Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 24

27 April 2022

CASE I:

Signalment:
Canine fetus and placenta. 40 days gestation, Dog, Welsh Corgi Pembroke (*Canis lupus familiaris*)

History:
Two fetuses and placenta. Previous litter same stud - 4 pups - normal gestation. No new dogs brought into kennel in past year. Another dam bred to same stud had a litter of 12 with 4 mummified fetuses, a third dam aborted 4 fetuses around day 40 gestation - bred to same stud as the other two females. No other previous history of abortion in kennel. Dam had temperature of 103 °F day after abortion. Physical examination of dam unremarkable. No vaccine history at clinic. Dogs will go next door and scavenge chicken carcasses from time to time.

Gross Pathology:
No significant external lesions were present on the puppies. Puppy A was enclosed in an intact placenta, which had no visible lesions.

Laboratory Results:
Microbiology: *Campylobacter jejuni* 3+ cultured from lung and placenta.

Microscopic Description:
Lesions present in the placenta are very subtle and involve the region of the marginal hematoma and chorioallantois. At the edge of the placental labyrinth at the region of the marginal hematoma there is proteinaceous material that is eosinophilic or hypereosinophilic and clumped. Around or within this are 20 µm diameter circular to oval structures with a defined outer boundary and containing multiple basophilic stippled material (trophoblasts filled with bacteria). Attached to and part of the non-labyrinthine chorion are trophoblasts with a distended cytoplasm and fine basophilic stippling (intratrophoblastic bacteria). In places there are no trophoblasts and there is basophilic stippling of the eroded surface. Neutrophils and or

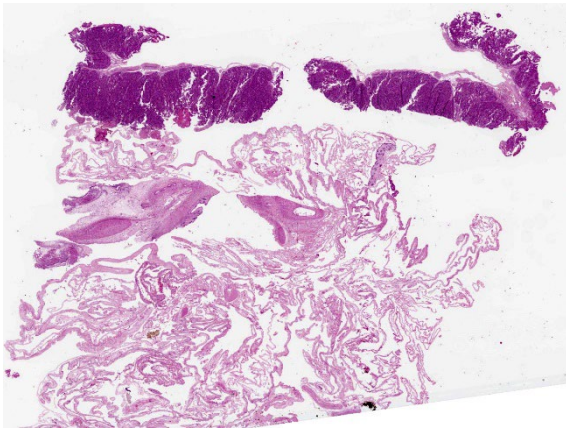

Figure 1-1. Placenta, dog. A section of zonary placenta is submitted with placental labyrinth at top. A clip of fetal eyelid is in the center of the folded membranes. (HE, 6X)

macrophages in the adjacent membrane are generally minimal. Throughout the chorio-allantois, in particular, and between amnion and allantois in the amniotic membrane are histiocytic cells that sometimes form clusters. There are extra cells within the walls of blood vessels or adjacent tissue. Some clumps of bacteria are within small blood vessels.

Contributor's Morphologic Diagnoses:
Intratrophoblastic bacteria of non-labryinthine placenta and marginal hematoma of placenta, and mild fibrinous, necrotic and histiocytic placentitis.

Contributor's Comment:
The lesions of bacterial failure of pregnancy, including abortion, in dogs are generally very subtle. The most important change is the presence of trophoblasts distended by bacteria. These are typically found at the tips of the labyrinthine villi and particularly in the region of the marginal hematoma. Inflammatory changes are usually mild and involve low numbers of neutrophils in the chorion near the distended trophoblasts, and mononuclear cells, particularly histiocytic cells, in other parts of the placenta particularly the allantois/amnion. The lesions in puppies can be variable, with multifocal neutrophilic pneumonia and hepatitis being reported.[1,3,4]

The lesions of *Brucella canis* are well reported in naturally acquired and experimental cases.[2] Even then, intratrophoblastic bacteria are the predominant change with minimal inflammation being present. There are a couple of individual case reports of *Campylobacter jejuni* as a cause of reproductive failure with abortion and perinatal mortality.[1,3,4] These cases may be accompanied by maternal illness including diarrhea, and diarrhea in human contacts. Acquisition of *Campylobacter jejuni* is reported from chicken carcasses or raw food diet, however subclinical persistent carriers do occur. The incubation period is typically short and of the order of several days. Bacteremia and localization in the placenta, phagocytosis by trophoblasts, fibrinous inflammation and then infection results in abortion, stillbirth or perinatal mortality.

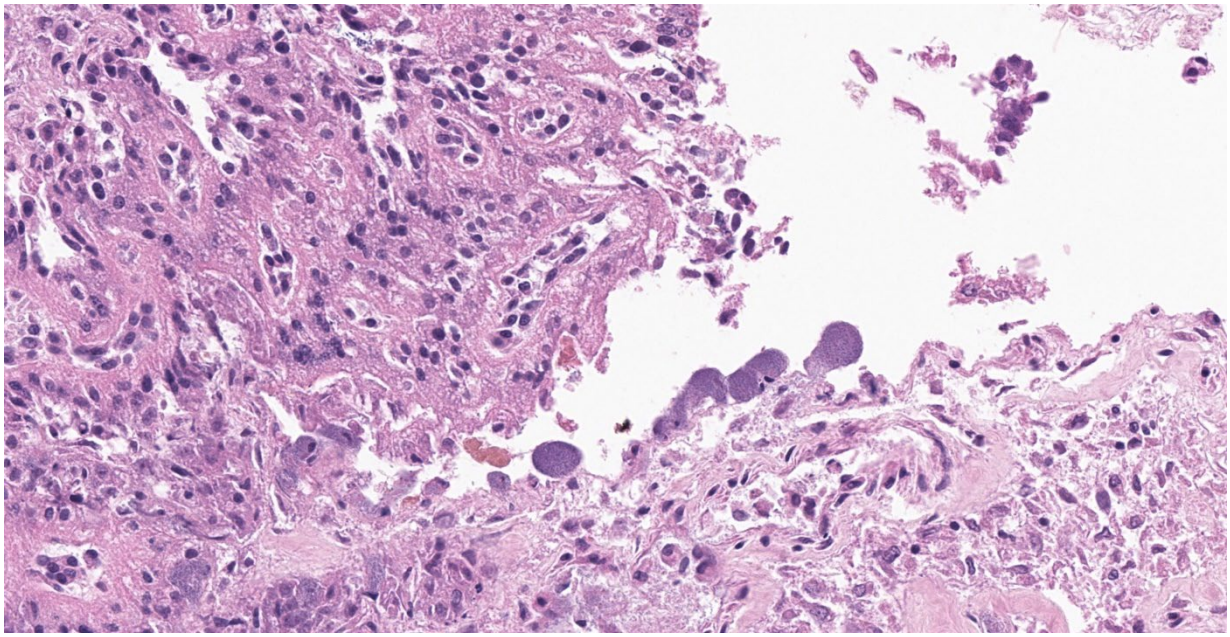

Figure 1-2. Placenta, dog. Trophoblasts lining placental villi immediately adjacent to the marginal sinus are swollen by large numbers of intracytoplasmic bacilli. (HE, 380X)

Contributing Institution:
Department of Pathobiology
Ontario Veterinary College
University of Guelph

JPC Diagnosis:
Placenta, edge of marginal hematoma: Placentitis, necrotizing, multifocal to coalesceing, mild, with intratrophoblastic, intravascular, and extracellular bacilli.

JPC Comment:
The contributor provides a concise overview of the histologic features and pathogenesis of canine abortion due to *Campylobacter jejuni*, an entity more commonly associated with reproductive failure in ruminants. *C. jejuni* is also the leading bacterial etiology associated with human foodborne gastroenteritis in both North America and Europe.[5]

Additional *Campylobacter* sp. associated with ruminant reproductive failure include *C. fetus* subsp. *fetus* and *C. fetus* subsp. *veneralis.* Although closely genetically related, there is significant variability between the two *C. fetus* subspecies in regard to their respective host range and epidemiologic features. *C. fetus* subsp. *veneralis'* distribution is essentially limited to the bovine reproductive tract and is the leading cause of venereally transmitted infectious infertility and embryotic death in this species. Conversely, both *C. fetus* subsp. *fetus* and *C. jejuni* have broad host ranges, often colonize the gastrointestinal tract of cattle, sheep, and goats as commensals, and are associated with abortion in sheep and cattle.[5]

McFadyean and Stockman first reported *Campylobacter*'s association with ovine abortion following its isolation from an aborted sheep fetus in 1906. The genus has since been identified as a worldwide cause of infertility and abortion in ruminants. *C. jejuni* and *C. fetus* subsp. *fetus* are associated with 10.3-25.2% of abortions in sheep, making *Campylobacter* one of the one of the leading causes of ovine abortion. Abortions typically occur during the last trimester and may affect up to 50% of ewes, with the disease colloquially known as epizootic abortion.

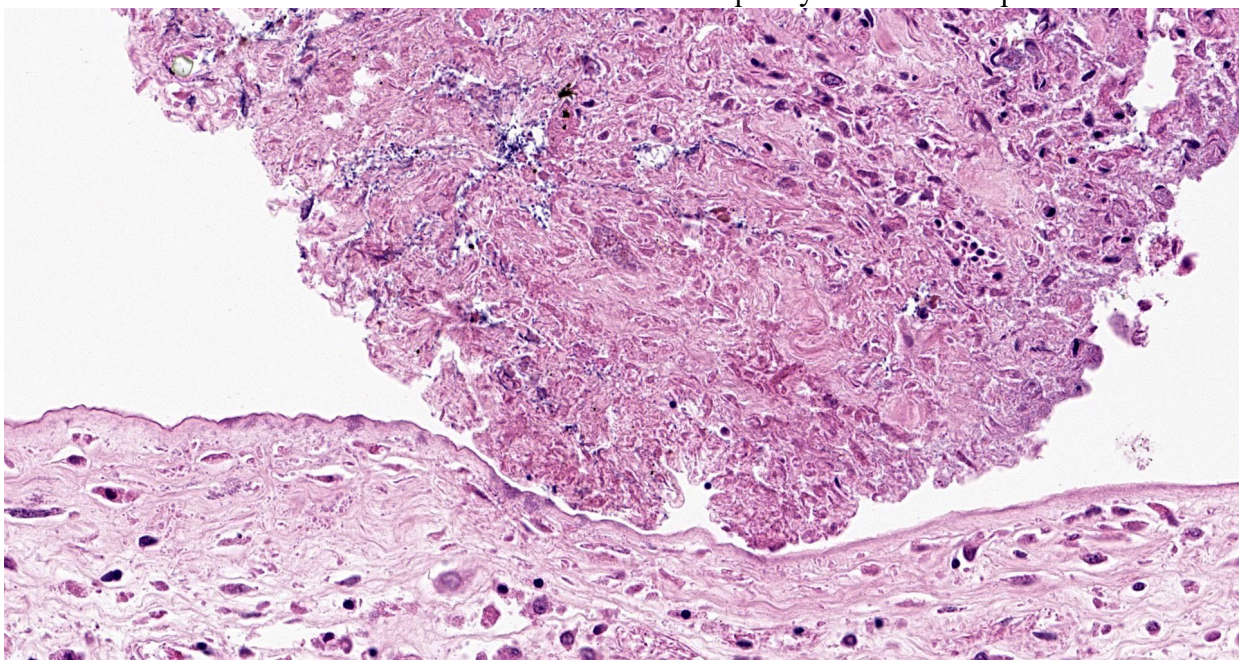

Figure 1-3. Placenta, dog. The marginal sinus contains proteinaceous material and cellular debris as well as free and intratrophoblastic bacilli. (HE, 380X)

Goats and cattle are more sporadically affected by *C. jejuni* and *C. fetus* subsp. *fetus*, with these entities being detected in 0.9-1.8% of aborted caprine fetuses and only 0.19% cattle fetuses were positive for *C. jejuni* during a study of approximately 9,000 aborted fetuses. However, abortion storms have also been reported in both goats and cattle.[5]

Historically, *C. fetus* subsp. *fetus* was the leading species of *Campylobacter* associated with ovine abortion worldwide. However, *C. jejuni* became the leading cause of *Campylobacter* abortion in ewes in the United States during the 2000's and is becoming increasingly common around the world. *C. fetus* subsp. *fetus* continues to be the primary species causing ovine abortions in the United Kingdom and New Zealand, although *C. jejuni* is also a major cause of ovine abortion in these countries as well.[5]

Campylobacter isolates associated with ovine abortion have historically demonstrated a high level of genetic diversity. However, recent studies have revealed the vast majority of ovine abortions in the United States are attributed to a single *C. jejuni* clone since identified as SA for "sheep abortion". A unique feature of this clone is its capability to cause both systemic infections and abortions, features that have been replicated

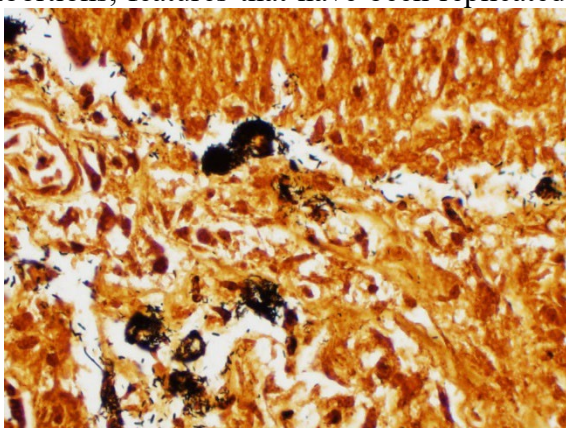

Figure 1-4. Placenta, dog. A silver stain easily demonstrates bacilli within trophoblasts. (WS 4.0, 400X)

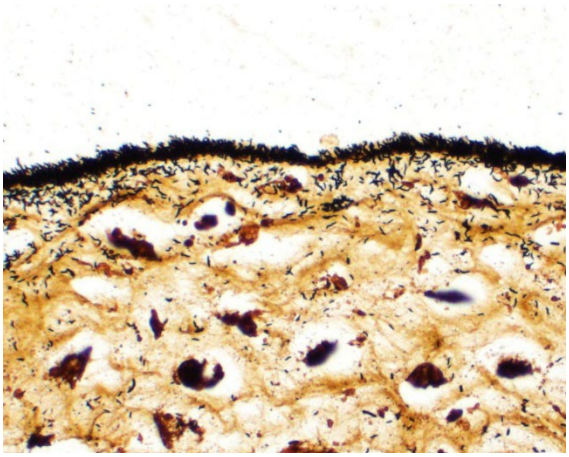

Figure 1-5. Placenta, dog. Numerous bacilli line the chorion in the region of the marginal sinus. (WS 4.0, 400X)

in a well-established laboratory model for *Campylobacter*-induced ovine abortions, the pregnant guinea pig.[5]

Ewes are commonly infected by *C. fetus* subsp. *fetus* and *C. jejuni* via the fecal-oral route as well as from aborted fetuses and fetal membranes. Most flocks are not affected by abortion storms despite *Campylobacter*'s presence on many farms. However, abortion storms are often associated with the introduction of animals from flocks with previous history of abortion into a naïve flock, which may affect up to 70% of ewes. Large numbers of *Campylobacter* are associated with the products of abortion, particularly the placenta and uterine discharge, and the bacteria may be continually shed in vaginal secretions for weeks afterward. Additional ewes often become infected after licking aborted fetuses. Affected flocks often have a history of one or two abortions followed by an abortion storm.[5]

Bulls asymptomatically harbor *C. fetus* subsp. *veneralis* in the epithelial crypts of the penis and prepuce, which is subsequently venerally transmitted cows during breeding. *C. fetus* subsp. *veneralis* infection does not prevent fertilization in infected cows, instead

resulting in endometriosis, salpingitis, and embryonic death most often between 15-80 days gestation, with a delayed return to estrus in up to 50% of cows in a herd. *C. fetus* subsp. *veneralis* may also result in abortions between 4-7 months of gestation in less than 10% of infected cows, likely due to smaller doses of inoculation or slower than typical bacterial replication. In contrast, neither *C. fetus* subsp. *fetus* nor *C. jejuni* are transmitted venerally by rams nor do they result in an ascending infection of the reproductive tract.

Many animals carry *C. fetus* subsp. *fetus* and *C. jejuni* in the intestinal tracts and gall bladder but do not demonstrate clinical disease. However, when animals carrying these *Campylobacter* sp. become immuno-compromised (such as pregnant ewes), the bacteria undergo translocation with systemic distribution and subsequent colonization of the uterus, resulting in placentitis, fetal infection, and abortion, most often during the final trimester.[5]

Macroscopic lesions of the placenta are often apparent but subtle, with intercotyledonary thickening, edema, congestion, and opacity while the cotyledons often have a superficial brown-red exudate. Gross findings in the fetus may include serosanguinous fluid within the body cavities with strands or mats of fibrin and there may be hepatic rupture with hemoabdomen. In addition, a very characteristic (although not pathognomonic) gross lesion associated with *Campylobacter* in sheep is a liver with multifocal, pale, circular to targetoid foci measuring up to 4cm in diameter. However, this lesion is only present in approximately 25% of cases and is also associated with *Flexispira rappini*, another commensal of the ovine gastro-intestinal tract associated with sporadic ovine abortion.[5]

The most common histologic lesions associated with *Campylobacter* abortion in sheep is placentitis (100%) which is often necrosuppurative and associated with placental vasculitis. As in this case, the most characteristic features are the presence of bacteria within trophoblasts, adjacent stroma, and within vascular channel remnants. Additional common findings include fetal pneumonia (92%), gastroenteritis (54%), serositis (41%), hepatitis (35%), and encephalitis (8%).[5]

The gold standard for diagnosis of *Campylobacter* abortion in ruminants is culture in conjunction with the previously described gross and histologic lesions. Optimal locations of culture collection include placenta and fetal stomach contents, followed by fetal lung, and fetal liver.[5]

In regard to canines, the first case report of *Campylobacter*-associated abortion origin-nated from the northwestern United States in 1984, with the second case report emerging a decade later in South Africa, both of which described late term abortions. Although the pathogenesis *C. jejuni*-induced abortion in canines is unclear, it is suspected to closely mirror the process previously described in sheep since both species are asymptomatic carriers of the pathogen and are affected by late term abortion.[4]

Based on the moderator's experience, *Campylobacter* sp. infection often presents with two unique histologic features in both canine and ruminant placenta; 1) the superficial aspect of the chorioallantois (most commonly the allantois in ruminants) has a "fuzzy" appearance due to myriad bacteria adhered to the superficial aspect; and 2) bacterial emboli within blood vessels. These findings are in addition to the presence of intratrophoblastic bacteria; however, this feature is also associated with other bacterial

etiologies known to cause canine abortion, including (but not limited to) *Brucella canis, Listeria monocytogenes,* and *Salmonella* spp. Feline abortion may also occur as the result of infection from the same etiologies, with the exception of *Brucella canis.* In addition, the moderator discussed felines being implicated as a source of zoonotic *Coxiella burnetii* infections, with the organism being isolated from the placenta from feline abortions.

The moderator discussed common features of bacterial canine placentitis. Inflammation is most often present in the non-zonary chorioallantois but is almost always mild and subtle, even in the face of dramatic infection. Key regions to evaluate for infected trophoblasts include those adjacent to the marginal hematoma and the surface of the zonary placenta.

Finally, participants noted the presence of haired skin within the submitted section of placenta. The moderator noted that since conjunctival lesions can be an indication of amnionitis, some institutions include fetal eyelids during regular tissue collection.

References:

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CASE II:

Signalment:
~1 day old neonatal calf; no sex provided; Angus-cross; bovine (*Bos taurus*)

History:
A rancher found a dead neonatal calf. It was the seventh neonatal death that year among 108 calves. The reported vaccination history was that dams received three products. These were a modified live polyvalent product containing BoHV-1, BRSV, BVDV and parainfluenza 3 virus; *Campylobacter fetus* bacterin; and a polyvalent inactivated product containing bovine rotavirus, bovine coronavirus, *Clostridium perfringens* type C, and *Escherichia coli.* No information was available about recent illness in the dam cohort.

Gross Pathology:
A veterinary practitioner did the necropsy. The laboratory received fresh and fixed tissues. No placental tissue was submitted.

Laboratory Results:
<10 ppm nitrate in aqueous humor (strip test)(within reference range); light growth of *E. coli* and β-hemolytic *Staphylococcus* sp. (lung; liver; small intestine; large intestine)(aerobic culture); *Ureaplasma* sp. detected (lung)(PCR); *Clostridium perfringens* type A, small intestine (anaerobic culture/PCR).

Contributor's Morphologic Diagnoses:
Lung: Interstitial pneumonia, mild, diffuse, with lymphoid hyperplasia of bronchus- and bronchiolar-associated lymphoid aggregates (BALT).

Contributor's Comment:
Other features in the submitted tissue are multifocal intra-alveolar hemorrhage, fibrin exudation in BALT, and histiocytic alveolitis. The detection by PCR of *Ureaplasma diversum* is significant, given the character of the pulmonary changes. Our laboratory did not receive some of the other tissues that help support a diagnosis of ureaplasmosis. Non-pulmonary lesions can include chorioamnionitis, erosive conjunctivitis with prominent goblet cells, and erosive arthritis.[4,7-9] Gross pulmonary lesions in bovine fetuses that are aborted due to ureaplasmosis are subtle or absent (firm, poorly aerated), and rarely recognized at necropsy. Most laboratory-diagnosed cases of abortion due to *U. diversum* occur in the third trimester. Some calves, as in this case, go to term and die shortly thereafter. Abortions can occur earlier in gestation. *U. diversum* also causes embryonic loss, although its importance is unclear.[9]

Our bacteriology laboratory tests lungs of aborted bovine fetuses for *U. diversum* by PCR whenever lesions suggestive of ureaplasmosis are found. Hallmark features are diffuse interstitial pneumonia and BALT hyperplasia, often in a minimally autolytic fetal carcass. Most such cases test positive by PCR. Placental and fetal pulmonary lesions have been reproduced experimentally following intra-amniotic challenge of cattle.[7] Amnionic lesions comprising multifocal necrosis with fibrosis and hemorrhage are said to be characteristic. According to one experienced diagnostic pathologist, *U. diversum* abortion in cattle is infrequent in the United States.[2] It is apparently more common in eastern and western Canada. Published surveys of bovine reproductive wastage found that ureaplasmosis accounted for 2.8% and 4.3% of losses.[3] Slaughterhouse surveys indicate that *U. diversum* is rarely found in fetuses and placentas from healthy cattle.

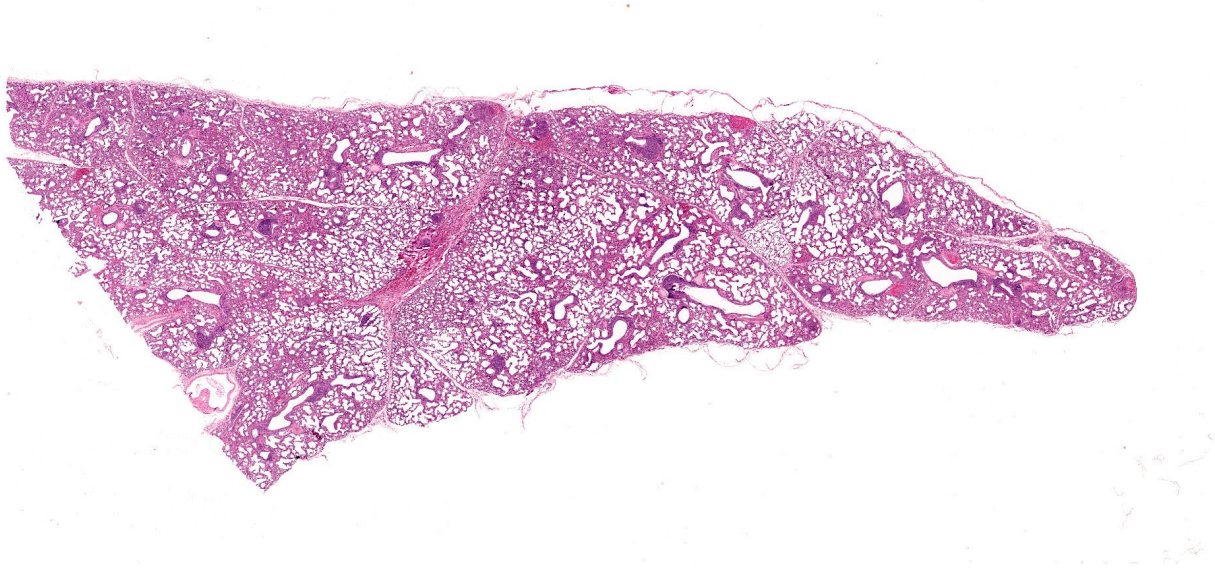

Figure 2-1. Lung, calf. There is diffuse expansion of the alveolar interstitium (interstitial pneumonia) affecting all lobules (HE, 6X)

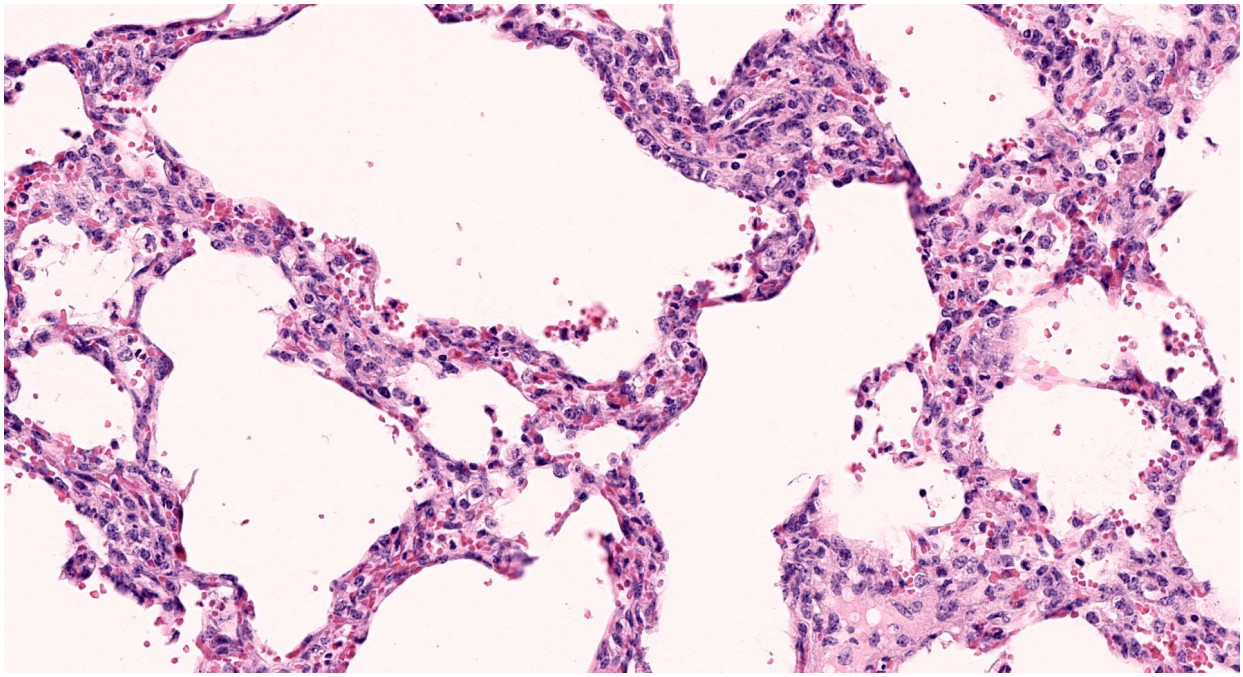

Figure 2-2. Lung, calf. Alveolar interstitium in multifocally and markedly expanded by variable numbers of macrophage, neutrophils, fibrin, edema and cellular debris. There is segmental type II pneumocyte hyperplasia. (HE, 300X)

In the past, laboratory diagnosis of *U. diversum* infections relied on culture. Successful isolation necessitated rapid transport of samples to the laboratory, specialized media, and expertise in ureaplasma culture and identification.[8] This is now largely suplanted by PCR using various published molecular protocols.[14] Controversy and uncertainty attend many aspects of ureaplasmosis in cattle due to the occurrence of multiple serotypes, the suspected existence of pathogenic and non-pathogenic strains, and failure of some experimental infections to induce disease.[1]

Ureaplasma are members of the family *Mycoplasmataceae,* order *Mycoplasmatales.* They are so named because they possess urease activity.[5] In the past they were called T-mycoplasmas, and presumed to be non-pathogenic. *U. diversum* is a common commensal in the reproductive tract of male and female cattle. It also colonizes nasal passages. *U. diversum* is considered one cause of bovine granular vulvitis syndrome. Other, less common conditions in cattle are endometritis, salpingitis, bovine seminal vesiculitis, and infertility. *U. diversum* is a common contaminant of bull semen, where it survives freezing for artificial insemination. Abortions due to *Mycoplasma* and *Ureaplasma* infection are sporadic with few accounts of herd outbreaks.[14] Based on experimental studies using caesarean-delivered, colostrum-deprived calves, *U. diversum* is also a low-grade pulmonary pathogen. It induces atelectasis, mild interstitial pneumonia, peribronchiolar lymphoid cuffs, and BALT hyperplasia.[11]

On a comparative note, related agents *U. urealyticum* and *U. parvum* are isolated from human amniotic fluid and placentas where they are associated with adverse pregnancy outcomes. These include preterm births, neonatal death, chorioamnionitis, low birth weight, pneumonia, and chronic lung disease.[12,13]

Contributing Institution:
Wyoming State Veterinary Laboratory; 1174 Snowy Range Road; Laramie; Wyoming 82070. http://www.uwyo.edu/wyovet/

JPC Diagnosis:
Lung: Pneumonia, interstitial, histiocytic, moderate, diffuse, with marked BALT hyperplasia.

JPC Comment:
The contributor provides a concise review of ureaplasmosis, an infrequent cause of reproductive failure in cattle associated with the key histologic features of interstitial pneumonia and BALT hyperplasia.

Ureaplasma is one of several genera within *Mollicutes*, a class composed wall-less bacteria from which their name is derived (mollis and cutis; Latin for "soft" and "skin", respectively). Composed of 14 genera and approximately 200 species, *Mollicutes* are considered to be the smallest self-replicating free-living organisms and are widely found in plants, animals, and humans. *Mollicutes* evolved from a Gram-positive precursor though a process known as genomic reduction, resulting in the loss of non-essential genes, such as those encoding the peptidoglycan cell wall and variable metabolic functions. Some *Mollicutes* are able metabolize carbohydrates (i.e. glucose fermenters) or amino acids such as L-arginine for energy via the arginine dehydrolase pathway. However, these pathways are defunct in other *Mollicutes* such as *Ureaplasma spp*. Thus *Mollicutes* metabolism is often tailored to specific microenvironments, which explains the narrow host ranges and niches of many species. *Mollicutes* that inhabit animals as commensals, saprophytes, or pathogens fall into three genera: *Mycoplasma, Ureaplasma,* and *Acholeplasma.*[10]

Interestingly, ATP production in *Ureaplasma* spp. occurs via an alternative metabolic pathway dependent on the hydrolysis of urea into ammonia via urease. This results in an intracellular accumulation of ammonia/ ammonium and the creation of an electrochemical gradient utilized to synth-

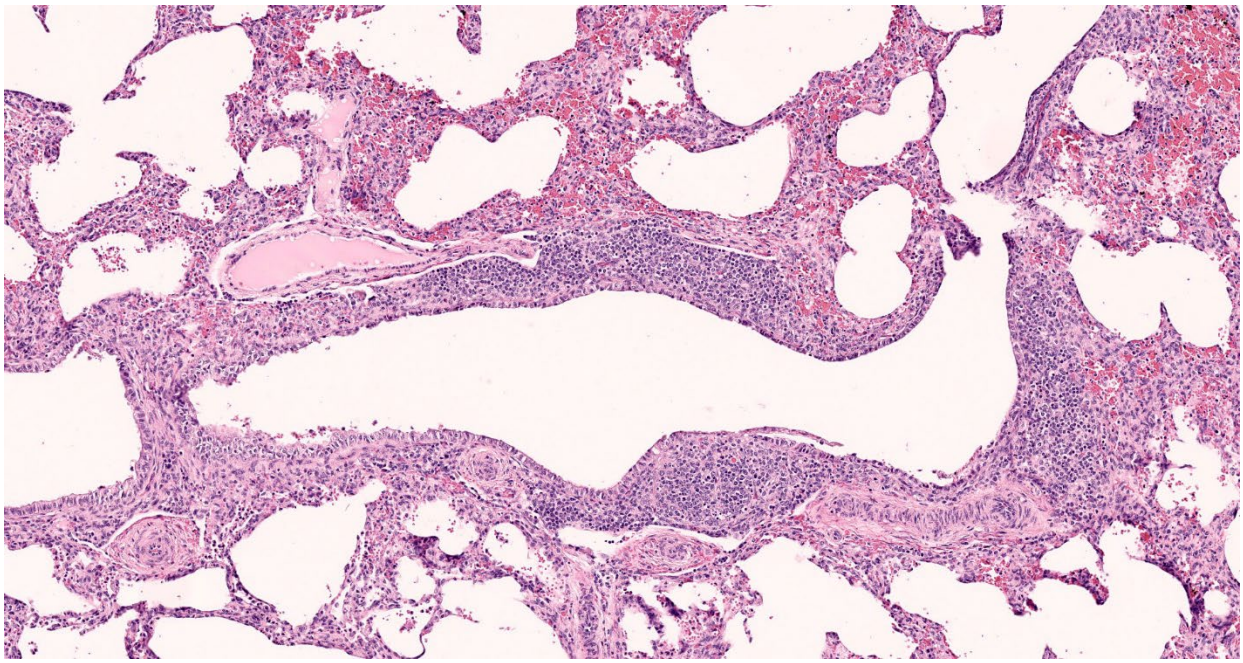

Figure 2-3. Lung, calf. There is marked BALT hyperplasia. (HE, 99X)

esize ATP via the combined actions of an ammonia transporter and a FOF_1-ATPase, both of which are bound to the trilaminated cell membrane. In addition, ureaplasmas also generate ammonia via degradation of L-histidine through the action of L-histidine ammonialyase. Regardless of its initial source, the released ammonia results in local irritation to mucous membranes in the infected urogenital and respiratory tract.[10] In addition to the local production of ammonia, other virulence factors of *Ureaplasma* spp. include the ability to adhere to and invade host cells, a range of lipid-associated membrane protein (LAMP) compounds, and the modulation of both prostaglandin synthesis and apoptosis.[10]

Endometrial cells infected with *Ureaplasma* spp. exhibit a significant reduction in the synthesis of prostaglandins E2 and F2a from arachidonic acid. This phenomenon is mediated by *Ureaplasma* spp. membrane phospholipases, such as phospholipase D. Given prostaglandins play a significant role in bovine embryo implantation and pregnancy maintenance, this modulation likely plays a major role in *U. diversum*'s association with premature bovine birth.[10]

Numerous LAMPs are encoded by the bovine ureaplasma genome, which are a mixture of cell-surface proteins and lipoproteins that interact with host cells and account for a significant amount of the cell's mass. LAMPs facilitate several key processes, including cellular adhesion and invasion, immunomodulation, inhibition or activation of apoptosis, and are the major pathogen associated molecular patterns associated with mollicute species. *U. diversum* undergoes internalization as quickly as one minute following its attachment to a target cell, which is soon followed by perinuclear localization. This process plays a key role in *Ureaplasma*'s survival in immunocompetent hosts, as the intracellular environment provides the organism with both nutrients and shelter from antimicrobials that do not penetrate the host cell. This survival mechanism coincides with *Ureaplasma*'s ability to inhibit apoptosis of infected cells.[10]

Ureaplasma diversum is capable of colonizing fetal lung in utero as well as newborn lung via endobronchial inoculation. As evident in this case, fetal pulmonary pathology is typically associated with lymphocytic infiltrates (i.e. BALT hyper-plasia) and is often accompanied by conjunctivitis. In most cases the fetus is relatively fresh while the placenta is often retained and inflamed.[10]

In addition to rare cases of mycotic infections, *U. diversum* is one of very few etiologies associated amnionitis, which macroscopically manifests as marked thickened of the amnion. In contrast, *U. diversum* does not cause lesions in the chorioallantois. Optimal tissue specimens for confirmatory testing include placenta, fetal lung and fetal abomasal fluid.[10]

Interestingly, *Ureaplasma diversum* has recently been isolated from swine with pneumonia. Isolates were only obtained from sick animals, which in contrast to cattle which often have asymptomatic infections of the respiratory tract. An additional study found *U. diversum* was only detectable in the lung tissue of affected swine, while also discovering that coinfection with *Myco-plasma* spp. such as *M. hyopneumonia* and *M. hyorhinus* to be common. Additional research is needed in regard to determining if *U. diversum* is simply an opportunistic pathogen or is a component of the Swine Respiratory Disease complex.[10]

Although not seen in this case, the moderator also discussed the presence of keratinocytes

within neonatal airways as an indication of fetal distress.

References:

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CASE III:

Signalment:
12-year-old female Dutch Warmblood horse (*Equus caballus*)

History:
The mare presented in late pregnancy with a preliminarily diagnosed of nocardioform placentitis and was treated with antibiotics, non-steroid anti-inflammatory drugs, and altrenogest for two weeks. She foaled on day 325 of gestation and fetal membranes were expelled 15 minutes post-partum.

Gross Pathology:
The chorionic surface had a large avillous region, extending from the base of the pregnant horn to the cervical star. Approximately 50% of the entire chorionic surface was coated by tan-brown, opaque, viscous exudate extending to the cervical

star. The amnion had diffuse mild opacity and edema. The chorioallantois weighed 3.8kg and the umbilical cord plus allantoamnion weighed 2.5kg. The total length of the umbilical cord is 80.5cm; with the total length of the amniotic segment being 45.4cm and the total length of the allantoic segment being 35.1cm.

Laboratory Results:
Aerobic culture of fresh chorioallantosis yielded moderate growth of bacteria in the *Mycobacterium smegmatis* group. 16S PCR sequencing of this isolate identified *Mycobacterium goodii*.

Microscopic Description:
Chorioallantois (placenta): Subgrossly, large mesoderm vessels in the chorionic plate have hyperplastic smooth muscle and are congested. The chorion has progressive loss, thickening, fusion and de-arborization of microcotyledon villous projections and concurrent thickening of the trophoblastic epithelium.

Vasculature of chorionic villi have obscured endothelium with severe marginization and perivascular cuffing by large numbers of neutrophils. The thickened sections of chorionic epithelium are composed of multilayered ballooned trophoblasts and syntrophoblasts packed with intracytoplasmic gram-positive, variably acid-fast positive, beaded bacilli. The chorionic surface has variable amounts of detached amalgamated degenerate trophoblasts with similar intracellular organisms. Chorionic connective tissues contain low numbers of infiltrating plasma cells, macrophages and neutrophils. Allantoic epithelium is multifocally eroded.

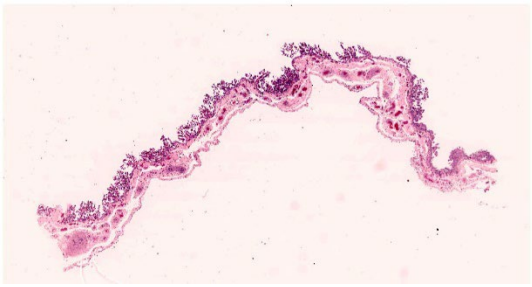

Figure 3-1. Placenta, horse. There are multifocal areas of villar blunting and loss. (HE, 5X)

Contributor's Morphologic Diagnoses:
Chorioplacentitis, degenerative, neutrophilic to lymphoplasmacytic, regionally extensive, chronic, severe, with intralesional intratrophoblastic, acid-fast bacilli, chorioallantois.

Contributor's Comment:
The focal distribution of equine nocardioform placentitis usually infects the chorionic surface of the body or horns sparing the cervical star. In this case, brown viscous exudate coating the chorionic lesion was characteristic of that observed in nocardioform placentitis that has been attributed to *Crossiella equi*, *Amycolatopsis*, and *Streptomyces spp.* and others.[3,4,8,13] Histologically, strongly acid-fast positive (Fite's method) intratrophoblastic bacilli were continuous with the body of the chorioallantois but had highest density in the cervical star, suggesting an ascending infection. Aerobic culture yielded moderate growth of bacteria in the *Mycobacterium smegmatis*-related group and 16S sequencing more specifically identified *Mycobacterium goodii* as the etiology.

In horses, mycobacterial placentitis has been attributed to various subspecies of *Mycobacterium avium*, specifically *hominissuis* and those in Runyon group IV.[5,7] *Mycobacterium smegmatis* has been implicated in experimental induction of mastitis and granulomatous mastitis in sheep and dairy cattle, systemic disease in immunocompromised canids and peritonitis in goldfish.[13] *M. smegmatis* has also caused nosocomial infections associated with post-surgical breast implants and osteomyelitis,

lymphadenitis, cellulitis, and aspiration pneumonia in humans.[1,4]

Taxonomic studies of *Mycobacterium smegmatis* have elucidated three subgroups with emerging clinical significance, one being *Mycobacterium goodii*.[1,4] This subgroup has been reported, in human medical literature, to have been cultured and implicated in the development of post-traumatic and/or post-surgical wound infections following surgical implantation of medical devices.[3,4,9] To the knowledge of the contributor, this is the first case of mycobacterial placentitis in an equid attributed to *Mycobacterium goodii*.

Contributing Institution:
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https://cdpm.vetmed.ufl.edu/

JPC Diagnosis:
Placenta, chorioallantois: Placentitis, necrotizing and neutrophilic, multifocal to coalescing, moderate, with numerous intratrophoblastic and extracellular bacilli.

JPC Comment:

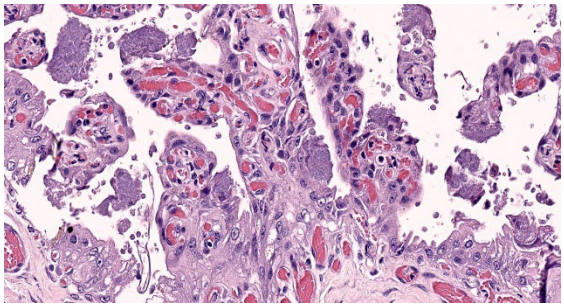

Figure 3-2. Placenta, horse. Multifocally, trophoblasts lining placental villi are swollen by an accumulation of numerous intracytoplasmic bacilli. There is multifocal rupture of affected trophoblasts, liberating bacilli into the intervillar space. (HE, 380X)

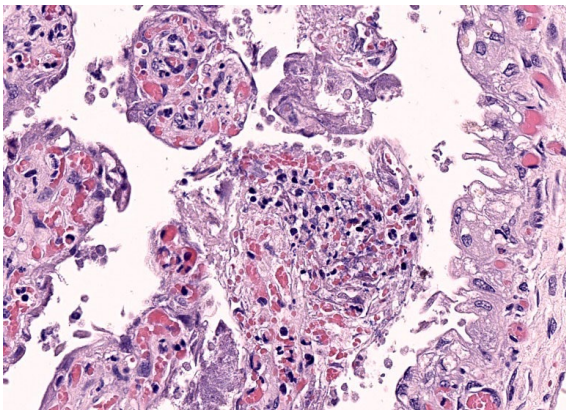

Figure 3-3. Placenta, horse. In areas of trophoblast infection and necrosis, there is infiltration of the fibrovascular core of the villi by neutrophils admixed with edema and necrosis. (HE, 380X)

The contributor provides a concise overview of etiologies associated with nocardioform placentitis and the emergence of *Mycobacteria goodii* as not only a cause of nosocomial infections in humans but also a cause of mycobacterial placentitis in mares. *Mycobacterium goodii* is a Gram-positive acid fast bacillus classified as a nontuberculous mycobacterium (NTM) that exhibits rapid growth (2-4 days) on most media, including blood, chocolate, trypticase soy, Middlebrook 7H10 or 7H11, and Lowenstein-Jensen agars; however, gene sequencing is required for differentiation from other rapidly growing NTM.[12] In addition to *M. goodii*, other rapidly growing mycobacterial species include those in the *M. smegmatis, M. fortuitum,* and *M. chelonae-abscessus* groups as well as other species such as *M. phlei* and *M. thermoresistible.* Rapidly growing mycobacterial species are ubiquitous in the environment and can be found in soil, dust, and drinking water.[2, 11] As noted by the contributor, *M. goodii* is closely related to *M. smegmatis,* an agent commonly associated with nosocomial infections.[12]

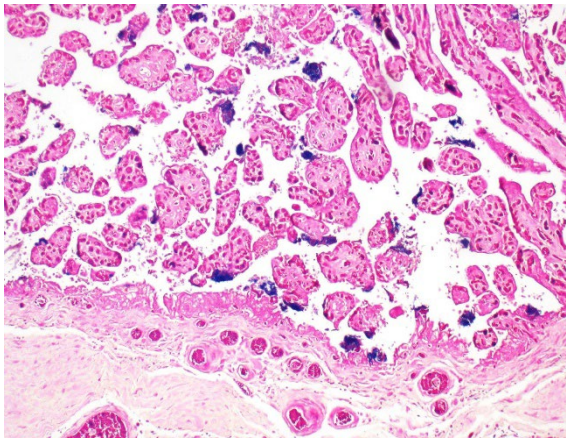

Figure 3-4. Placenta, horse. A tissue gram stain demonstrates intratrophoblastic gram-positive bacilli. (HE, 380X). (Brown-Brenn, 400X) (Photo courtesy of: University of Florida, College of Veterinary Medicine Department of Comparative, Diagnostic, and Population Medicine https://cdpm.vetmed.ufl.edu/)

In 1999, Brown et al. reclassified *M. smegmatis* into three species (*M. smegmatis sensu stricto, M. wolinskyi,* and *M. goodii)* based on genetic sequence and antimicrobial resistance. Each species may be identified with up 90% accuracy based on its sensitivity to tobramycin, with M. *smegmatis sensu stricto* being most susceptible, followed by *M. goodii* with intermediate susceptibility, and *M. wolinskyi* being resistant.[2]

As previously noted, *M. goodii* is commonly associated with nosocomial infections in human healthcare settings following invasive procedures. Implantations such as prosthetic joints and cardiac pocket devices such as pacemakers and automated implantable cardiac defibrillators are most commonly affected.[12]

Treatment typically entails a complex combination of implant removal, surgical debridement, and prolonged appropriate antibiotic therapy (up to 12 months). Many cases are initially treated empirically with clarithromycin and rifampin, both of which *M. goodii* is inherently resistant due to its overexpression of the *Wag31* gene and presence of the *erm* gene. *Wag31* reduces *M. goodii*'s permeability to lipophilic medications such as rifampin by increasing the thickness of the peptidoglycan layer while the *erm* gene alters the ribosomal binding site targeted by macrolides such as clarithromycin. The most effective medications for the treatment of *M. goodii* in humans are sulfamethoxazole /trimethroprim and ethambutal. However, allergies and renal toxicity often limit the use of sulfamethoxazole/trimethroprim, necessi-tating the use of alternatives such as doxycycline and ciprofloxacin or a combination of amikacin and meropenem for more serious infections.[12]

Infection caused by rapidly growing mycobacterial species have also been reported in cats and less commonly in dogs. These infections most commonly result in panniculitis and are often preceded by some form of trauma, such as bite wounds, penetrating foreign bodies, or surgical manipulation. These lesions vary from single to multiple, firm, often painless, subcutaneous nodules with multiple draining tracts to subcutaneous abscesses. Dogs are often affected in regions associated with bite wounds or subcutaneous injections, such as the dorsum, flank, dorsal neck, and shoulder,

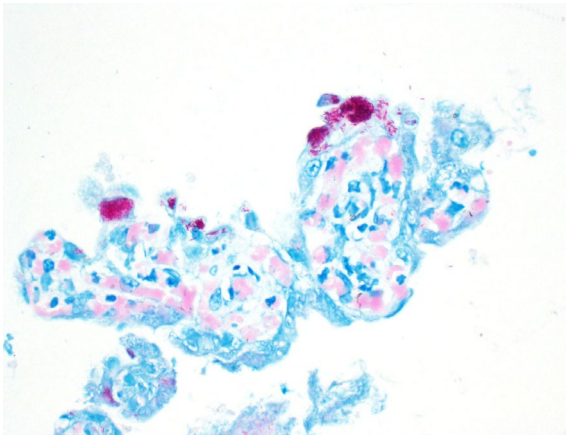

Figure 3-5. Placenta, horse. A Fite-Faraco acid-fast stain demonstrates intratrophoblastic acid-fast bacilli. (Fite-Faraco, 400X)

in addition to surgical or intravenous catheterization sites. The inguinal region is most commonly affected location in cats.[2]

In mares, placentitis is a common cause of pregnancy loss, which occurs due to inhibition of nutrient and fetal waste transfer across the placenta. As previously noted by the contributor, some cases equine placentitis may be categorized as "nocardioform" or "mucoid", implying a nonascending placentitis. In these cases, the cervical star region of the chorioallantois is spared while bacterial foci are distributed to the body and/or horns of the chorioallantois. In contrast, ascending placentitis occurs as the result of pathogens crossing the cervical barrier, classically resulting in inflammation, thickening, and separation of the chorioallantois at the region on the cervical os (i.e. cervical star). *Sreptococcus equi* subsp. *zooepidemicus* is the most common bacterial isolate associated with ascending placentitis, followed by *Escherichia coli, Pesudomonas* spp., and *Klebsiella* spp. As previously discussed by the contributor, etiologies associated wtih nocardioform placentitis include common soil-borne bacteria such as *Crossiella equi*, *Amycolatopsis* sp., *Streptomyces sp.,* and *Cellulosisimicrobium cellulans.*[6]

A similar case of an equine abortion is reported in a 2014 report by Johnson et al.[6], which describes a case of mycobacterial placentitis with intratrophoblastic acid-fast bacteria in the cervical star chorion without villous necrosis, inflammation, or hyper-plasia of trophoblasts. In the case of the 2014 report, culture revealed a pure growth of *Mycobacterium* Runyon IV, which are comprised of fast-growing, saprophytic, acid fast, nontuberculous bacilli, which encompasses all mycobacteria outside the *Mycobacterium tuberculosis* complex, including *M. goodii*. Sequence analysis

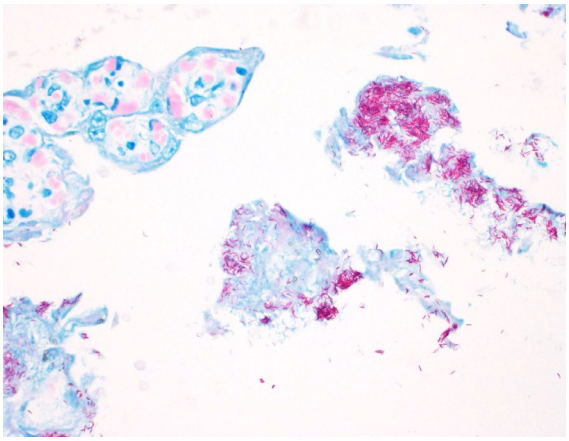

Figure 3-6. Placenta, horse. A Fite-Faraco acid-fast stain demonstrates the filamentous nature of the acid-fast bacilli within debris in the intervillar space. (Fite-Faraco, 400X)

using the 16S ribosomal RNA gene and the *rpoβ* gene found the isolate contained a 5% difference in the *rpoβ* gene compared to known species at the time, indicating a novel species. Five similar cases of mycobacterial abortion reported in Kentucky thoroughbred mares between 2002 and 2006 were also attributed to an unknown species of *Mycobacterium.*[6] Considering the present case is the first identified case of *M. goodii* placentitis, a retrospective analysis of tissue samples from the previously described cases may be warranted.

Although *Mycobacteria* spp. are rarely associated with placentitis and abortion, mycobacteriosis should be considered in cases where more common bacteria are not isolated. Furthermore, use of acid-fast stains should be considered in cases of equine placentas exhibiting atypical placentitis lesions.[6]

Conference participants discussed the absence of microcotyledons at the lateral aspect of the tissue section and its significance. Although the exact location of placenta was not specified, the absence of microcotyledons indicates the tissue section most likely includes a portion of the cervical

star. As noted in the contributor's comment, thickening of the cervical star is consistent with an ascending infection.

Finally, the moderator and the majority of attendees were of the opinion that this case is inconsistent with the strict definition of nocardioform placentitis. Although the presence of brown exudate is a feature of nocardioform placentitis, the hyperplastic chorioallantois in the region interpreted to be the cervical star is more consistent with an ascending infection, and this agent is not considered a significant player in nocardioform placentitides.

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CASE IV:

Signalment:
9 year old, Male, Canine; Miniature Schnauzer (*Canis lupus familiaris*)

History:
Presumed acute abdomen. Chronic cryptorchid. Some feminization. Two abdominal masses that communicated were resected. One was at the base of the right kidney. The second seemed to be a prostatic cyst, markedly distended with exudate.

Gross Pathology:
None submitted.

Laboratory Results:
None submitted.

Histopathological Description:
Testis:
Blood vessels in the pampiniform plexus are moderately dilated. The testis is widely effaced by a highly cellular, nonencapsulated, multilobulated neoplasm composed of polyhedral cells arranged in tubules, islands, and cords separated and surrounded by dense bands of fibrous connective tissue. The neoplastic cells have moderate amounts of pale eosinophilic, variably foamy (lipid droplets) cytoplasm with indistinct cell margins and have a round to oval nucleus with coarse chromatin and up to two distinct nucleoli. The neoplastic cells occasionally palisade along the basement membrane of the tubules. Cellular and nuclear pleomorphism are mild. Mitotic figures are rare.

Uterus:
Adjacent to the testis, partially within and expanding the tunica albuginea, possibly into the tunica vaginalis, and also representing the separate tissue specimen, there is a

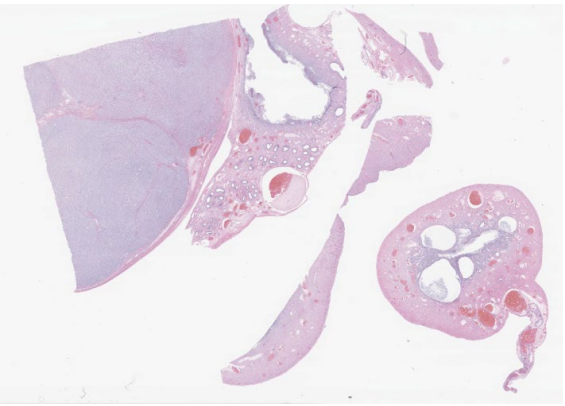

Figure 4-1. Gonad and adjacent tissue, dog. A section of testis and multiple sections of uterus are submitted from a dog (not two dogs, just one dog, and that's a problem in itself!) (HE, 5X)

smooth muscle lined tubular structure with a lumen lined by cuboidal to low columnar epithelial cells and with glandular structures within submucosal stroma, consistent with uterus. Within the lumen, infiltrating the stroma, and within multifocal variably dilated glands, there are neutrophils, plasma cells, lymphocytes, and foamy pale eosinophilic fibrillar material. There are areas of squamous metaplasia and erosion.

Contributor's Morphologic Diagnoses:
Testis: Sertoli cell tumor
Uterus: Uterus with pyometra/endometritis

Condition: Persistent Müllerian Duct Syndrome

Contributor's Comment:
The most common testicular tumors in dogs are: seminomas, interstitial cell tumors (Leydig cell tumors), Sertoli cell tumors, and mixed germ cell-stromal tumors. Cryptorchidism is associated with the development of seminomas, Sertoli cell tumors, and mixed germ cell-stromal tumors.[1,2] Sertoli cell tumors most commonly occur in the right testis[1] which is also more commonly cryptorchid than the left testis. The most common clinical signs/lesions associated with Sertoli cell tumors are gynecomastia, behavior changes,

alopecia, and bone marrow suppression.[1,6] Sertoli cell tumors can be hormonally active and secrete hormones including Estrodiol 17B[6] but not all patients with signs of feminization have elevated estrogen levels.[1] The most common age at presentation is 10 years old, however, patients with tumors in a cryptorchid testis may present at a younger age.[6] Grossly, Sertoli cell tumors are firm (due to abundant fibrous connective tissue within the mass), demarcated, variably nodular masses that can grow to very large size.[1] The majority of Sertoli cell tumors are benign neoplasms[1] but malignant forms can occur and may be more common in a retained testis. Histologically, intratubular and diffuse forms occur.[1] Miniature Schnauzer dogs a predisposed to Persistent Müllerian Duct Syndrome presenting with cryptorchidism (approximately 50% cases)[5], Sertoli Cell Tumor, and pyometra[1], as seen in this case.

Sexual differentiation in dogs occurs in 3 consecutive events- chromosomal sex determination at fertilization, gonodal sex development (in utero), and phenotypic sexual determination.[3,9] Testicular development in males requires Müllerian Inhibiting Substance (MIS) resulting in Müllerian duct regression and testosterone resulting in development of the vas deferens and epididymides.[3] Persistent Müllerian duct syndrome (PMDS) is considered a

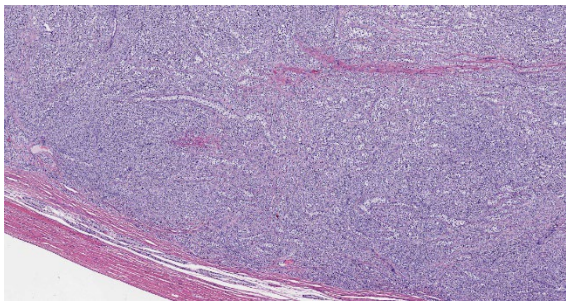

Figure 4-2. Testis, dog. 95% of the testis is effaced by a densely cellular neoplasm often arranged in tubules. Several compressed atrophic seminiferous tubules are present at lower right. (HE, 40X)

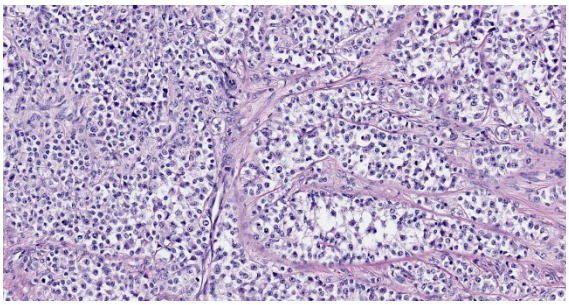

Figure 4-3. Testis, dog. Neopalstic Sertoli calls are arranged in sheets (left) and tubules (right). (HE, 232X)

phenotypic sex abnormality or disorder of sexual development (DSD) and is also a type of male pseudohermaphroditism.[9] Persistent Müllerian Duct Syndrome occurs in Miniature Schnauzers[1] and other less commonly reported dog breeds[5,9] and may rarely occur in cats. PMDS is due to a defect in production or function of MIS or mutated MIS receptor. A transition mutation resulting in a dysfunctional MIS receptor is the cause of the autosomal recessive condition in minature Schnauzers.[7] The affected males have a normal male karyotype and male gonads, but also have a uterine body, uterine tubes, and cranial vagina.[9]

Pyometra is either acute or chronic inflammation of the uterus resulting in a purulent exudation. It can be open (open cervix with secondary bacterial infection) or closed and aseptic. Male patients with PMDS have no cervix or caudal vagina so the pyometra is aseptic and may at least in part be due to failure of drainage of luminal fluids secreted by variably dilated endometrial glands. Hormonal influence from a functional Sertoli cell tumor and variable cystic endometrial hyperplasia can contribute to the pyometra as well.

Contributing Institution:

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JPC Diagnosis:
1. Testis: Sertoli (sustentacular) cell tumor.
2. Uterus: Persistent paramesonephric duct, with marked suppurative endometritis and cystic endometrial hyperplasia.

JPC Comment:
The contributor provides a concise review male sexual development, factors associated with the development of the Persistent Müllerian Duct syndrome (PMDS), and sequela often associated with this disorder of sexual development (DSD), including testicular tumors and cryptorchidism, both of which were a subject of discussion during 21-22 WSC 14, case 1.

PMDS was first described as a rare form of male pseudohermaphroditism characterized by the presence of Müllerian Duct derivatives in humans by Nilson in 1939.[8] Canine PMDS was first reported in 1976 by Brown, Bure, and McEntee, who described three cases of male pseudohermaphroditism and cryptorchidism miniature schnauzers.[10] Although this XY disorder of sexual development (DSD) is most prevalent miniature schnauzers, it has since been reported other canine breeds, cats, cattle, goats, and a beluga whale.[4,11]

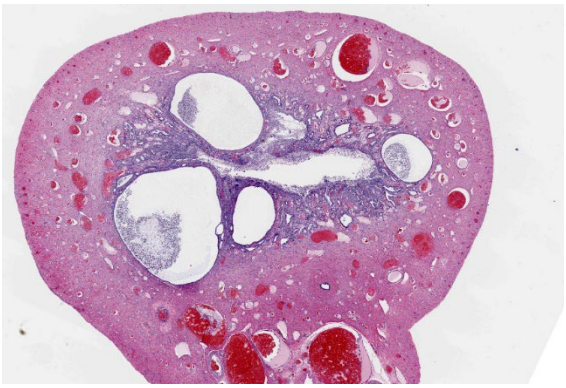

Figure 4-4. Persistent paramesonephric duct. Sections of the persistent paramesonephric duct with tortuous and dilated endometrial glands. (HE, 12X)

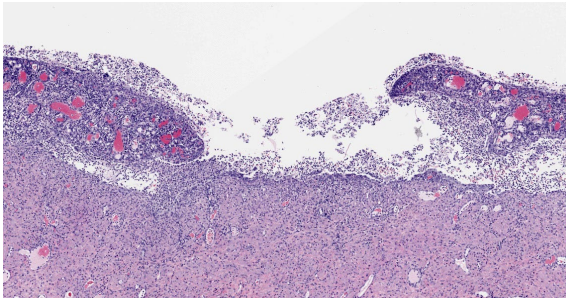

Figure 4-5. Persistent paramesonephric duct. There is marked suppurative endometritis with segmental endometrial necrosis and loss. (HE, 65X)

PMDS may arise due to either lack of anti-Müllerian hormone or a faulty receptor. In miniature schnauzers, PMDS is most commonly attributed to a cytosine to thymine nonsense autosomal recessive mutation in exon 3 of the gene encoding the anti-Müllerian hormone type II receptor (*AMHR2).*[7,10] Homozygous dogs therefore lack functional receptors for anti-Müllerian hormone, resulting in failure of Müllerian duct regression and the formation of derivatives such as oviducts, uterus, cervix, and a cranial vagina, which may insert into the prostate. However, these female reproductive tract structures are internal and the dog exhibits a male phenotype.[10]

As noted by the contributor, approximately 50% of cases are either uni- or bilaterally cryptorchid. Despite being fertile, unilateral cryptorchid males are generally removed from the breeding pool. However, hetero- and homozygous female carriers, in addition to the remaining 50% of homozygous males with normal descended testicles are fertile.[10]

A 2018 study[10] investigating the prevalence of the *AMHR2* mutation in miniature schnauzers found 1.9% of 216 randomized samples were homozygous for the *AMHR2* mutation, while 27.3% (n=59) were heterozygous. However, it is worth noting all the DNA samples used in this study were obtained from a single regional laboratory

and may therefore reflect prevalence in a single geographic region rather than amongst the breed as a whole.[10]

PMDS is associated with significant morbidities, including increased risk of testicular tumors in cryptorchid males, as well as pyometra, both of which are demonstrated in this case. Additional pathologic conditions associated with PMDS include hydrometra, urinary tract infections, and prostatitis. Responsible breeding programs are therefore desirable in regard to the prevention of whelping additional puppies affected by PMDS. However, implementation of this concept would be a challenge given 100% of female and heterozygous males and approximately 50% of homozygous males are phenotypically normal. Therefore, development of a commercially available test for the *AMHR2* mutation may be beneficial for the future of diagnosing the particular syndrome in this breed.[10]

From an evolving nomenclature standpoint, we have included the non-eponymous (but not widely used) terminology for a Sertoli cell tumor in this case (sustentacular cell tumor)

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