolinska bodies or signet ring formation in only 3 of 38 cases, perhaps indicating that this histologic finding is less frequent in feline cases compared with canine UC. Mitotic rate was variable with occasional bizarre mitotic figures.[17]

Neoplastic cells are typically arranged in similar patterns, such as trabeculae, cords, and islands, as their canine UC counterparts; however, feline UCs frequently (9/38) contain a glandular histomorphology that appears similar to a histologic variant seen in humans, but not dogs, and researchers suggest that this unique morphology should be described in pathologic reports of feline UC to aid in identifying metastatic disease that may show this unexpected pattern.[17] Feline UC neoplastic cells are present on variable stroma that ranges in quantity and morphology; stroma may be sparse or abundant, and may be characterized as mucinous, myxomatous, or scirrhous. Inflammatory infiltrates, when present, are primarily composed of lymphocytes with fewer plasma cells and neutrophils.[17]

There are some reported associations between feline UC and urinary tract infection, with concurrent infections reported in up to 70% of

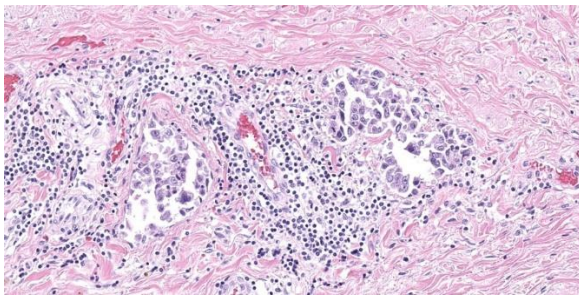

Figure 4-4. Urinary bladder, dog. High magnification of lymphovascular invasion of neoplastic urothelium. There are lymphocytes and fewer plasma cells surrounding ectatic lymphatics containing neoplastic cells. (HE, 237X)

feline UC patients.[7,17] In the most recent case series, approximately 28% of patients for whom clinical history was available had cystitis, stones or bladder crystals at the time of diagnosis.[17]

Though data is sparse, feline UC is thought to be highly recurrent, and metastasis at the time of diagnosis, typically to the regional lymph nodes or lungs, has been reported in 12-50% of cases. As the contributor notes, the prognosis for canine UC is strongly associated with the TNM staging system; however, TNM staging is typically not performed with feline UCs and prognostic information has therefore not been established.[17]

As in the previous case, Dr. Meuten chose to discuss this classic neoplasm in the context of synoptic reporting. Participants discussed whether infiltration, grade, or both should be reported. Dr. Meuten noted, as does the contributor, that there is no veterinary research that ascribes prognostic or clinical meaning to UC grading, and he would therefore be inclined to omit this data. This lead to a broader discussion of how synoptic reporting could potentially be tailored to meet the needs of the intended recipient, as information considered useful by a general practitioner is likely different than what would be useful for a veterinary oncologist. Conversation broadened to discuss which stakeholders are best positioned to generate synoptic reporting templates. Should it be the pathologists, who are not necessarily up to date on the latest prognostic, therapy-determinative factors for all tumors in veterinary oncology, or should it be the oncologist, who are more knowledgeable on those points and who are, after all, the end users. Dr. Meuten felt strongly that collaborative effort was needed between veterinary oncologists and pathologists to ensure that any synoptic report templates are targeted, useful, and relevant to the widest audience possible.

As discussed above, the authors of the most recent descriptive feline UC study noted that a particular histomorphologic feature (glandular morphology) should be reported in veterinary reports, not because it had prognostic value, but because it had potential utility in identifying metastatic disease with an unexpected histomorphology. This data point, not prognostic and not backed up by extensive research yet nonetheless useful, gets at the heart of many conference participants' concerns over the trend toward synoptic reporting. A checklist, "just the facts" style of report, while perhaps efficient, easy to read, streamlined, and standardized, feels as if it strips the profession of something fundamental: the ability to communicate impressions and findings, born not solely from metrics, but from experience, that others may find useful when practicing the art of medicine. As synoptic reporting continues to gain popularity, discussions such as these should continue to ensure that nuanced evaluation and communication are not sacrified on in the interest of data-driven efficiency.

On a more prosaic note, conference participants preferred to omit grading from the diagnosis as, per conference discussion, the histologic grade currently has no prognostic significance. Conference participants noted the contributor's classification of the UC as non-papillary; however, in the section examined at conference, participants felt that the dominant pattern was papillary, and this view is reflected in the morphologic diagnosis.

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