Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

Conference 14

12 January 2022

CASE I: N-521/18 (JPC 4137931)

Signalment:
3 year-old. Female. Rasa Aragonesa. Ovine (*Ovis orientalis aries*)

History:
The animal presented with agalactia to the clinical service for ruminants (Servicio Clínico de Rumiantes – SCRUM) of the University of Zaragoza two months after parturition. At clinical examination, the animal showed induration of the mammary gland and mild diffuse increase of respiratory sounds.

Gross Pathology:
At necropsy, the sheep showed body score of 3/5. Mammary gland presented mild diffuse enlargement and severe increase in firmness. Lungs were increased in size and weight and showed greyish discoloration. Mediastinal and tracheobronchial lymph nodes were enlarged.

Laboratory Results:
The animal was seropositive to small ruminant lentivirus (SRLV, Elitest MVV/CAEV, Hyphen Biomed).

Microscopic Description:
Diffusely, affecting up to the 90% of the section, there are inflammatory and degenerative changes in the acini, inter- and intralobular ducts and intersititium that efface the normal structure. Multifocally, mammary interstitium is infiltrated by abundant lymphocytes and fewer plasma cells, with occasionally formation of large follicular structures (not in all slides). There are lesser numbers of histiocytes and rare neutrophils. Multifocally, acini are reduced in size (atrophy) and show attenuated, disorganized, swollen and/or vacuolated epithelium (degeneration), with occasional acinar collapse with pyknosis and karyorrhexis (necrosis). Lumina of acini are rarely filled with an eosinophilic proteinaceous fluid, with occasional clear lipidic droplets and abundant foamy macrophages with occasional multinucleated giant cells. Diffusely, inter- and intralobular septa are prominent and infiltrated by mild numbers of the previously-described inflammatory cells, generally in a perivascular location as well as moderate numbers of plump fibroblast intermingled with immature collagen fibers (intralobular fibrosis).

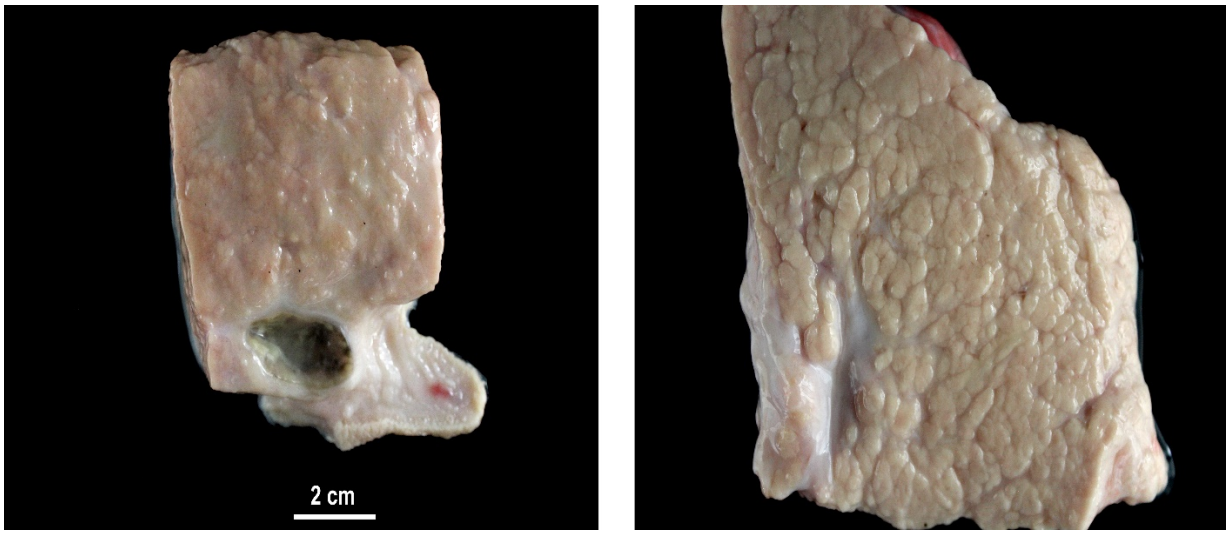

Figure 1-1. Mammary gland, sheep. The mammary gland was firm to the touch. (*Photo courtesy of:* Facultad de Veterinaria, Departamento de Patología Animal, University of Zaragoza, https://patologiaanimal.unizar.es)

Contributor's Morphologic Diagnoses:
Mammary gland: Mastitis, interstitial, lymphoplasmacytic, diffuse, severe, with lymphoid follicle formation, acinar atrophy and degeneration and mild intralobular fibrosis.

Contributor's Comment:
This is the mammary form of small ruminant lentiviruses (SRLV). This chronic disease is caused by a group of non-oncogenic exogenous retroviruses of the genus lentivirus that target immune cells, mainly macrophages.

The SRLVs are a group of highly related single stranded RNA viruses with high mutational potential that affect sheep and goats.[11] These viruses induce chronic inflammation that usually remains subclinical. When it becomes clinical, the disease can express mainly in four different locations: lung, joints (mainly carpus), central nervous system and mammary gland.[7] The expression of each form as well as the severity of the lesion depends on the host immune response and the viral factors.[3,9]

The mammary form is difficult to detect and mainly consist of mammary gland induration, progressive bilateral atrophy and milk production loss that leads to deficient lamb growth.[6] Both mammary glands are usually symmetrically affected. Microscopic lesions are characterized by abundant lymphocytes and plasma cells that infiltrate the interstitial and periductal connective tissue and even form lymphoid nodules. In advanced lesions, degeneration and loss of acinar and ductular epithelium may happen as a consequence of inflammation rather than direct virus effect. Strikingly, interstitial fibrosis is a controversial histologic finding of lentiviral mastitis.[2,7,13] As far as contributors are concerned, interlobular fibrosis is hard to determine because of microscopic appearance of the mammary septa is highly variable according to the physiological condition of the animal. However, intralobular fibrosis is more challenging to interpret and authors question if the moderate intralobular fibrosis present in this case has been induced by the viral infection or is the reflection of the acinar atrophy and collapse. In goats affected by the SRLV, mastitis is not commonly detected.

The respiratory form is the most common presentation of the disease in sheep and consist of progressive pneumonia with marked dyspnea and weight loss.[1] Lungs are characterized by showing a rubbery firm texture and lack of collapse when thorax is opened. Indeed, this animal presented with a diffuse interstitial pneumonia with BALT hyperplasia (not submitted). The neurologic form is characterized by demyelinating leukoencephalomyelitis that leads to ataxia and profound weight loss.[10] This form progresses faster than the respiratory one and usually occurs in adult sheep and goat kids (2-4 months). Articular form is characterized by arthritis affecting mainly the carpal joints.[8]

The main route of transmission is aerosol, particularly under intensive housing. Colostrum transmission plays also an important role.[7] The virus infects a variety of cell types (mammary epithelium, fibroblast, endothelial cells, monocytes, choroid plexus) but its replication mainly occurs in mature macrophages.[11] Lentiviral infected macrophages produce cytokines that recruit and activate other leukocytes but also enhance lentiviral replication. Indeed, clinical signs and histopathological lesions are the result of the inflammatory process instead direct viral damage to the organ. There is neither treatment nor commercial vaccines for the disease, what makes challenging the immunization of the ovine population against the virus.[12]

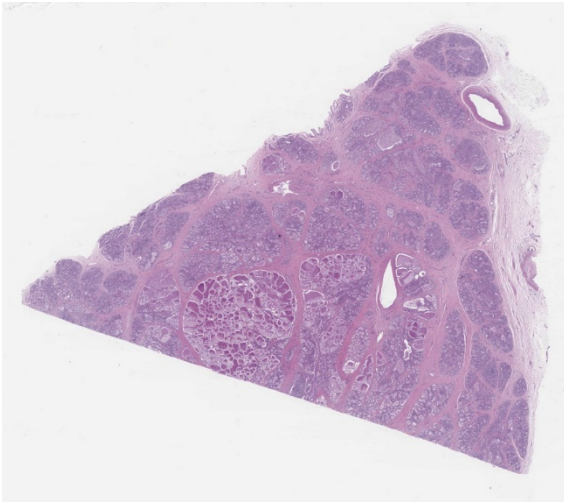

Figure 1-2. Mammary gland, sheep. There is a cellular infiltrate within approximately 66% of lobules of acinar tissue. (HE, 4X)

The main differential diagnosis for this kind of mastitis in the Mediterranean countries is contagious agalactia caused by *Mycoplasma agalactiae*, a septicemic pathogen with high mortality rates that can cause also polyarthritis and keratoconjunctivitis. *Staphylococcus aureus, Mannheimia haemolytica* and *Corynebacterium pseudotuberculosis* should also be considered, although unilateral involvement and suppurative and/or necrotizing mastitis should be expected with these pathogens.[13]

Contributing Institution:
Universidad de Zaragoza. Departamento de Patología Animal
https://patologiaanimal.unizar.es

JPC Diagnosis:
Mammary gland: Mastitis, lymphohistiocytic and neutrophilic, lobular, multifocal, marked, chronic, with fibrosis.

JPC Comment:
The contributor provides a concise review of the pathogenesis and multiple clinical manifestations associated with small ruminant lentiviruses (SRLVs), an economy-ically significant group of viruses distributed worldwide, with notable exceptions including Iceland, New Zealand, and Australia.[15]

SRLVs are a group of viruses in the genus *Lentivirus* in the Retroviridae family that include caprine arthritis and encephalitis virus (CAEV), maedi-visna virus (MVV), and ovine progressive pneumonia virus (OPPV). Lifelong infection associated with

a slow onset (*lentus;* Latin for "slow") of clinical signs is a key feature of SRLVs.[5] These entities are aptly named given MVV is associated with chronic respiratory disease (maedi = shortness of breath) and neurologic disease (visna = wasting due to meningo-encephalitis) while CAEV is classically associated with arthritis and/or encephalitis. Although these viruses were historically believed to be host specific, with CAEV infecting goats while MVV/OPPV infected sheep; however, interspecies transmission has been reported.[5]

SRLVs are divided into five genetically diverse genotypes (A-E). There is 25-37% nucleotide sequence variation between types as the result of common point mutations (largely due to a lack of proofreading ability of the viral transcriptase) and recombination events mediated by coinfections. As previously alluded to in regard to interspecies transmission, most genotypes and associated subtypes have been reported in both sheep and goats. However, only the E genotype has been reported in sheep while six A subtypes have only been reported in goats. These reports may be indicative of host specificity of some subtypes and a genotype, though it is also possible interspecies transmission has not yet occurred or simply has not yet been identified.[5]

SRLVs can present a challenge for producers to control given its ability to undergo transmission both vertically via infected colostrum and milk as well as horizontally via secretions/excretions from the respiratory, urogenital, and digestive tracts. In addition, only 30% of SRLV infected animals develop clinical disease whereas the remaining animals may remain subclinically infected for life. Control measures involving neonates include separation of the newborn from the dam prior to suckling, feeding pasteurized colostrum, and subsequently hand/artificially rearing the animal. Strict biosecurity measures, including screening of animals prior to herd/flock entry, lifelong separation of carriers, and elimination or disinfection of shared fomites (e.g. automatic

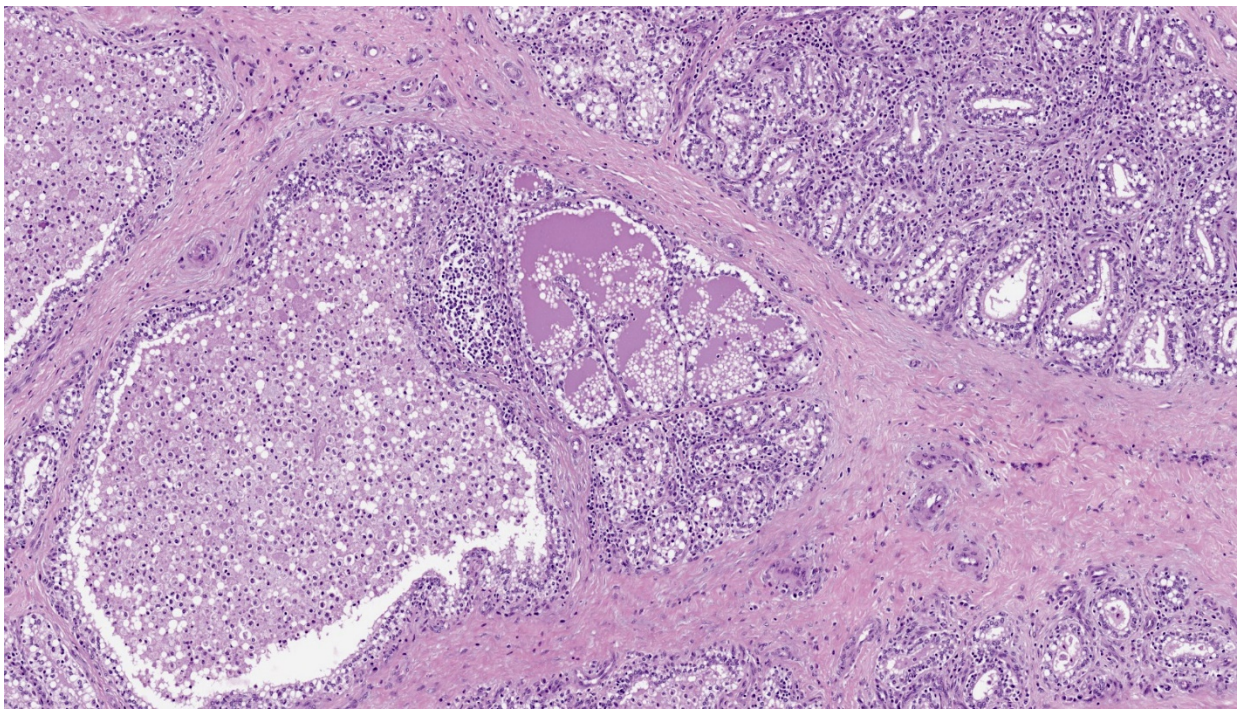

Figure 1-3. Mammary gland, sheep. Interglandular septa are expanded by large numbers of lymphocytes, macrophages and neutrophils, which infiltrate adjacent acini. (HE, 85X)

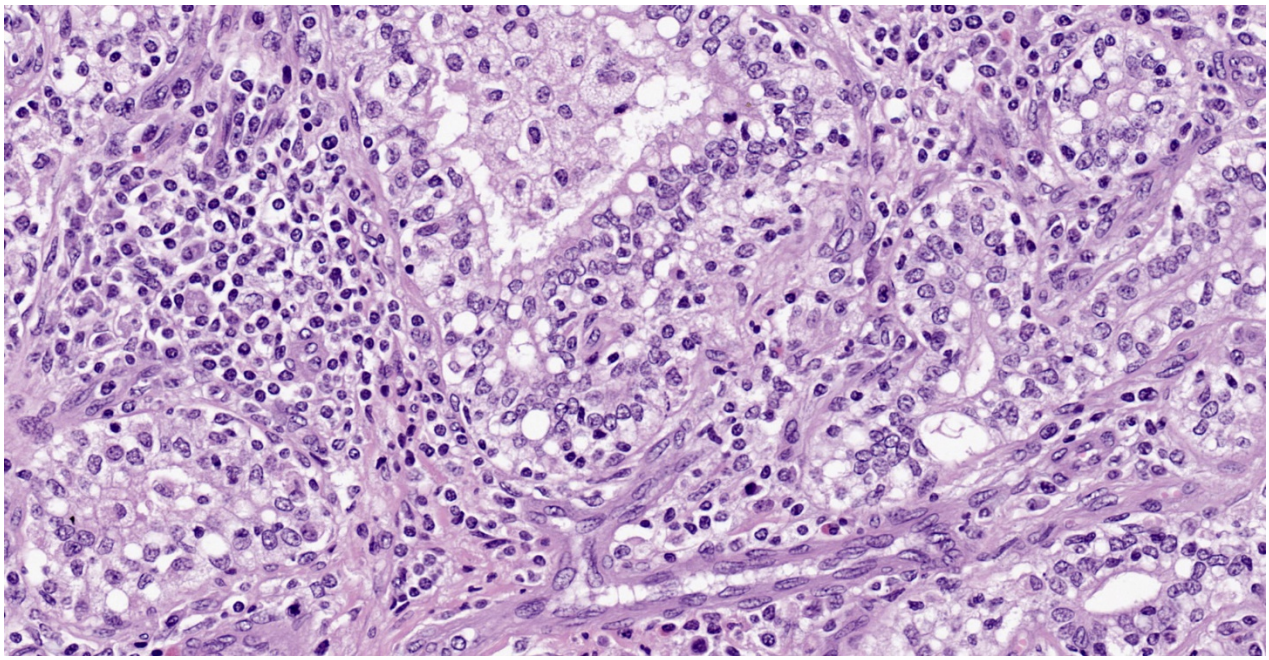

Figure 1-4. Mammary gland, sheep. Higher magnification of inflamed/necrotic acini. A small lymphoid nodule is present at left. (HE, 365X)

milkers), and testing programs are utilized to control transmission.[5]

Many producers are unaware ewes or does have developed mastitis until the affected dam's offspring are found to be malnourished or have died. Clinically, the udders appear bilaterally normal but are firm without pitting edema upon palpation and only a small amount of milk can be manually expressed. One study found weaning weights of lambs reared by SRLV-infected ewes were decreased (3.86-4.95kg) when compared to those reared by SRLV-negative ewes, which correlates to significant financial loss for a producer over the ewe's lifetime.[6,15] Affected animals are typically culled; however, SRLV negative offspring can be reared from SRLV positive dams as previously discussed. Although the intensive hand rearing process is a costly alternative, it provides an option to preserve valuable bloodlines.[15]

Testing is an essential tool utilized by producers to eliminate SRLV infected animals subsequently maintain SRLV free flocks. Commonly tested animals include weaned replacement lambs, yearlings that have not yet intermixed with mature ewes, and ewes (and rams) that are of an unknown status prior to herd entry. Serum-based ELISA testing is the most common method, which is highly sensitive, though false positives do occur. Upon testing positive, seropositive animals are segregated from seronegative animals and are commonly marked (e.g. bright ear tag) to prevent inadvertent intermixing. Newly purchased animals, as well as those returning from shows with contact with other small ruminants are tested upon arrival, quarantined, and tested again 60 days later prior to release. Unfortunately, early infections cannot be detected using ELISA. It is therefore common to test these animals again at two-to three-month intervals until two to three consecutive whole-group tests have been completed. In addition to false positives, false negatives have also been reported with ELISA testing. This situation typically becomes apparent when a flock is seronegative over consecutive tests but

seropositive animals are identified in future whole-flock tests. One protocol studied to overcome this phenomenon combined the use of highly sensitive ELISA testing with PCR to detect proviral SRLV sequences integrated into the animal's genome once every three months. The leader-gag sequence was selected for RT-PCR due to being highly conserved despite SRLV's high rate of mutations. PCR testing identified some infected animals prior to becoming seropositive, facilitating the study herd's return of to a SRLV negative status after three rounds of testing at two- to three-month intervals. Although effective, cost is a significant disadvantage of this approach.[15]

An additional control strategy under investigation is the use of host genetics given some genotypes/polymorphisms have been associated with SRLV resistance or low viral loads, which have been shown to correlate with decreased lesion severity. In addition, it is also likely decreased viral loads present a lower risk of transmission. One potential gene found to be associated with decreased SRLV susceptibility or lower viral load in sheep is the transmembrane 154 gene (*TMEM154*), which encodes a protein of an unknown function. Although selective breeding programs are a potentially effective component of an overall control strategy, purpose-bred resistance may be overcome as the result of rapid mutations associated with SRLVs.[5]

Any mention of MVV or CAEV would be remiss without discussion of typical clinical manifestations of neurologic disease associated with ages of the infected animals. Although older goats may also be affected with the neurologic form of CAEV, it most frequently occurs in kids between one to six months of age whereas visna affects adult sheep. Affected kids initially develop a pelvic limb paresis that progresses to paralysis of all four limbs. Visna presents as two forms in the sheep, depending on the region of the CNS affected. Lesions affecting the brain arise in the lateral ventricles and result in a head tilt and circling toward the affected side. Sheep with lesions in the spinal cord classically present with unilateral knuckling of a pelvic limb while the contralateral limb retains the ability to bear weight.[15]

During the conference, the moderator discussed the importance of subclinical animals acting as reservoirs within herds. This was further highlighted in a recent study[14] conducted in Italy evaluating subclinical mastitis which 89 macroscopically healthy sheep and goat udders were cultured and submitted for histologic evaluation. Infectious agents were identified in 64 (71.9%) cases. Coagulase-negative staphylocci were the most prevalent bacteria isolated (46.2%), followed by environmental opportunistic bacterial pathogens (34.7%). 75% of infected mammary glands had co-infections and a total of 138 different bacteria were isolated. In addition, lentiviruses were detected in 39% of infected udders.[14] Histologically, 45/89 udders demonstrated features of chronic non-suppurative mastitis, followed by chronic mixed mastitis (12/89), and acute suppurative mastitis (4/89). Only 28/89 udders were histologically normal. Therefore, small ruminants with macroscopically healthy udders may act as reservoirs for microbial agents within herds and flocks[14], further necessitating herd health surveillance programs such as previously discussed.

References:

1. Blacklaws BA. Small ruminant lentiviruses: Immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. *Comp Immunol Microbiol Infect Dis*. 2012;35(3):259–269.
2. Bolea R, Monleón E, Carrasco L *et al*. Maedi-visna virus infection of ovine

mammary epithelial cells. *Vet Res*. 2006; 37: 133-144.
3. Gayo E, Polledo L, Balseiro A, *et al*. Inflammatory Lesion Patterns in Target Organs of Visna/Maedi in Sheep and their Significance in the Pathogenesis and Diagnosis of the Infection. *J Comp Pathol.* 2018;159:49–56.
4. Gayo E, Polledo L, Magalde A, *et al*. Characterization of minimal lesions related to the presence of visna/maedi virus in the mammary gland and milk of dairy sheep. BMC *Vet Res*. 2019;15:109. 1-9.
5. Highland MA. Small Ruminant Lentiviruses: Strain Variation, Viral Tropism, and Host Genetics Influence Pathogenesis. *Vet Pathol*. 2017;54(3):353-354.
6. Keen JE, Hungerford LL, Littledike ET, et al. Effect of ewe ovine lentivirus infection on ewe and lamb productivity. Prev Vet Med 1997;30:155–69.
7. Minguijón E, Reina R, Pérez M, *et al*. Small ruminant lentivirus infections and diseases. *Vet Microbiol*. 2015;181(1–2):75–89.
8. Pérez M, Biescas E, Reina R, et al. Small Ruminant Lentivirus–Induced Arthritis. *Vet Pathol*. 2015;52(1):132–139.
9. Pinczowski P, Sanjosé L, Gimeno M, *et al*. Small Ruminant Lentiviruses in Sheep. *Vet Pathol*. 2017;54:413–424.
10. Polledo L, González J, Benavides J, *et al*. Patterns of Lesion and Local Host Cellular Immune Response in Natural Cases of Ovine Maedi-Visna. *J Comp Pathol*. 2011;147(1):1–10.
11. Pritchard GC, McConnel I. Maedi-Visna. In: Aitken ID, editor. *Diseases of sheep. Fourth Edition*. Oxford (UK): Blackwell Publishing; 2007. 217–223.
12. Reina R, de Andrés D, Amorena B. Immunization against small ruminant lentiviruses. *Viruses*. 2013;5:1948–1963.
13. Schlafer D, Foster R. Female Genital System. In: Maxie GM, ed. *Pathology of Domestic Animals. Volume 3*. 2016:358–465.
14. Spuria L, Biasibetti E, Bisanzio D, et al. Microbial agents in macroscopically healthy mammary gland tissues of small ruminants. *PeerJ*. 2017;5:e3994. Published 2017 Nov 13.
15. Wolf C. Update on Small Ruminant Lentiviruses. *Vet Clin North Am Food Anim Pract*. 2021;37(1):199-208.

CASE II: MB19-317 (JPC 4166552)

Signalment:
An 8-year-old, 28.5 kg, castrated male, boxer dog (*Canis lupus familiaris)*

History:
The intact male dog was presented for bilateral perineal hernia and right unilateral cryptorchidism. The left testicle was descended in the scrotum. Abdominal ultrasound noted prostatomegaly, sublumbar lymphadenomegaly, minimal abdominal effusion, and an intraabdominal mass suspected to be an enlarged retained right testicle (cryptorchid). Surgical intervention included bilateral perineal hernia repair, left orchiectomy with scrotal ablation, and right cryptorchidectomy. Noted during the celiotomy, the peritoneum was diffusely thickened and multinodular with increased peritoneal effusion. The left testicle with scrotum, the intraabdominal enlarged right testicle, and portions of the multinodular peritoneum were submitted for pathological examination.

Gross Pathology:
Received 3 labeled jars with tissue fixed in 10% neutral-buffered formalin. Jar 1, "body wall", has one tissue (peritoneum) that is 1.4 x 0.9 x 0.5 cm, is tan and irregularly rectangular. Jar 2, "intraabdominal wall testicle", has one tissue that is 5.5 x 4 x 3.5 cm with features that are consistent with testicle (right). The epididymis and an area of

the tunica albuginea are roughened, tan, and granular. The portion of the tissue that corresponds to testicle measures 4.5 x 3.5 x 3.5 cm. On cross-section, the testicle is firm, tan, and multilobular traversed by haphazard and arborizing subtle tan streaks. Jar 3, "scrotum descend testicle", has one tissue composed of testicle (left) and skin (scrotum) that measures 4.5 x 3 x 1 cm. The elliptical portion of skin has a 2 cm long and 0.5 cm diameter tan fibrous stalk that attaches to the teste. The testicle measures 3.5 x 2.5 x 2 cm and on section the tunica albuginea is diffusely thickened and firm up to 0.5 cm and this tissue melds with the previously described stalk.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
"Intraabdominal wall testicle", enlarged cryptorchid right testicle: Nearly diffusely replacing tissue architecture, associated with few atrophied remnant seminiferous tubules that contain only Sertoli cells, and extending into the tunica albuginea there is a moderately well-demarcated, unencapsulated, infiltrative, and highly cellular testicular germ cell tumor that is arranged in intratubular to diffuse sheets of densely packed round cells. Arrangements are supported by marked haphazard arborizing fibrous connective tissue. Cells are round to polygonal, have moderate eosinophilic cytoplasm, and large round to oblong vesiculated nuclei with prominent up to 4 central magenta nucleoli and occasional nuclear cytoplasmic inclusions. There is frequent individual cell apoptosis/necrosis with suspected phagocytosis ("starry-sky" pattern). Mitoses are 13 per 2.4 mm^2 (equivalent to 10 FN22/40X fields) and some are atypical. Multifocally in the stroma are lymphocyte and plasma cell aggregates. There is moderate to marked anisocytosis and anisokaryosis. There is karyomegaly and occasional multinucleation. Multifocal vessels contain luminal tumor cells (unclear if this represents true vascular invasion or transposed cells that occurred during trimming). Tumor cells extend into the tunica albuginea (invasion). Lining the serosa of the epididymis, the ductus deferens, the tunica albuginea, and the vascular adventitia are multifocal to coalescing pyogranulomas. Centrally, admixed with neutrophils and cell debris, there are round, <30 µm diameter organisms that have a refractile capsule that surround vacuolated basophilic wispy material or that contain packed 3 µm round basophilic structures (developing spherules, variably with endospores) (*Coccidioides* spp.). This is followed by epithelioid macrophages, few multinucleated giant cells, lymphocytes, plasma cells, fibrosis, neovascularization, and reactive hypertrophied mesothelium. At one aspect of the tissue, architecture is smudged and coagulated with nuclear streaming (cautery artifact).

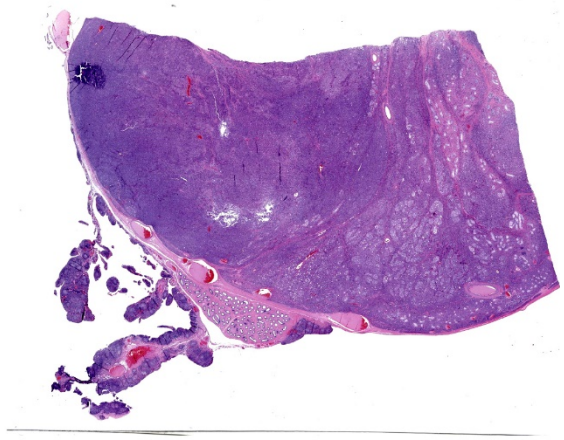

Figure 2-1. Testis, dog. A section of a cryptorchid testis is presented for examination. There is loss of normal architecture, and a cellular infiltrate expands the spermatic cord and fibrous tags projecting from the vaginal tunics. (HE, 6X)

Body wall (peritoneum) (not submitted): Thickening the peritoneum, admixed with fibrosis, reactive hypertrophied mesothelium, and above regions of skeletal muscle bundles there are pyogranulomas containing

fungal spherules with occasional endosporulation (*Coccidioides* spp.), as previously described.

Descended testicle (left) with scrotum (not submitted): Multifocally thickening and infiltrating the tunica albuginea and tunica vaginalis, among portions of the epididymis and supported by fibrosis, there are pyogranulomas containing fungal spherules with occasional endosporulation (*Coccidioides* spp.), as previously described. The testicular parenchyma is variably compressed by the peripheral enflamed fibroplasia, and seminiferous tubules have reduced cellularity without spermatids (atrophy) with mild interstitial fibrosis.

Contributor's Morphologic Diagnosis:

1. Testicle, intraabdominal cryptorchid, right: Seminoma, intratubular and diffuse types, with invasion of the tunica albuginea.
2. Testicle, intraabdominal cryptorchid, right: Periorchitis, pyogranulomatous, lymphoplasmacytic, marked, chronic, with intralesional fungal spherules (*Coccidioides* spp.).
3. Abdomen and descended testicle (not submitted), left: Peritonitis and periorchitis, pyogranulomatous, lymphoplasmacytic, marked, chronic, with intralesional fungal spherules (*Coccidioides* spp.).

Contributor's Comment:

The dog was diagnosed with two simultaneous but unrelated disease processes, one neoplastic and one infectious. The cryptorchid right testicle was enlarged by a seminoma with features of both, intratubular and diffuse type patterns.[2] Definitive vascular invasion was lacking, but neoplastic cells did regionally invade the tunica albuginea. The biopsy of peritoneal tissue, the intraabdominal testicle's capsule, and the descended testicle's capsule and peripheral space (tunica vaginalis) were diffusely thickened by chronic inflammation dominated by pyogranulomas containing fungal spherules with variable endosporulation, consistent with *Coccidioides* spp. The fungal

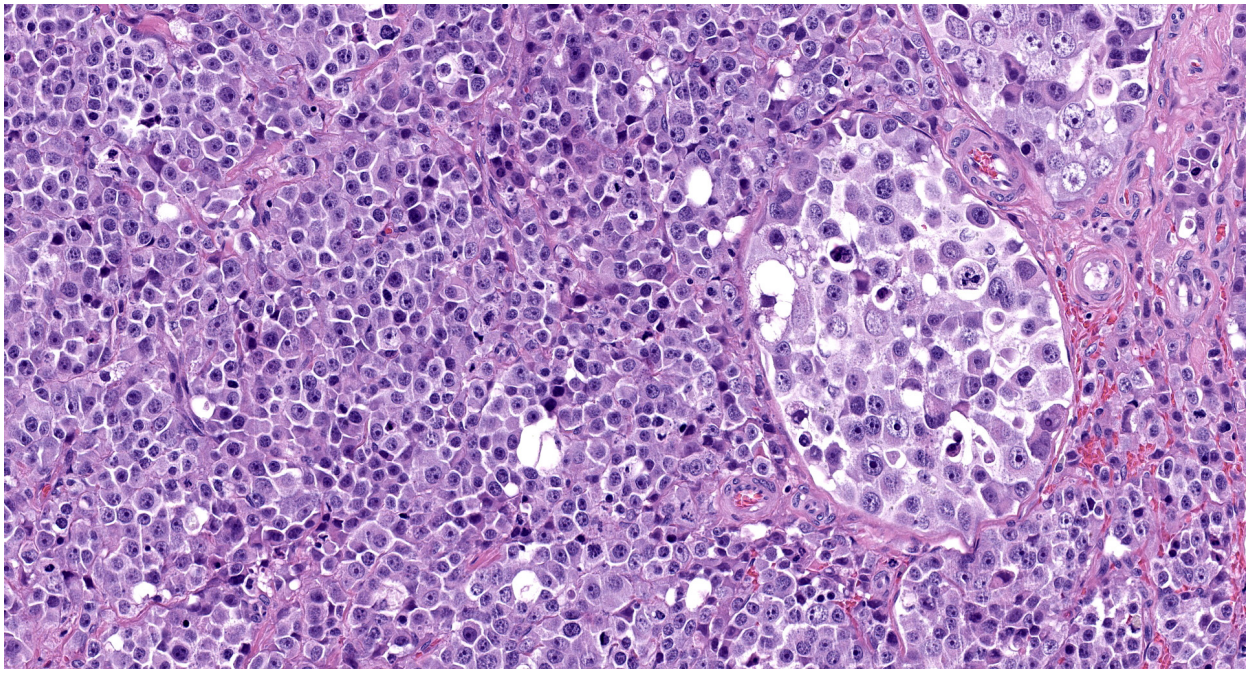

Figure 2-2. Testis, dog. Neoplastic germ cells fill the few remaining seminiferous tubules and sheets of similar cells efface the remaining tubules and interstitial cells. (HE, 228X)

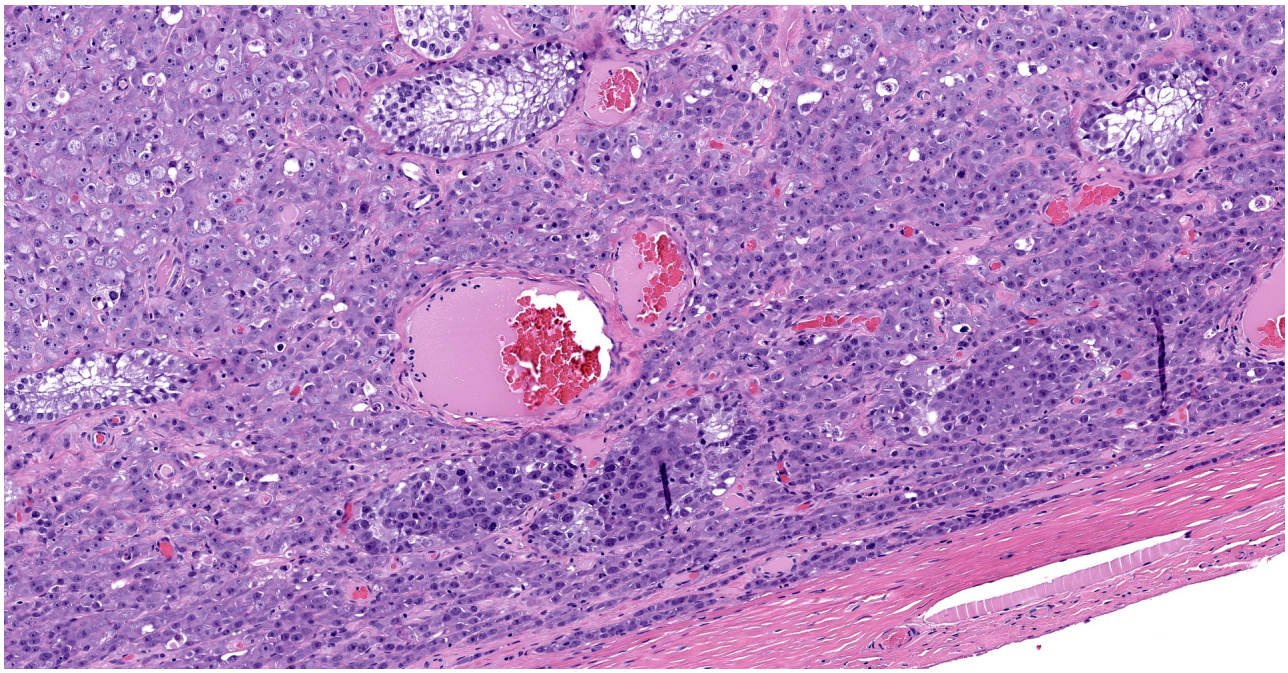

Figure 2-3. Testis, dog. Few atrophic tubules (total absence of spermatogonia and a single layer of Sertoli cells – left and center top) are scattered at the periphery of the testis. Neoplastic germ cells (bottom center) infiltrate the inner layers of the tunica albuginea. (HE, 129X)

periorchitis of the descended left testicle was likely the extension of the peritoneal infection to the vaginal process, since the latter, demarcated by the parietal and visceral vaginal tunics, is a continuation of the abdominal cavity. A regional intrascrotal fungal inoculation of the left testis is another possibility, though less likely given the dog's marked fungal peritonitis.

Common testicular tumors of the dog are Sertoli cell tumor, interstitial or Leydig cell tumor, and seminoma.[2,8] Typically, these testicular tumors are benign; however, their malignancy is difficult to determine based solely on cell morphology. Therefore, a definitive designation of malignancy requires recognizing metastasis in the spermatic cord, regional lymphatics, sentinel lymph nodes, or in distant sites.[2] Seminomas are the most common germ cell tumor of older dogs, and these tumors are typically hormonally inactive and prevalent in cryptorchid testes.[2] Grossly, seminomas are lobulated, white to gray-white, soft, bulge on section, and may have hemorrhage.[2] Microscopic features are characteristic, and include the growth of densely packed sheets of polygonal round cells that may arrange in tubules or diffuse pattern or a combination.[2,8] As in humans, the expression of c-KIT and PLAP in dog seminomas may allow further differentiation between spermatocytic or classical seminoma, which may have prognostic implications.[8]

Coccidioidomycosis is a localized to systemic infection caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*.[5] These fungi are saprobic and endemic in dry climates of the southwestern United States (US), Mexico, Central America, and South America.[9] Current climate change scenarios predict that by 2100 *Coccidioides* spp. endemicity in the US will expand northward into drying western states accompanied by a 50% increase in human infections.[4] In addition to humans, these fungi infect a wide range of mammals with varying susceptibility.[1,5,11] The primary route of infection is through the inhalation of environmental arthrospores or less likely,

following direct cutaneous inoculation.[5] Once inhaled, the infection may be limited to the lung (pneumonia) and draining lymph nodes (lymphadenitis) or the infection may become disseminated, spreading hematogenously or lymphogenously to distant sites.[5] Lameness resulting from fungal granulomatous osteomyelitis is a common indication of disseminated disease.[5]

Young, large breed dogs spending time outdoors in endemic areas, especially as working or sporting dogs or roaming deserts, are at increased risk for coccidioidomycosis.[5] Infected dogs display a variety of clinical signs and disease severity, which renders definitive diagnosis difficult.[5,7,9] Because of the inhalation route of infection, clinical signs attributable to fungal pneumonia are common; however, dogs with disseminated disease may not always have history of respiratory morbidity.[5] Moreover, the highly diverse patterns of systemic dissemination preclude a case definition and result in atypical infections, such that in endemic areas coccidioidomycosis is often a differential diagnosis in ill dogs. *Coccidioides* spp. can disseminate anywhere in the body, including central nervous system, bone, pericardium, eye, reproductive tissue, parenchymal organs, endocrine tissue, gastrointestinal tract, and, as in this case, peritoneum.[9] Where a clinical suspicion of coccidioidomycosis exists, serology for immunoglobin (Ig) G and IgM is an available screening tool in dogs, albeit imperfect.[7] A retrospective study found that the sensitivity of a commercially available agar gel immunodiffusion assay (AGID) serologic test for canine coccidioidomycosis was 87% for IgG and 46% for IgM.[7] The advent of an enhanced enzyme immunoassay to detect *Coccidioides* spp. antibodies shows promise to be more sensitive and specific than AGID.[7] Multiple studies suggest that elevated IgM occurs with acute disease, while elevated IgG indicates chronic or disseminated disease or both.[7]

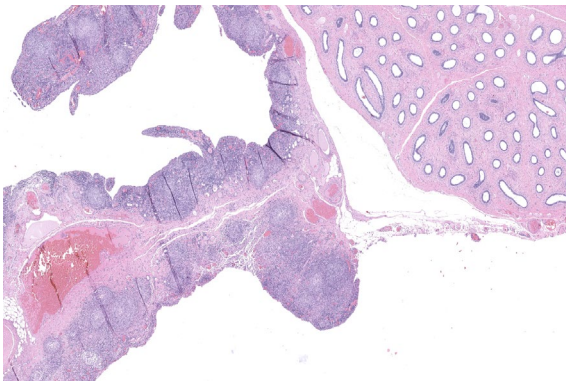

Figure 2-4. Spermatic cord, dog. There is expansion of the tunics lining the spermatic cord by an inflammatory infiltrate composed of pyogranulomas separated by lymphocytes and plasma cells. Epididymis at right. (HE, 26X)

This dog's diagnosis of disseminated coccidioidomycosis was fortuitous. The primary complaint was perineal hernia and unilateral cryptorchidism with likely neoplasia. This dog did not present with morbidity concerning for *Coccidioides* spp. infection. However, all biopsies detected peritoneal coccidioidomycosis. Serum collected the day after the biopsies yielded positive *Coccidioides* spp. IgM (1:4) and IgG (1:32) titers. It is probable that this patient's coccidioidomycosis involved more than the peritoneal cavity, but additional diagnostics, including thoracic radiographs were not pursued. The patient's sublumbar lymphadenomegaly could represent fungal lymphadenitis or perhaps seminoma metastasis or may simply be immunologic lymphoid hyperplasia. Following the *Coccidioides* spp. diagnosis, the dog was prescribed fluconazole per os twice daily. Six months later, upon titer reevaluation, the patient continued to have positive *Coccidioides* spp. IgM (1:4) and IgG (1:8) titers. The decreasing IgG titer is likely a result of treatment.[5] More than a year later, the patient is alive, though the extensiveness of its coccidioidomycosis and the possibility

of seminoma metastasis was never fully investigated.

Contributing Institution:
Midwestern University, College of Veterinary Medicine
Diagnostic Pathology Center
5725 West Utopia road
Glendale, Arizona 85308
https://www.midwestern.edu/

JPC Diagnoses:
1. Testis: Seminoma.
2. Testis: Hypoplasia and aspermatogenesis, diffuse, severe.
3. Testis, vaginal tunics and spermatic cord: Pyogranulomas, multiple, with endosporulating spherules, etiology consistent with *Coccidioides* sp.

JPC Comment:
The contributor provides an excellent review of both entities presented in this patient that presented for what was anticipated to be a "routine" cryptorchid castration.

Cox, Wallace, and Jessen first suggested a hereditary basis for cryptorchidism in dogs in 1978; however, Gubbels, Scholten, Janss, and Rothuizen provided convincing evidence in 2009 following the evaluation of data from 11,230 litters in 12 purebred dog breeds. Matings involving presumed carriers (parent of at least one previously identified cryptorchid) were associated with a 24.1% rate of cryptorchidism compared to 2.1% in the overall population.[10]

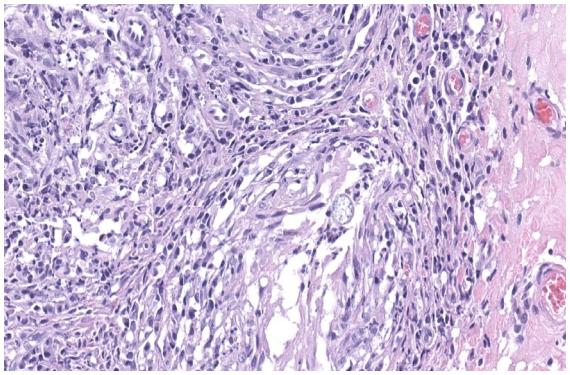

Figure 2-5. Spermatic cord, dog. Pyogranulomas are centered on 10-30 µm eondosporulating yeasts (arrow), consistent with *Coccidioides* sp. (HE, 381X)

Cryptorchid dogs are at increased risk for testicular neoplasia (especially Sertoli cell tumors and seminomas) and spermatic cord torsion. In addition, cryptorchidism has been found to be associated with umbilical and inguinal hernias, hip dysplasia, patellar luxation, penile and preputial defects, and occurs in up to 50% of dogs with persistent Müllerian duct syndrome (PMDS).[10, 12] PMDS is rare form of male pseudohermaphroditism characterized by the existence of a hypoplastic oviduct, uterus, and cranial vagina and is associated with an autosomal recessive pattern of inheritance most commonly observed in miniature schnauzers.[12]

Testicular neoplasia commonly occurs in dogs, with one study[6] reporting 62 (27%) of 232 male dogs between 80 days and 15 years (mean 10.7 years) of age presented for necropsy to be affected. Many affected animals had more than one testicular tumor, with a total of 110 identified. Although seminomas are commonly reported to be the most common testicular neoplasm, interstitial cell tumors were the most commonly reported testicular neoplasm in this study (50%), followed by seminomas (42%), and Sertoli cell tumors were the least common (8%). Of the 62 dogs with testicular tumors, 19 had more than one tumor type while two had all three.[6]

Testicular tumors typically have unique macroscopic and histologic features, with those of seminomas being previously described by the contributor. Macroscopically, interstitial cell tumors are typically yellow to brown, soft, nodular masses with hemorrhagic foci. Histologically, interstitial cell tumors are composed of round to

polygonal cells with finely vacuolated eosinophilic cytoplasm, an eccentric round nucleus, and have rare mitotic figures. Sertoli cell tumors typically present as grey to white, firm, multinodular masses that are clearly demarcated from surrounding parenchyma. Histologically, Sertoli cell tumors may demonstrate diffuse, intra-tubular, or a combination of growth patterns and are characterized by elongated cells with faintly eosinophilic cytoplasm often arranged in palisades lining well-delineated tubular structures associated with abundant fibrous stroma and rare mitotic figures.[6]

Dr. Myrnie Gifford, an assistant health officer in Kern County, California was the first physician to identify *Coccidioides* spp. as the etiologic agent of "San Joaquin Valley Fever" in 1934. Shortly thereafter, between 1945 and 1951, Drs. Phyllis Edwards and Carroll Palmer conducted an extensive seroprevalence study using over 110,000 Navy recruits, student nurses, and college students, resulting in the first known map of the disease's distribution across The United States.[3]

As noted by the contributor, the environment plays a significant role in this pathogen's growth and life stages. Thought to proliferate during short-lived wet conditions in an otherwise arid environment, *Coccidiodes* subsequently autolyzes into infective arthro-conidia when stressed by hot and dry conditions, which are then dispersed by wind. As a result, the climate of preceding seasons has been shown to play a significant role in the incidence of coccidiomycosis. For example, higher rates of human coccidio-mycosis have been reported in the San Joaquin Valley of California during autumns following cooler and wetter springs.[3]

Between 7000 and 9000 protein coding genes exist in the *Coccidioides* genome, which as a whole is poorly understood. Less than 10 genes have been functionally characterized so far, many of which are related to virulence. Interestingly, ammonia production appears to play a role in virulence during spherule development and rupture. Deletion of urease gene (*URE*) resulted in partial reduction of ammonia and increased the survival of mice by 60%, whereas double deletion of urease and ureidoglycolatehydrolase (*Ugh*) genes resulted in even lower ammonia levels and increased the survival of mice to 90%.

Studies have shown the host's innate immune response to *Coccidioides* spherules relies on TLR2, myeloid differentiation factor 88 (MyD88) and Dectin-1. In addition, T cells have been shown to play a crucial role in the body's defense against *Coccidioides,* as Th1 responses associated with IL-12 and IFN-γ and Th17 responses activated by IL-17 and IL-22 are both critical for protection against coccidiomycosis.[3]

During the conference the moderator emphasized the risk posed to laboratory personnel by *Coccidioides* spp. Infected animals are not contagious; however, infective arthroconidia formed when the organism is cultured can be inhaled if proper safety protocols are not followed. Therefore, laboratory personnel should always be notified when samples from potential cases of coccidiomycosis are submitted for culture as a professional courtesy.

References:

1. Adaska JM. Peritoneal Coccidioidomycosis in a Mountain Lion in California. J Wildl Dis 1999;35(1):75-77
2. Foster RA. Male Genital System. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. St. Louis, MO; 2016:465-510.

3. Gorris ME, Caballero Van Dyke MC, Carey A, Hamm PS, Mead HL, Uehling JK. A Review of *Coccidioides* Research, Outstanding Questions in the Field, and Contributions by Women Scientists [published online ahead of print, 2021 Aug 2]. *Curr Clin Microbiol Rep*. 2021;1-15.
4. Gorris ME, Treseder KK, Zender CS, Randerson JT. Expansion of coccidioidomycosis endemic regions in the United States in response to climate change. *GeoHealth* 2019:308-327.
5. Graupmann-Kuzma A, Valentine BA, Shubitz LF, et al. Coccidioidomycosis in Dogs and Cats: A Review. *J Am Anim Hosp Assoc* 2008;44(5):226–235.
6. Grieco V, Riccardi E, Greppi GF, Teruzzi F, Iermanò V, Finazzi M. Canine testicular tumours: a study on 232 dogs. *J Comp Pathol*. 2008;138(2-3):86-89.
7. Gunstra A, Steurer JA, Seibert RL, Dixon BC, Russell DS. Sensitivity of Serologic Testing for Dogs Diagnosed with Coccidioidomycosis on Histology: 52 Cases (2012–2013). *J Am Anim Hosp Assoc* 2019;55(5):238–242.
8. Hohšteter M, Artuković B, Severin K, et al. Canine testicular tumors: two types of seminomas can be differentiated by immunohistochemistry. *BMC Vet Res* 2014;10:169
9. Izquierdo A, Jaffey JA, Szabo S, et al. Coccidioides posadasii in a Dog With Cervical Dissemination Complicated by Esophageal Fistula. *Front Vet Sci* 2020;7:285.
10. Khan FA, Gartley CJ, Khanam A. Canine cryptorchidism: An update. *Reprod Domest Anim*. 2018;53(6):1263-1270.
11. Koistinen K, Mullaney L, Bell T, et al. Coccidioidomycosis in Nonhuman Primates: Pathologic and Clinical Findings. Vet Pathol. 2018;55(6): 905–915.
12. Park EJ, Lee SH, Jo YK, et al. Coincidence of Persistent Müllerian duct syndrome and testicular tumors in dogs. *BMC Vet Res*. 2017;13(1):156. Published 2017 Jun 2.

CASE III: P-2021 169 (JPC 4168027)

Signalment:
A 4-year-old, male neutered, crossbreed dog (*Canis familiaris*)

History:
The dog was imported to the UK from Cyprus in April 2016. At that time the dog was in contact with a bitch with CTVT and in October 2020 the dog showed a firm swelling along the caudal prepuce in the ventral midline. The biopsy from the penis confirmed a CTVT. The dog was treated with vincristine with a modest reduction in size of the mass, however, the reduction was incomplete, and doxorubicin was given, but without a measurable response. Radiotherapy was given in March 2021, with some shrinkage of the penile mass. An ulcerated mass developed on the skin of the right ventral neck a month later. The dog was referred to the QVSH, University of Cambridge. On presentation, the dog was dull but responsive with a BCS 3/9. Both the penile lesion and the neck mass were evident. Abdominal ultrasound showed several, ill-defined, rounded to multilobulated, hypoechoic masses distributed throughout the liver lobes, measuring 1.56cm - 3.46cm. The penis glands showed a hypoechoic, symmetrical structure surrounding the base of the penis. Cytology of the liver nodules confirmed CTVT cells and histology of the penile glands and the skin mass confirmed CTVT. Following deterioration in the dog's condition and the onset of anorexia and vomiting, euthanasia was performed on humane grounds.

Gross Pathology:
Presented for necropsy was a 4-year-old male neutered cross breed dog. The animal was in body condition 3/9. There was a 2.5x3.5x2.5cm tumor on the skin of the right ventral cervical region. The mass was firm and had a central cavitated opening (1.4x1x1cm). The mass was mobile from the underlying subcutaneous tissues. The mass was homogeneously light cream to pink when the surface was cut, with a central 0.5x0.7x 1.1cm cavity. There was a tumor at the base of the penis 4.2x3.5x3cm on average. The tumor was firm and with an overlying intact mucosa. There was a multinodular firm tumor at the base of the penis approximately 4.2x3.5x3cm on average, well-demarcated, partially encapsulated, but locally infiltrative towards the caudal part of the base of the penis. The tumor was lined by a smooth pink to cream mucosal surface. The tumor was homogenously cream to white. The rest of the penis had a smooth white to pink mucosal surface. The liver had multiple distinctive firm white nodular tumors with a smooth surface on the right medial lateral lobe and left lateral lobe. The masses vary from 0.3x0.5x0.4cm to 1.3x1.5x1.1cm. The masses were well-demarcated, partially encapsulated, firm, homogeneously white to light pink on cut surface. The right mandibular lymph node was 1.5x fold from the left mandibular lymph node, with a homogeneous white parenchyma on cut surface. The rest of the organs were unremarkable.

Laboratory Results:
In October 2020 biochemistry revealed an elevated ALKP 1595IU/L (reference interval 23-212). Serum biochemistry and hematology were performed; alkaline phosphatase remained elevated at 1700iu/L (26-107). There was a lymphocytopenia 0.7 x $10/^{9}$/l (reference interval 1-4.7) and an erythrocytosis RBC 9.16 X $10/^{12}$/L (5.5-8.5),

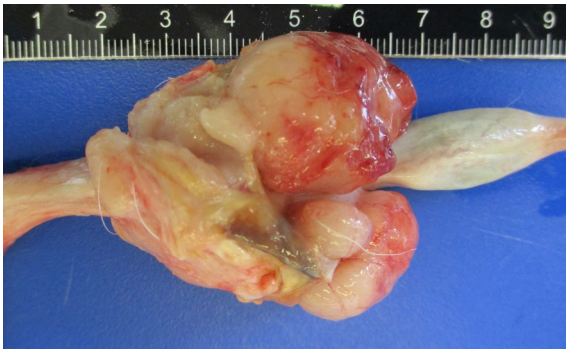

Figure 3-1. Penis, dog. A lobulated mass is present at the caudal part of the penis. (*Photo courtesy of:* Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB30ES, UK https://www.vet.cam.ac.uk)

Hb 22.4 (12-18) HCT 60.9% (37-55) which was unexplained.

Microscopic Description:
Present within the liver parenchyma is a densely cellular, locally infiltrative, mostly encapsulated neoplastic mass. Neoplastic cells are arranged in densely packed sheets that are supported by a thin collagenous stroma or more prominent fibrovascular connective tissue stroma. Individual neoplastic cells are round to oval, with scant to moderate amount of eosinophilic to light basophilic cytoplasm with variably distinct cell borders. Nuclei are large, oval to round with coarsely aggregated chromatin and variable prominent central nucleolus. Mitoses are 20 per 10 HPFs ($2.37mm^2$), with some being bizarre. Present within a variable thick fibrous connective capsule are variable numbers of new blood vessels, lymphocytes, plasma cells and fewer neutrophils, occasional bile ducts and hepatocytes and rare giant cells. The adjacent parenchyma is compressed, with smaller hepatocytes than expected (atrophy), aggregates of lymphocytes and scattered plasma cells. Portal triads exhibit variable amounts of fibrosis, moderate biliary hyperplasia, and macrophages with deposits of golden-brown intracytoplasmic material (presumed lipogranulomas). Diffusely there is moderate

to severe hyperplasia and hypertrophy of hepatic stellate (Ito) cells.

Present beneath the mucosa of the penis (not submitted) is a densely cellular, moderately well-demarcated with a thin fibrous connective tissue capsule or a pseudocapsule neoplastic mass, which in some areas forms lobules. Neoplastic cells are arranged in densely packed sheets that are supported by thin collagenous or fibrovascular stroma. Neoplastic cells are similar to the ones described above. Peripheral to the mass are small numbers of plasma cells and fewer numbers of lymphocytes. The epidermis overlying these sections of haired skin is diffusely ulcerated (not submitted). Present within the sections is a densely cellular, moderately well demarcated, non-encapsulated neoplastic mass that compresses the adjacent dermis, subcutaneous tissues, and underlying skeletal muscle. Neoplastic cells are arranged in densely packed sheets that are supported by a thin collagenous stroma or more prominent fibrovascular connective tissue stroma. Individual neoplastic cells are very similar as the ones described above. Mitoses are 29 per 10 HPFs (2.37mm^2), with some being bizarre. Scattered small to moderate sized areas of necrosis and neutrophil accumulation and fibrin deposits are present throughout the mass. Multifocally peripherally blood vessels to the mass exhibit small number of plasma cells and fewer lymphocytes. The ulcerated area exhibits moderate number of viable and degenerated neutrophils, cellular debris, and fibrin deposits. The rest of the overlying epidermis and adjacent dermis is within normal limits.

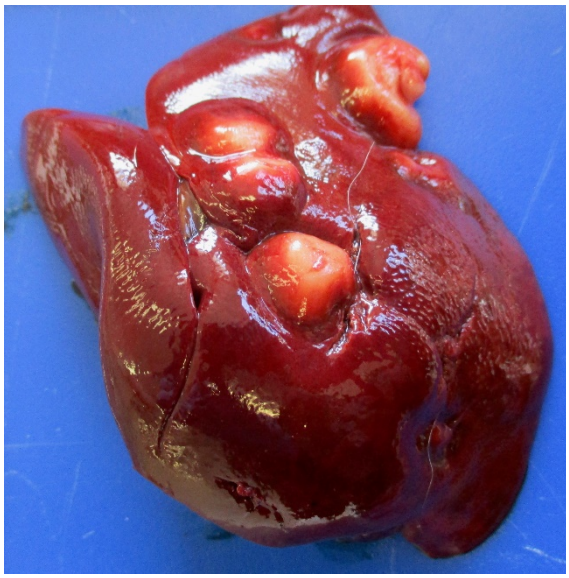

Figure 3-2. Liver, dog. There are multiple discrete firm white smooth nodular masses. The masses are well-demarcated, and partially encapsulated. (*Photo courtesy of:* Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB30ES, UK https://www.vet.cam.ac.uk)

Immunohistochemistry showed neoplastic cells positive staining for vimentin. Neoplastic cells did not exhibit positive staining for CD3, CD79a, and IBA1. Neoplastic cells were negative for toluidine blue staining.

Contributor's Morphologic Diagnoses:
Metastatic canine transmissible venereal tumor to liver.

Contributor's Comment:
Canine transmissible venereal tumor (CTVT), also called Sticker's sarcoma, transmissible venereal sarcoma, venereal granuloma and infectious sarcoma, is a contagious tumor that propagates naturally in dogs usually during coitus, licking, sniffing, and other interaction between an affected dog and another dog.[2,6,7] The other contagious known tumors are the Tasmanian devil facial tumor disease[14] and a similar tumor in Syrian Hamsters.

CTVT is a naturally occurring allogenic tumor that is transmitted by living neoplastic cells from dog to dog.[3,7,13] The number of diploid chromosomes in the dog is 78, whereas there are 58 or 59 chromosomes in cells of CTVT.[3] Cells of the CTVT can be recognized by their chromosomes.[11] CTVT is the oldest known somatic cell lineage, that arose in a single dog over 11,000 years ago and spread across continents some 500 years ago.[3] CTVT may have first arisen within a genetically isolated population of early dogs whose limited genetic diversity facilitated the cancer's escape from its hosts immune systems.[7] The first known report of this tumor was made in 1810 by a London veterinary practitioner. CTVT cells commonly harbor a retrotransposon upstream from the *c-myc* oncogene that is a molecular fingerprint for the tumour.[3]

CTVT has been unusual in many regards, however during the last few years more cases have been seen in countries that used to have low case numbers. CTVT has been reported from all continents except Antarctica.[2] CTVT is a rare tumor in household pet dogs, but relatively common in stray dog populations in some countries, especially in tropical and subtropical regions.[3]

CTVT is most often present on the external genitalia, but it can be implanted on the oral cavity, nasal mucosa, conjunctival mucosa, and skin. The tumor usually develops locally in affected areas and metastases are rare, but can occur in approximately 1.5 to 6% of infected dogs[12] to inguinal and iliac nodes, brain, and other viscera[3] and underlying tissues such as skeletal muscle. Extragenital presentations of CTVT can occur without genital lesions. CTVT cells grow typically in the submucosa of the external genitalia, which may be ulcerated and bleeding from the site can occur. Tumors vary in size, up to 10cm in diameter[3,13] and may invade underlying tissues. Regression of tumors can occur when a rich lymphocytic infiltrate is present throughout the mass within 6 months.[15] However, in immunologically compromised dog's tumors can persist and metastases to distant organs can occur. Vincristine is known to be an effective treatment of CTVT, and in some cases a combination with surgery, doxorubicin or radiotherapy has been used.[13] In this case unfortunately the dog failed to respond to treatment and euthanasia was performed. CTVT cells are arranged in sheets and cords, are round or oval with scant cytoplasm that is sometimes vacuolated and have variably distinct cell borders.[1] Nuclei are central, large, oval or round, coarsely aggregated chromatin and a single prominent nucleolus. Mitotic figures are common and many are bizarre.[9]

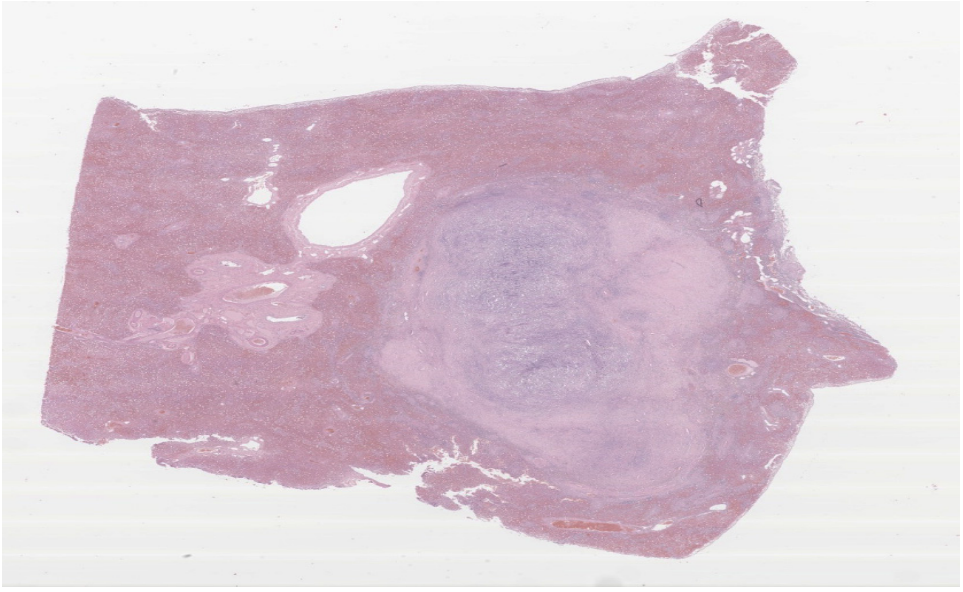

Figure 3-3. Liver, dog. An encapsulated neoplasm is present within the liver. (HE, 4X)

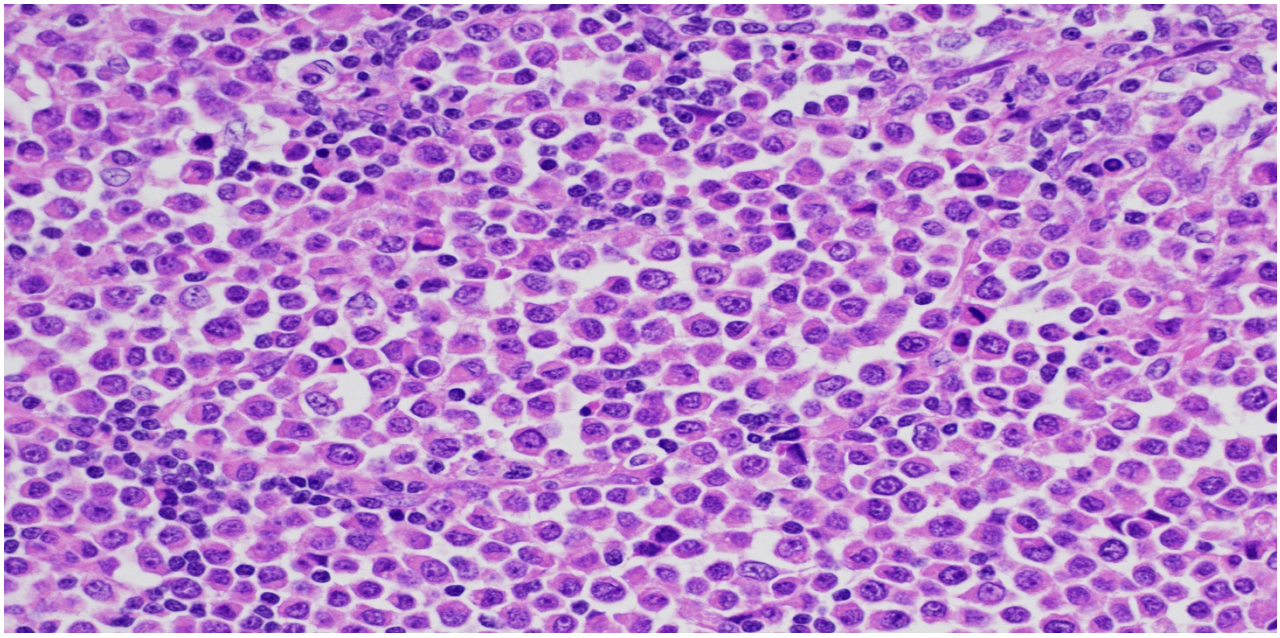

Figure 3-4. Liver, dog. Neoplastic cells are arranged in nets and packets on a fine fibrovascular stroma Individual neoplastic cells are round, with scant to moderate amount of eosinophilic to light basophilic cytoplasm with distinct cell borders. Nuclei are large, oval to round with coarsely aggregated chromatin and a central nucleolus. (HE, 400X) (*Photo courtesy of:* Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB30ES, UK https://www.vet.cam.ac.uk)

The supporting fibrovascular stroma is usually scant at early stages, but if the tumor is under regression the stroma is more abundant and T lymphocytes[5] and other inflammatory cells are evident throughout the neoplastic tissue.

The histogenesis of CTVT cells is still poorly defined and incompletely characterized.[3] Neoplastic cells are variably labelled for vimentin and CD18, further suggesting a histiocytic origin.[3,9] Immunoreactivity for lysozyme and alpha-1-antitrypsin can be detected in some CTVT cells.

On routine H&E sections differential diagnoses of CTVT cells are other round cell tumors, including histiocytoma, lymphoma, and mast cell tumor, and cytological features can be subtle.[1,2,3,5,6,9,11,13] Less common differential diagnoses are amelanotic melanomas and poorly differentiated carcinomas. CTVT cells usually have low nuclear-to-cytoplasm ratios and distinct small cytoplasmic vacuoles.[4] Histology and cytology examination can be diagnostic for CTVT however, it is crucial to consider the tumor site and clinical history for diagnosis.[2,3,4,9,11,12,13,14] The current case is an unusual presentation of a CTVT in liver and lymph nodes, being the primary neoplastic site the base of the penis. The combined use of a panel of antibodies in conjunction with microscopic assessment could facilitate the diagnosis of extragenital CTVT and difficult cases where no obvious primary tumor is present.

Contributing Institution:
Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge. Cambridge CB3 0ES, UK.

JPC Diagnosis:
Liver: Metastatic transmissible venereal tumor.

JPC Comment:
The contributor provides an excellent synopsis of canine transmissible venereal tumor (CTVT), a naturally occurring parasitic allograft. This transmissible neoplasm is commonly associated with canines with free outdoor access (such as strays) and is endemic in more than 90 countries, most commonly in tropical and subtropical regions.[9] Although CTVT only occurs naturally in dogs, experimental infection has been reported in other canids such as foxes, coyotes, and jackals.[2]

Clinically, CTVT typically exhibits a predictable cycle characterized by an initial growth phase (P phase) over 4-6 months, followed by a stable phase, and finally a regression phase (R phase), although not all tumors regress. Histologically, high numbers of mast cells and small caliber blood vessels are commonly observed at the leading margin of progressing tumors while regressing tumors have increased numbers of lymphocytes, predominantly composed of T cells. During the P phase, tumor cells typically exhibit a round cell morphology, while cells in the stable phase undergo transition from round cells to spindle cells, which become vacuolated and are associated with increased collagenous matrix during the regression phase. Unsurprisingly, mitotic counts and telemerase activity is higher during the P phase.[2]

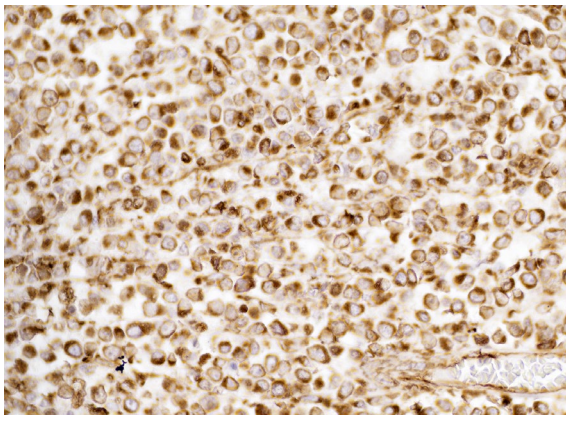

Figure 3-5. Liver, dog. Neoplastic cells stain strongly immunopositive for vimentin (antivimentin, 400X) (*Photo courtesy of:* Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB30ES, UK https://www.vet.cam.ac.uk)

Spontaneous tumor regression is predominantly mediated by the host's immune response, namely as the result of IgG antibody formation that begins after approximately 40 days of tumor growth, which also prevents reinfection in immunologically competent hosts. In addition, newborn pups born to immune dams are also resistant and achieve remission more quickly than those born to non-immune dams.[2]

CTVTs evade the host immune response via several mechanisms, including down regulation of their *MHC* genes, resulting in complete inhibition of MHC II while approximately 10% of MHC I genes are expressed. This pattern of MHC suppression without complete inhibition is crucial in regard to the neoplasm's ability to evade detection by the host's immune system as complete masking of MHC I genes tends to result in the upregulation of natural killer cell activity while a low "background" level of MHC I expression prevents this activation while also effectively masking the cells from the host's immune system. Additionally, CTVT suppresses the immune system by secreting TGF-β1 and circulating monocytes are frequently depressed up to 40%. In addition, the CTVT is associated with impaired efficiency in which mature dendritic cells are generated from monocytes and immature dendritic cells.[2]

As noted by the contributor, CTVT can be histologically difficult to differentiate from other round cell tumors, such as histiocytoma, lymphosarcoma, and mast cell tumors. In addition to vimentin, lysozyme, and α-1-antitrypsin CTVT cells may also demonstrate immunoreactivity for glial

fibrillary acidic protein while being negative for cytokeratins, S100, and muscle markers.[2]

The Tasmanian devil facial tumor (TDFT) is another transmissible tumor that was first noted in 1996 and is restricted to the island of Tasmania, the only natural habitat of Tasmanian devils. Estimates from 2004-2007 estimated the disease was endemic to 70% of the island, decimating up to 90% of Tasmanian devil populations in some regions. This rapid spread is likely attributable to multiple behavioral characteristics of devils, which are non-territorial, roam up to 27km^2, and also frequently bite others during feeding, fighting, and mating, facilitating transfer of transmissible tumor cells. In contrast to CTVTs which often stabilize and/or regress, TDFTs frequently occur on the face and mouth resulting in severe disfigurement and do not regress; approximately 65% undergoing metastasis to visceral organs, most commonly to the lungs.[8]

Histologically, TDFTs are composed of round cells and are characterized as undifferentiated, infiltrative mesenchymal neoplasms within a pseudocapsule supported by fibrovascular stroma and are often ulcerated. Initially, TDFTs were thought to be of neuroendocrine origin due to expression neuron specific enolase, synaptophysin, and vimentin. However, recent research has found tumor cells also produce periaxin, a protein specific to Schwann cells; periaxin is now considered a sensitive and specific diagnostic marker for these tumors. Given both Schwann and neuroendocrine cells are derived from the neural crest, it is likely TDFTs originated from a precursor neural crest cell.[8]

DFTD presents a serious risk to Tasmanian devils, which may be extinct in the wild in as few as 35 years. Given their behavioral characteristics and limited geographic distribution, few options exist to prevent their extinction short of isolating unaffected animals and culling those affected. However, recent development of a potential vaccine may offer a new approach toward thwarting the extinction of this unique species.[8]

References:

1. Champour M, Ojrati N, Nikrou A, Rhahimian H, et al. First report of diffuse cutaneous transmissible venereal tumor in Iran. *Comp Clin Pathol.* 2015;**24**:741-744.
2. Ganguly B, Das U, Das AK. Canine transmissible venereal tumour: a review. *Vet Comp Oncol.* 2013;**14**(1):1-12.
3. Hendrick MJ. Mesenchymal tumors of the skin and soft tissues. In: *Tumors in Domestic Animals.* 5th ed. Wiley Blackwell; 2017.
4. Henson KL. Reproductive system. In *Atlas of Canine and Feline Cytology*. W.B. Saunders Co; 2001.
5. Mizuno S, Fujinaga T, Hagio M. Role of lymphocytes in spontaneous regression of experimentally transplanted canine transmissible venereal sarcoma. *J Vet Med Sci.* 1994;**56**(1):15-20.
6. Mozos E, Mendez A, Gomez-Villamandos JC, et al. Immunohistochemical characterization of canine transmissible venereal tumor. *Vet Pathol.* 1996;**33**:257-263.
7. Murchison E, Wedge DC, Alexandrov LB, et al. Transmissible dog cancer genome reveals the origin and history of an ancient cell lineage. *Science.* 2014;**343**:437-440.
8. Ostrander EA, Davis BW, Ostrander GK. Transmissible Tumors: Breaking the Cancer Paradigm. *Trends Genet*. 2016;32(1):1-15.
9. Pashkevych I, Strybel V, Soroka N. Diagnostic methods of canine

transmissible venereal sarcoma. *Vet Med Sci.* 2018;**3**:67-76.
10. Pimentel PAB, Oliveira CSF, Horta RS. Epidemiological study of canine transmissible venereal tumor (CTVT) in Brazil, 2000-2020. *Prev Vet Med*. 2021;197:105526.
11. Prier JE. Chromosome pattern of canine transmissible sarcoma cells in culture. *Nature.* 1966;**212**:725-726.
12. Rogers KS, Walker MA, Dillon HB. Transmissible venereal tumor: a retrospective study of 29 cases. *J Am Anim Hosp Assoc.* 1998;**34**(6):463-470.
13. Strakova A, Murchison E. The changing global distribution and prevalence of canine transmissible venereal tumour. *BMC Vet Res.* 2014;**10**(1):168-168.
14. Welsh JS. Contagious cancer. *Oncologist.* 2011;**16**:1-4.
15. Yang TJ, Jones JB. Canine transmissible venereal sarcoma: transplantation studies in neonatal and adult dogs. *J Natl Cancer Inst.* 1973;**51**(6):1915-1918.

CASE IV: 21-199 (JPC 4167919)

Signalment:
Male, Warmblood, fetus, ~ 180-210 days gestation (estimated)

History:
Fetus found in the morning with mare having aborted overnight. No preceding clinical signs. Mare had Potomac horse fever in the fall. No other recent changes, closed herd.

Gross Pathology:
Gross examination of the submitted fetus (estimated gestational age of 180 to 210 days) and associated placenta revealed evidence of enlarged/non-collapsed, mildly firm, lungs and faint, generalized, myocardial pallor. The liver is enlarged and pale and dotted with faint, pin-point foci of pallor. Lymphoid follicular hyperplasia was within the splenic white pulp accompanied by generalized lymphadenomegaly and an enlarged pale thymus.

Laboratory Results:
EHV 1 and 4 PCR (pooled lung, liver, and spleen) – Negative
Potomac Horse Fever PCR (pooled lung, liver, and placenta) - Positive

Microscopic Description:
Heart, left and right ventricles: Sections of left and right ventricular myocardium are examined, and both contain similar changes. The sections are dotted with moderately cellular and often vasocentric foci of inflammation. The perivascular to interstitial infiltrates consist of lymphocytes, macrophages, fewer plasma cells and rare neutrophils. The inflammatory cells often infiltrate the vessel walls (interpreted as lymphohistiocytic vasculitis) and are accompanied by mild to moderate expansion

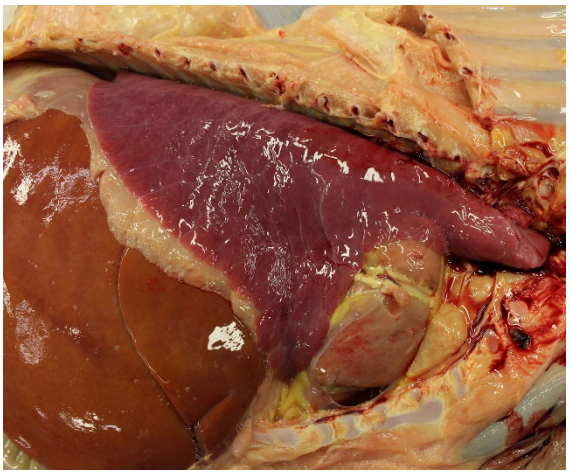

Figure 4-1. Viscera *in situ*, equine abortus. The lungs are enlarged and firm. The myocardium demonstrates diffuse pallor and there are scattered pale areas within the liver. (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

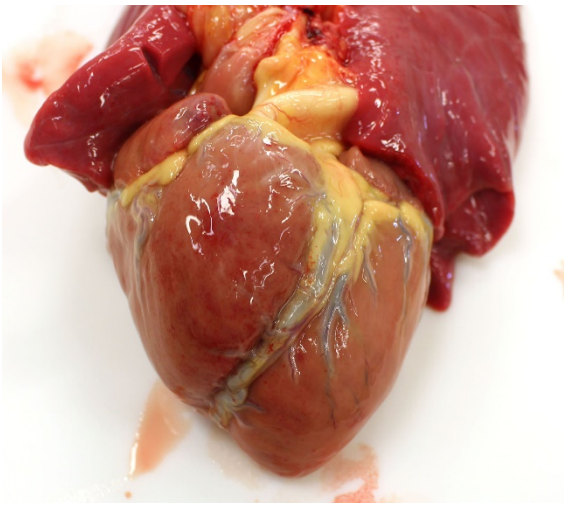

Figure 4-2. Heart, equine abortus. The myocardium has retiform areas of pallor which bulge minimally outward on presentation. (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

of the vascular adventitia, and adjacent myocardial interstitium, by clear edema fluid. The endothelial cells lining affected vessels, and interstitial fibroblasts, are plump with vesicular nuclei (interpreted as reactive change). The cardiomyocytes separated by inflammatory cells and attendant edema are frequently pale with perinuclear clearing to vacuolation and plump/vesicular nuclei (degenerative change suggestive of cardiomyocyte injury). Similar inflammatory infiltrates are noted within the lining epicardium.

Lymph node, mediastinal: The capsule as well as capsular and hilar vessels are expanded and infiltrated by variable dense inflammatory infiltrates composed predominantly of lymphocytes and macrophages. Occasional vessels contain luminal aggregates of eosinophilic fibrillar material (interpreted as fibrin) accompanied by marginated and subintimal inflammatory cells. The cortices are dotted with prominent secondary follicles (follicular hyperplasia) similar edema and variably dense lymphohistiocytic infiltrates noted within the supporting capsule, interstitium, and vascular adventitia. The subcapsular and medullary sinuses are moderately distended with mixtures of acute hemorrhage, circulating mononuclear cells, and foamy to debris-laden macrophages. Subjectively, increased numbers of plasma cells distend the medullary cords. Similar vasocentric foci of inflammation are noted amongst the surrounding adipose tissue. Some sections of lymph node also contained extensive regions of acute necrosis.

Additional histopathological findings:
Small intestine: Lymphohistiocytic enteritis, moderate, subacute
Lung: Interstitial pneumonia, lymphohistiocytic, moderate, subacute, diffuse
Liver: Lymphohistiocytic portal hepatitis, mild, diffuse
Kidney: Lymphohistiocytic interstitial nephritis, moderate, diffuse with acute tubular injury and necrosis
Adrenal gland: Lymphohistiocytic adrenalitis, perivascular and interstitial, moderate, subacute
Spleen, white pulp: Follicular hyperplasia, moderate

Contributor's Morphologic Diagnoses:

1. Heart, left and right ventricle: Interstitial myocarditis and vasculitis, lymphohistiocytic, multifocal, subacute, moderate with perivascular and interstitial edema.
2. Lymph node, mediastinal: Vasculitis and lymphadenitis, lymphohistiocytic, multifocal, subacute, with cortical follicular hyperplasia.

Contributor's Comment:

The lesions found within the heart, liver, lymph node, and intestine of this aborted fetus are characteristic pathological findings described in fetuses aborted due to an underlying *Neorickettsia risticii* infection (Potomac horse fever). Typical necropsy findings found non-pregnant horses dying from Potomac horse fever (PHF) include enterocolitis with abortion occurring in pregnant mares following clinical recovery. Abortions due to PHF have been reported to occur anywhere from between 166- to 226-days gestation, with clinical illness being reported anywhere from 68- to 143-days of gestation. Clinical signs reported in the pregnant mares, prior to abortion, are similar to those reported in nonpregnant horses (gastrointestinal hypermotility or diarrhea, fever, dehydration, depression, tachypnea, tachycardia, and anorexia). *Neorickettsia risticii* is not included in the listed causes of equine abortion in several large case reviews[5,6,8] and textbooks[15] related to equine abortion and neonatal loss, despite the few reports being found in the literature.[3,9]

Neorickettsia spp. bacteria replicate intracellularly within host cells (within a phagolysosome) and often use trematode hosts. Various trematode species (affecting all levels of the life cycle) found throughout Canada and the United States act as hosts for this bacterium with the trematodes often being situated within their own insect (e.g. damselflies, caddisflies, stoneflies, mayflies, etc.) or snail hosts.[11,12,13] Infection is thought to occur most commonly in horses housed around creeks or other bodies of water through accidental ingestion of snails or aquatic insects harboring *N. risticii*-infected trematodes. Following ingestion, the incubation period is approximately 9 to 14 days and diarrhea typically 1 to 3 days following the onset of fever. Many cases are subclinical with some horse developing clinical disease of PHF. It is thought that this and similar rickettsiae have an affinity for monocytes and intestinal epithelial cells[4] with infection sometimes resulting in cellular injury/necrosis and attendant lympho-histiocytic inflammation. The mechanism through which the bacteria inoculate the target cells (monocytes and enterocytes) is not known but may resemble that of *Neorickettsia helmintheca* in cases of salmon poisoning disease in dogs. Infection via inoculation of enterocytes and circulation monocytes may account for the bacteria dissemination with enteric and vasocentric

Figure 4-3. Spleen, equine abortus. There is marked hyperplasia of the splenic white pulp. (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

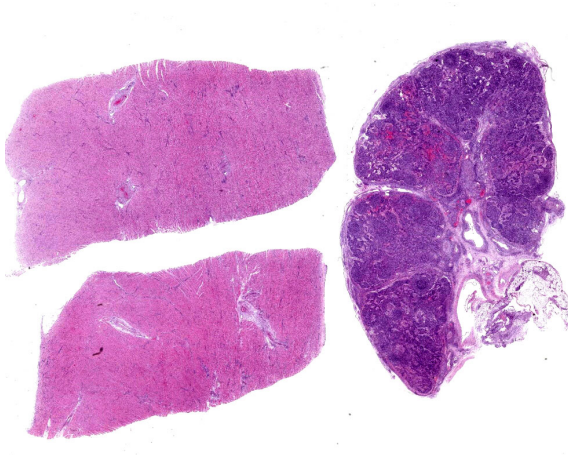

Figure 4-4. Heart, lymph node, equine abortus. At subgross magnification, there are multifocal cellular infiltrates within the myocardium (left), and normal nodal architecture is disorganized with multifocal mild sinusoidal hemorrhage (right). (HE, 6X)

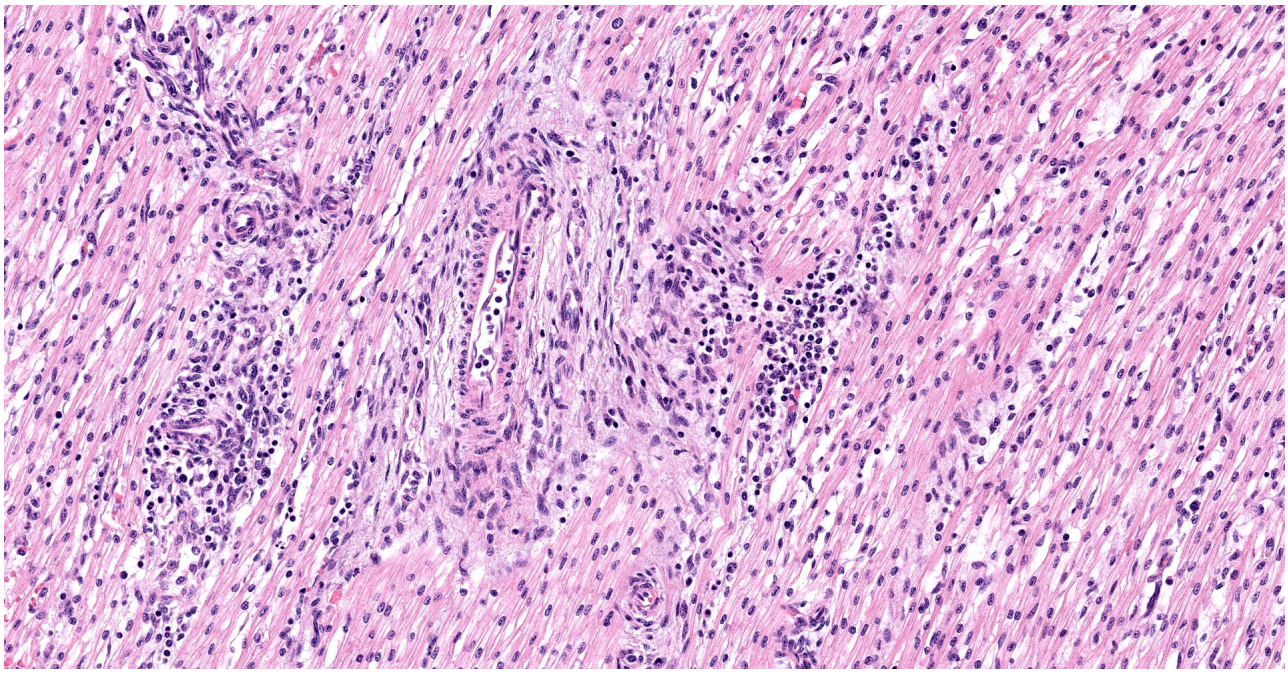

Figure 4-5. Heart, equine abortus. Moderate numbers of lymphocytes and macrophages expand the interstitium (right), and are present in perivascular areas (left). (HE, 195X*J*)

lesions found in the intestine and a variety of organ tissues, including the heart, liver, lung, spleen, lymph nodes, and adrenal gland in fetal tissues. Demonstration of intracellular bacteria and morula in affected organ tissues can be difficult using special stains (e.g. Romanowsky stain) and confirmatory diagnosis often relies on PCR testing and sequencing.

Natural infections caused by *N. risticii* have been infrequently reported in cats and dogs with clinical signs (including anorexia, depression, lethargy, fever, vomiting, diarrhea, lymphadenomegaly, weightloss, lameness, polyarthritis) often resembling those of *N. risticii* infection in horses and *N. helmonthica* in dogs.[7]

Contributing Institution:
Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary.
https://vet.ucalgary.ca/

JPC Diagnoses:

1. Heart: Pancarditis and vasculitis, lymphohistiocytic, multifocal to coalescing, severe, with multifocal myofiber degeneration, necrosis, and loss.
2. Lymph node: Reactive hyperplasia, diffuse, mild to moderate with lymphocytolysis, medullary plasmacytosis, and lymphohistocytic vasculitis.
3. Perinodal fat: Steatitis, lymphohistiocytic, multifocal, mild.

JPC Comment:
"Potomac horse fever" (PHF) was first recognized as a clinical entity in horses in 1979 by veterinarians in Montgomery County, Maryland and Fairfax and Loudon counties, Virginia. Most cases occurred in horses located "within a mile or two" of a six mile long segment of the Potomac River. Between 1982 and 1986, cases dramatically increased in the same region, leading to speculation of a new disease, which was initially known as "Acute

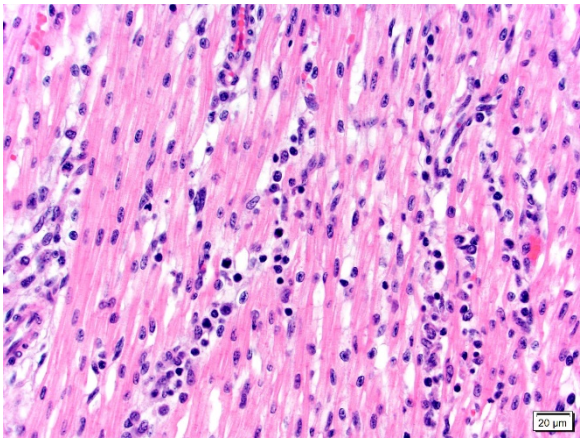

Figure 4-6. Heart, equine abortus. High magnification of the interstitial myocardial infiltrate. (HE, 400X) (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

Equine Diarrhea Syndrome" (AEDS). Although first thought to be localized to a focal area adjacent to the Potomac River, PHF has since been recognized in 43 states of the United States, three provinces of Canada, and has been documented in France, the Netherlands, and Brazil.[2,10] In addition to PHF, this entity is also known as "equine ehrlichial colitis" (EEC), "equine monocytic ehrlichiosis" (EME), "Shasta River Crud", "churrio", and "churrido equino".[2] Interestingly, the term "Potomac horse fever" is credited to a reporter covering the initial outbreak in Maryland, though the term "equine neorickettsiosis" may be more appropriate given its distribution.[2,14]

In 1984, a rickettsial organism was identified in tissue from a pony experimentally infected with PHF by researchers using transmission electron microscopy. The organism was suggested to belong to genus *Ehrlichia* given its round shape and location within histiocytic cell vacuoles and was named *Ehrlichia risticii*. The bacterium was subsequently renamed *Neorickettsia risticii* in 2001. *N. risticii* is a gram-negative coccus and stains dark blue to purple with Giemsa and Romanowsky's stains. Serologic diagnosis using indirect fluorescent antibody (IFA) testing is available but can pose a diagnostic challenge for clinicians. Although a 4-fold titer increase is considered diagnostic, antibody levels reach a high point within a few days of infection, requiring collection of serum samples early in the disease process. Previously exposed horses may have a titer of 1:640 for over a year, therefore IFA test titers collected after the initial antibody spike are unable to distinguish between active or past infection, nor vaccination.[2] Isolation of *R.* risticii in cell culture is the gold standard for diagnosis, though this method is both time consuming and often unavailable as a commercial service.[1] Confirmatory diagnosis therefore often relies on detection of *N. risticii* DNA via PCR testing and sequencing as previously noted by the contributor.[1,2] A recent report noted feces were the optimal sample utilized for PCR detection, followed by blood.[1]

PHF typically occurs on a seasonal basis, with the majority of infections occurring during the late summer months, with nearly 70% of the 904 cases reported during the 1982 and 1986 outbreak occurring during July and August. Acute infection in adult horses is typically characterized by of

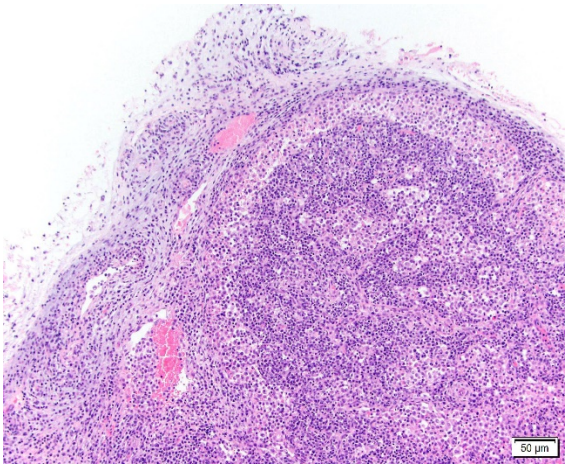

Figure 4-7. Lymph node, equine abortus. There is disorganized cortical hyperplasia with numerous tingible body macrophages. Sinuses contain numerous macrophages. (HE, 400X) (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

depression, anorexia, and fever followed by moderate to severe diarrhea in approximately 60% of horses that may persist for up to 10 days with a consistency ranging from profuse and watery to "cow-pie-like". Additional clinical signs may include laminitis in 15-30% of PHF cases, subcutaneous edema along the ventral abdomen and limbs, and congested mucous membranes with elevated heart and respiration rates as the result of cardiovascular compromise secondary to both toxemia and dehydration. Overall, the mortality rate of PHF ranges between 17% and 36%, with some cases requiring euthanasia due to secondary complications such as laminitis.[2]

Experimental cases of PHF are associated with a leukopenia (<5.0 x 10^9/L) between 8-19 days post infection in up to 90% of horses, followed by a leukocytosis (>14.0 x 10^9/L) between 13 to 30 days post infection in approximately 50% of horses. Clinical biochemistry results are non-specific.[2]

Although thought to be a focal entity affecting the eastern United States in 1979, the disease now known as PHF was likely described over half a century earlier by Dr. Frank Schofield in 1924 while investigating a locally endemic disease in Kent and Essex counties of Ontario. The disease was colloquially known by farmers as "cholera", "horse cholera", and "abdominal typhoid". Dr. Schofield described nearly identical clinical signs in horses affected with PHF, which occurred from the middle of July through the beginning of August. Schofield also astutely noted the disease's geographic pattern of distribution, observing, "There is a definite relationship between the water front and the occurrence of disease" and "…one might say that the zone of infection extends from the water front to a distance of about five miles inland". In addition, Schofield noted mayflies (*Ephemerida*) were found in

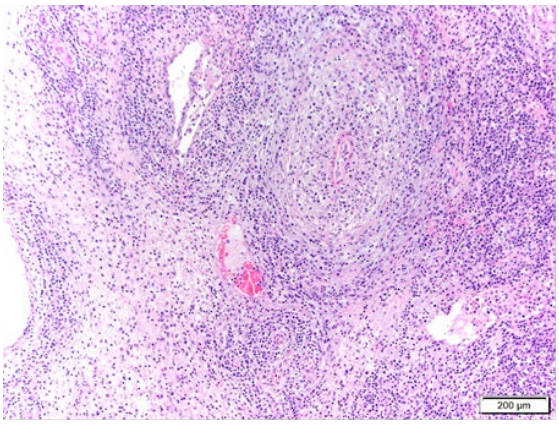

Figure 4-8. Lymph node, equine abortus. The infiltration of arteriolar adventitia by macrophages and lymphocytes is especially profound in the submitted node. (HE, 400X) (*Photo courtesy of* Department of Veterinary Clinical and Diagnostic Sciences within the Faculty of Veterinary Medicine, University of Calgary. https://vet.ucalgary.ca/)

unusually high numbers in areas with the highest prevalence of disease, stating "…the disease always appears a few days after the May flies appear, and is at its worst by the time the May flies disappear."[2]

As noted by the contributor, the life cycle of *N. risticii* is predominantly dependent on trematodes. This infection is thought to be a commensal or mutualistic relationship as infected trematodes do not appear to be negatively affected. In the eastern United States, the maintenance host of *N. risticii* is *Acanthatrium oregonese*. This digenic trematode has a complex life cycle, including miracidia and procytes in snail hosts, free-swimming cercariae, metacercariae in aquatic insects (e.g. caddis and mayflies) and egg laying adults that reside in the intestinal lumen of insectivorous bats. *N. risticii* undergoes vertical transmission with transo-varial passage in trematodes. Follow-ing a rise in water temperatures, infected snails release *N. risticii* infected cercaria into the water, which develop into metacercariae, and subsequently infect second intermediate hosts such as fish, amphibians, birds, reptiles, and mammals. In one study using PCR and sequence analysis in an *N. risticii* endemic

region, the pathogen was detected in immature and adult caddisflies (Trichoptera), mayflies (Ephemeroptera), damselflies (Odonata, Zygoptera), dragonflies (Odonata, Anisoptera), and stone flies (Plecoptera) with an overall prevalence of 31.9%.[2] Following their emergence, mayflies have been reported to travel as far as 8km inland, which may explain infections of animals lacking access to contaminated rivers and lakes.[1]

In 1991, a novel species of *Neorickettsia* also capable of also causing PHF was identified in a horse from Findlay, Ohio and is now known as *N. findlayensis.* In 2017, this entity was identified in two symptomatic horses in Ontario via culture that were PCR negative for *N. risticii.* Therefore, *N. finlayensis* infection may present as a potential confounder when PHF is suspected but PCR testing is negative.[1]

Additional causes of equine abortion discussed by the moderator included numerous bacterial, mycotic, and viral etiologies in addition to toxins, genetic and nutritional disorders, twinning, umbilical cord torsion, and placental separation.

Both the moderator and conference participants believed the histologic lesions in the submitted tissues were non-specific and definitive diagnosis of equine neorickettsiosis without the use of additional diagnostics, such as PCR in this case, would be challenging. Additional differentials discussed associated with similar lesions in fetal tissue include *Neospora* sp., *Toxoplasma gondii*, equine herpesvirus-1, equine arteritis virus, and *N. risticii.* Differentials for similar lesions in adult tissues included *Anaplasma phagocytophilium, Lesimania sp., Trypanosoma sp., Neospora sp.,* equine herpesvirus-1, and *Toxoplasma gondii.*

References:

1. Arroyo LG, Moore A, Bedford S, et al. Potomac horse fever in Ontario: Clinical, geographic, and diagnostic aspects. *Can Vet J*. 2021;62(6):622-628.
2. Baird JD, Arroyo LG. Historical aspects of Potomac horse fever in Ontario (1924-2010). *Can Vet J*. 2013;54(6):565-572.
3. Coffman EA, Abd-Eldaim M, Craig LE. Abortion in a Horse Following Neorickettsia Risticii Infection. *Journal of Veterinary Diagnostic Investigation*. 2008;20**:** 827-830.
4. Cordes DO, Perry BD, Rikihisa Y, Chickering WR. Enterocolitis caused by Ehrlichia sp. in the horse (Potomac horse fever). *Vet Pathol*. 1986;23**:** 471-477.
5. Donahue JM, Williams NM. Emergent Causes of Placentitis and Abortion. *Veterinary Clinics of North America: Equine Practice*. 2000;16**:** 443-456.
6. Giles RC, Donahue JM, Hong CB, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). *J Am Vet Med Assoc*. 1993;203**:** 1170-1175.
7. Greene CE. *Infectious diseases of the dog and cat*. St. Louis, Mo.: St. Louis, Mo. : Elsevier/Saunders 4th ed.; 2012.
8. Hong CB, Donahue JM, Giles RC, et al. Equine Abortion and Stillbirth in Central Kentucky during 1988 and 1989 Foaling Seasons. *Journal of Veterinary Diagnostic Investigation*. 1993;5**:** 560-566.
9. Long MT, Goetz TE, Whiteley HE, Kakoma I, Lock TE. Identification of Ehrlichia Risticii as the Causative Agent of two Equine Abortions Following Natural Maternal Infection. *Journal of Veterinary Diagnostic Investigation*. 1995;7**:** 201-205.
10. Paulino PG, Almosny N, Oliveira R, et al. Detection of Neorickettsia risticii, the agent of Potomac horse fever, in horses from Rio de Janeiro, Brazil [published

correction appears in Sci Rep. 2020 Jul 29;10(1):13001]. *Sci Rep*. 2020;10(1):7208. Published 2020 Apr 29.
11. Pusterla N, Madigan JE, Chae J-S, DeRock E, Johnson E, Pusterla JB. Helminthic Transmission and Isolation of *Ehrlichia risticii*, the Causative Agent of Potomac Horse Fever, by Using Trematode Stages from Freshwater Stream Snails. *Journal of Clinical Microbiology*. 2000;38**:** 1293-1297.
12. Pusterlaa N, Leuteneggera CM, Sigristb B, Chaea J, Lutzb H, Madigana JE: Detection and quantitation of Ehrlichia risticii genomic DNA in infected horses and snails by real-time PCR. 2000
13. Teymournejad O, Lin M, Bekebrede H, et al. Isolation and Molecular Analysis of a Novel Neorickettsia Species That Causes Potomac Horse Fever. *mBio*. 2020;11.
14. Uzal FA, Plattner BL, Hostetter JM. Alimentary System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. Philadelphia, PA: Elsevier; 2016:200.
15. Williams NM: Disorders of Horses, pp. 147-171. Oxford, UK: Wiley-Blackwell, Oxford, UK, 2012