Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #25 01 May 2024

CASE I:

Signalment:
Female goat (*Capra hircus*), age and breed unspecified.

History:
The owners had 3 goat does that aborted premature and full-term fetuses after being purchased 4 months prior. These goats were dewormed a week before sample submission. Submitted for necropsy evaluation are 2 fetuses and one placenta.

Gross Pathology:
No major gross lesions were observed in the placenta.

Laboratory Results:
Q-fever PCR at referral laboratory – Positive for *Coxiella burnetti*.

Microscopic Description:
Placenta (chorioallantois): In regionally extensive areas, numerous intracytoplasmic pleomorphic (cocci, coccobacillary, and bacillary morphologies), gram negative bacterial microorganisms are observed within hypertrophied syncytiotrophoblasts on the chorionic villi. The chorioallantois is severely expanded by edema and numerous inflammatory infiltrates, predominantly composed of plasma cells, lymphocytes and hofbauer cells

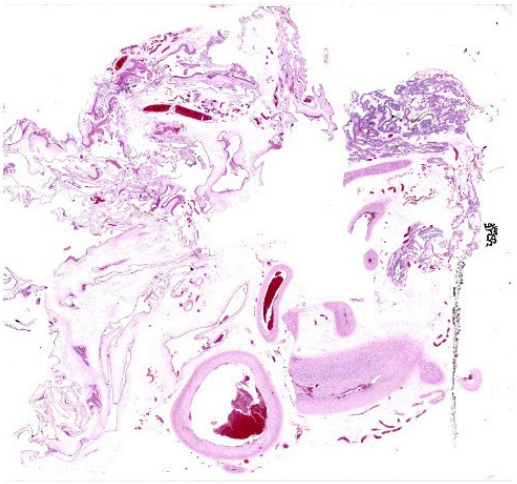

Figure 1-1. Placenta, goat. Sections of the cotyledonary (upper right) and inter-cotyledonary placenta (upper left) are submitted for examination. (HE, 5X)

(placental macrophages). Small caliber vessels in the chorionic stroma are moderately dilated by congestion.

Contributor's Morphologic Diagnosis:
Placenta: Placentitis, lymphoplasmacytic and histiocytic, acute to subacute, regionally extensive, severe with intratrophoblastic bacteria (*Coxiella burnetti*).

Contributor's Comment:
Q fever, the disease caused by the bacterial organism *Coxiella burnetti*, an obligate intracellular pleomorphic gram-negative bacterial

microorganism, is considered a potential bioterrorism agent in many countries, including the United States. A worldwide presence has been reported, except in New Zealand.

The US Centers For Disease Control has classified *C. burnetti* as 'category B' – moderately easy to disseminate with moderate morbidity and mortality. Previous findings indicate that *C. burnetti* has a low infectious dose for initiating a disease process i.e., up to 90% probability for a single bacterium to initiate a disease process.[9]

Coxiella spp. belong to class Gammaproteobacteria, order Legionellales and family Coxiellaceae. *C. burnetti* has been identified in multiple species including domestic ruminants, birds, and some insects.[2,6,11,16] Domestic ruminants are frequently considered as reservoir hosts for *C. burnetti*.[7,13] This pathogen is zoonotic to humans, and can cause a wide array of clinical findings and lesions in different species, that includes abortions, stillbirths, hepatomegaly, splenomegaly, endocarditis, and encephalitis.[2]

C. burnetti has been identified in the gut cells of ticks (*Dermacentor* sp., *Haemaphysalis* sp., *Ixodes* sp., *Hyalomma* sp., *Ornithodoros* sp.,) implicating these ticks as one of the major modes of transmission for this pathogen.[3] Other recorded routes of transmission include aerosolization, ingestion of contaminated placenta (in dogs and cats), exposure to infected milk and other dairy products, horizontal (person-to-person), exposure to fomites, and contact with infected hide or wool from animals.[1] Transmission via fomites, reproductive tissues, and contaminated, unpasteurized dairy products are the most prominent causes for pathogen transmission across different species.

A biphasic growth pattern has been reported for this obligate intracellular gram-negative microorganism, characterized by two major cell types – a spore-like small cell variant (SCV) and an actively dividing large cell variant (LCV).[2,7,12,14] This spore-like SCV has a higher concentration of peptidoglycan, providing resistance to a wide array of physical and chemical stressors, thus permitting the pathogen to survive in the environment. SCVs are usually shed into the environment via placental fluids, mammary secretions, urine, feces, and fomites during the parturition process. By inhalation and close contact, these SCVs then spread to others in the immediate vicinity. Following inhalation, *C. burnetti* is commonly observed within macrophages in various organs including lungs, which leads to inhibition of cell death by impairing phagolysosome formation and macrophage function.[12,14] The exact mechanism for tropism towards trophoblasts is still definitively undetermined, although it is believed that mild immunosuppression during pregnancy may be one of the causes for pathogen localization in the placenta.[18] Maturation and development of SCV in the placenta following a biologically active LCV phase will lead to further spread of the pathogen through shedding into the environment.

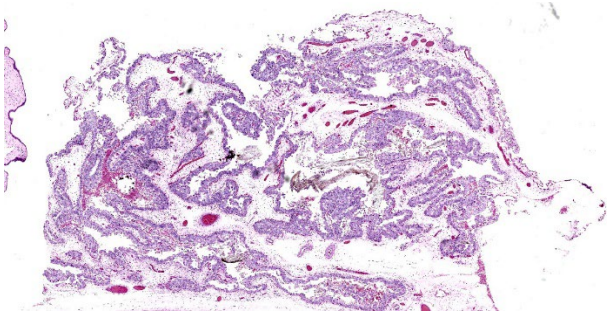

Figure 1-2. Placenta, goat. Cotyledonary epithelium (trophoblasts) are markedly and diffusely expanded. The chorioallantoic stroma is diffusely edematous. (HE, 23X)

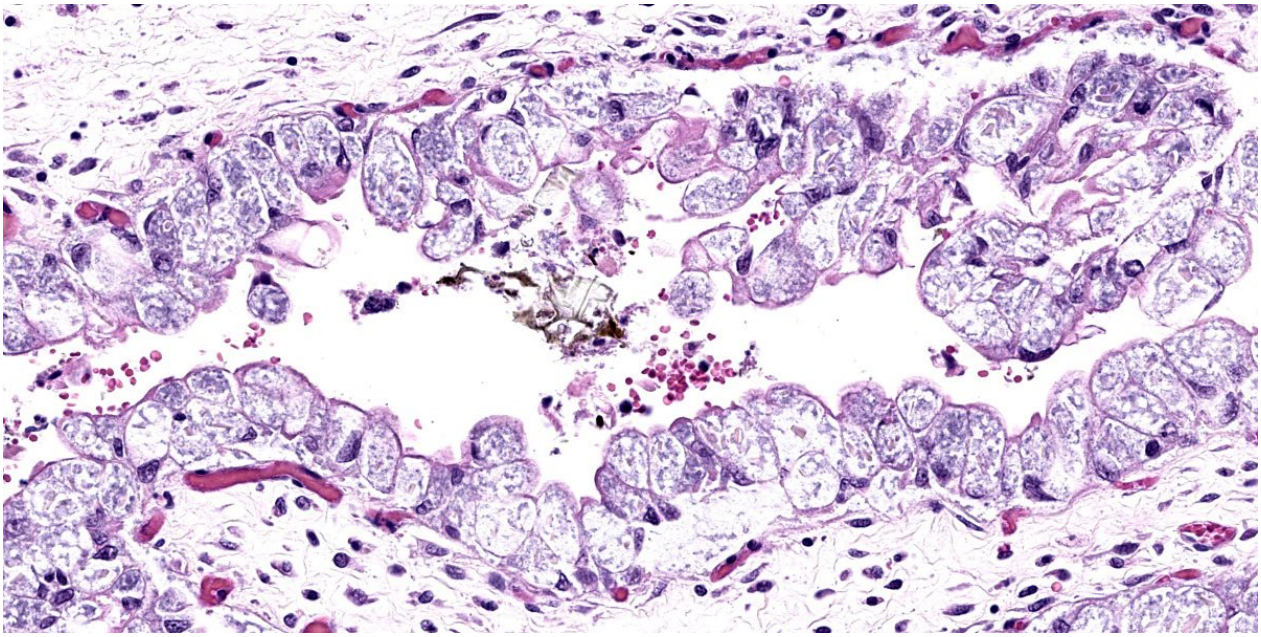

Figure 1-3. Placenta, goat. Higher magnification of trophoblasts. Almost every trophoblast contains numerous 1µm rickettsia within their cytoplasm. This foamy cytoplasmic appearance is characteristic of *Coxiella burnetti.* (HE, 381X)

In humans, *C. burnetti* can result in the following: self-limiting flu-like clinical signs, severe atypical pneumonia, hepatitis with severe hepatomegaly and icterus, maculopapular exanthema, pericarditis, myocarditis, aseptic meningitis, seizures, polyradiculoneuritis, optic neuritis, transient hypoplastic anemia, and/or lymphadenopathy, as well as other manifestations.[8] The chronic form of Qfever has been reported in 5% of all clinical reports and the most commonly reported lesions in chronic disease are endocarditis, hepatic fibrosis, osteoarthritis, osteomyelitis, as well as other manifestations.[8]

One previous study has reported strong placental tropism of *C. burnetti* at 2-4 weeks after inoculation in pregnant goats, and higher numbers of microorganisms isolated from these infected goats at the time of kidding.[15] In domestic ruminants, histological findings from reproductive tissues (such as the placenta) are a histiocytic and lymphoplasmacytic placentitis with numerous intrahistiocytic and intratrophoblastic bacterial microorganisms. Other commonly used diagnostic techniques includes immunoperoxidase staining for highlighting bacterial microorganisms, immunohistochemistry using monoclonal antibodies against *C. burnetti*, PCR amplification of different DNA targets including 16S and 23S, and serological techniques such as ELISA, complement fixation, western blotting, dot blotting, and microagglutination.[8]

Q-fever is still poorly understood and is zoonotically important due to its multiple attributes, including low infectious dose and environmental persistence. Subsequent screening of all abortions in domestic ruminants, decontamination, biosecurity and treatment are strongly recommended to curb the spread of this pathogen.

The main findings in this case are: 1) lymphoplasmacytic and histiocytic placentitis, 2) the presence of large numbers of intratrophoblastic gram negative bacterial organisms,

and 3) PCR positive detection of *C. burnetti*. Altogether, these findings are consistent with *Coxiella burnetti* infection, resulting in abortion in this case.

Contributing Institution:
Oklahoma State University
Department of Veterinary Pathobiology
College of Veterinary Medicine
Stillwater, OK 74078 USA
www.vetmed.okstate.edu

JPC Diagnosis:
Placenta: Placentitis, lymphohistiocytic, diffuse, mild, with innumerable intratrophoblastic coccobacilli.

JPC Comment:
Coxiella burnetii, like any self-respecting intracellular pathogen, must find a way to solve an existential problem: how to survive inside a cell that is trying to kill it. *C. burnetii* employs a variety of weapons to this end, some of which are well-characterized, and some of which remain poorly understood.

A key weapon in its arsenal is a Type IV secretion system (T4SS), one of several types of such systems used by microorganisms to transport macromolecules across cell membranes.[17] Once *C. burnetti* is phagocytosed by a macrophage, it uses its T4SS to modify host cellular processes to develop a *C. burnetti*-containing vacuole (CCV) in which it can thrive and replicate. The CCV is characterized by "promiscuous fusogenicity," prominent size, temporal stability, and the ability to promote *C. burnetti* replication, all of which distinguish the CCV from a typical lysosome.[10] The T4SS is critical to the formation and maintenance of this CCV intracellular niche and to thwarting the host cell death pathways which are typically arrayed against it.[4]

The *C. burnetii* T4SS transfers over 130 bacterial proteins, very few of which have been characterized, from the CCV into the macrophage cytoplasm.[4] Among those bacterial proteins with known functions, one, AnkG, binds mitochondrial p32 which inhibits the intrinsic apoptosis pathway. Another, inhibitor of caspase activation (IcaA), frustrates pyroptosis by inhibiting the NLRP3 inflammasome and caspase-1 activation.[4]

The effects of translocated bacterial proteins can be appreciated, even if the mechanism of action or the specific protein cannot be identified. One such example relates to the nutrient-sensing mammalian target of rapamycin complex 1 (mTORC1) which homeostatically inhibits autophagy but is itself inhibited in times of nutrient deprivation. Inhibition of mTORC1 typically leads to autophagy, the catabolism of cellular organelles and the repurposing of the liberated macromolecules as metabolic substrates. Inhibition of MTORC1 leads to the formation of large, fusogenic lysosomal organelles that anticipate the surge of incoming catabolic cargo.[10]

Research has demonstrated that *C. burnetti* inhibits mTORC1 by an unknown mechanism in the face of nutrient sufficiency, leading to the development of the large, fusogenic CCV that accommodates hundreds of replicative organisms.[10] The CCV fuses with autophagic vesicles and accumulates autophagy-related proteins such as beclin-1, LC3, and p62. The critical role of T4SS in this process, and by extension, the critical role of translocated bacterial proteins, is evidenced by experimental studies that disrupt T4SS function and inhibit nutrient-dependent au-

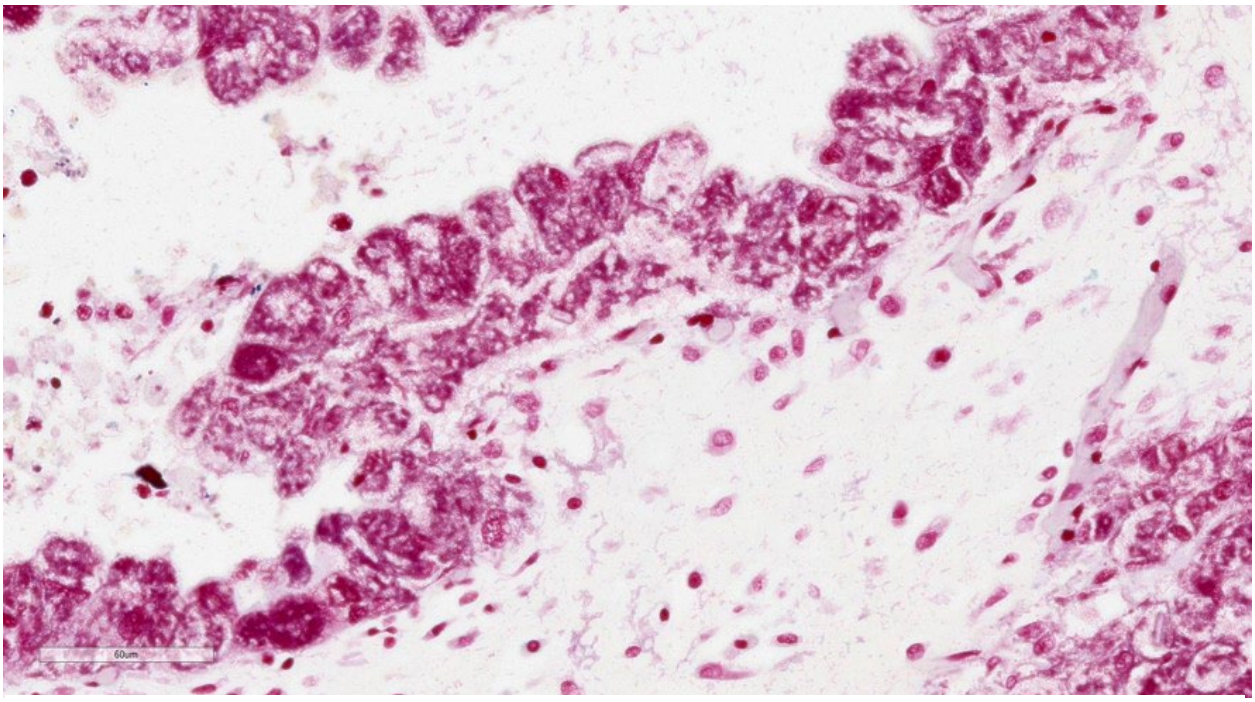

Figure 1-4. Placenta, goat. Gram-negative intratrophoblastic bacterial organisms, identified as *Coxiella burnetti* by PCR. (Gram, 400X) (*Photo courtesy of:* Oklahoma State University College of Veterinary Medicine, Stillwater, OK. https://www.vetmed.okstate.edu)

tophagy and bacterial replication in *C. burnetti* infected cells.[10] Researchers have speculated that *C. burnetti* activates autophagic catabolism via mTORC1 inhibition to provide replication-supporting nutrients within the CCV.[10]

C. burnetti likely has many more undiscovered virulence factors which are the subject of active research due to its highly infectious nature and unique mechanisms of intracellular survival. A nice summary of the current understanding of *C. burnetti* virulence factors can be found in a 2020 review by Dragan and Voth.[5]

Our moderator this week was Dr. Susan Bender, Assistant Professor of Clinical Pathobiology ar the University of Pennsylvania School of Veterinary Medicine. Participants seemed to approach this slide with some trepidation and initial discussion focused on the basics of tissue identification and proper terminology. Dr. Bender noted that the challenge was made even more difficult by the tremendous distention of the trophoblasts by *Coxiella* organisms.

Participants also discussed an area of the tissue that appeared to contain squamous metaplasia, which provoked some discussion as this would be an unusual finding in the chorioallantois (though not unprecedented; this change can be observed in fungal abortions in goats). Dr. Bender noted that these most likely represent amniotic plaques, a normal finding in the amnion, a portion of which is likely in the slide along with the chorioallantois. A few participants also noted possible thrombi in a a few vessels; however, these are likely not significant without accompanying vascular injury, which is not seen in section and is

not an expected finding for this disease entity.

The remarkable expansion of the trophoblasts in this case is a key histologic feature, which Dr. Bender noted should bring a differential list to mind. While the blue frothy material is a classic appearance for *Coxiella burnetii, Chlamydia abortus* should also be on the differential list. Other differentials include *Brucella* spp., *Listeria* spp., and *Campylobacter* spp., though those agents do not typically cause such massive trophoblast expansion. Dr. Bender ended discussion by reminding participants that Q fever is a reportable diseaes which, if encountered, should be promptly reported to state authorities with no bleating around the bush!

Participants noted the severe inflammation described by the contributor; however, in the section assessed in conference, participants felt the inflammation was rather mild and predominantly lymphohistiocytic, leading to some minor tinkering with the morphologic diagnosis.

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CASE II:

Signalment:
5-year-old female breed unspecified goat (*Capra aegagrus hircus*).

History:
Livestock farm with ecological and extensive goat production. After the introduction of new animals, blindness, polyarthritis, and chronic mastitis only affecting postpartum and lactating goats were observed. The process reached up to 20% mortality.

Gross Pathology:
Lesions were consistent with mastitis, characterized by a firm consistency of the mammary gland parenchyma and abnormal turbid milky discharge admixed with fibrinous material. Joints showed fibrino-purulent content in the joint space and significant thickening of the joint capsule, compatible with chronic polyarthritis. Unilateral keratitis with mild corneal opacity and bilateral blepharoconjunctivitis were also observed.

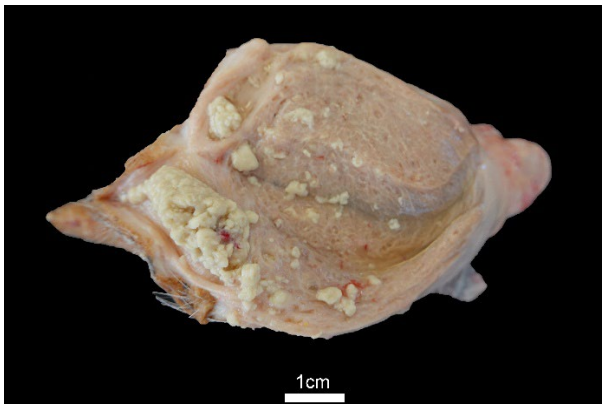

Figure 2-1. Mammary gland, goat. The mammary gland is firm with an abnormal turbid milky discharge admixed with fibrinous material. (*Photo courtesy of:* Department of Animal Pathology, Universidad de Zaragoza, Spain. http://patologiaanimal.unizar.es/)

Laboratory Results:
Hematology and biochemical analysis revealed marked leukocytosis, neutrophilia, hyperglobulinemia, and increased gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST). *Mycoplasma agalactiae* was identified in mammary gland and synovial fluid swabs by Real Time Polymerase Chain Reaction (RT-PCR).

Microscopic Description:
Mammary gland: A severe, chronic active, multifocal inflammatory process comprising 70% of section appears to infiltrate and efface mammary gland lobules and acini. At higher magnification, inflammation is composed of abundant lymphocytes, macrophages, and plasma cells, admixed with fewer neutrophils and scattered eosinophils. Occasionally associated with mammary ducts and acini, lymphocytes and macrophages form round aggregates (tertiary lymphoid follicles). Epithelial ductal cells show one or more of the following changes: intracytoplasmic vacuoles and attenuation (degeneration), shrunken and hypereosinophilic cytoplasm with pyknotic to no nuclei (necrosis)

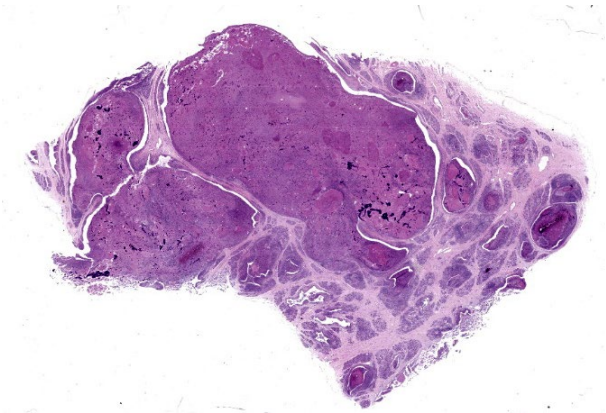

Figure 2-2. Mammary gland, goat. A section of mammary gland is submitted. Lactiferous ducts are markedly ectatic and filled with necrotic debris. (HE, 5X)

or appear sloughing towards the ductal lumen. Ductal lumens are distended and partially or totally occluded by an abundant amorphous eosinophilic material (proteinaceous secretion), admixed with viable and degenerate neutrophils, macrophages, lymphocytes, and karyorrhectic and cellular debris. Within this secretion, there are occasionally amorphous basophilic granules (mineralization). Diffusely expanding the interlobular interstitium and mammary lobules, there is an increased number of mature collagen fibers (fibrosis).

Contributor's Morphologic Diagnosis:
Mammary gland: Severe, chronic, multifocal to coalescing, lymphoplasmacytic and histiocytic mastitis with fibrosis and necrosis.

Contributor's Comment:
Contagious agalactia (CA) is a notifiable disease distributed worldwide that affects goats and sheep.[8] The etiological agent in sheep is *Mycoplasma agalactiae* (Ma), whereas in goats *M. mycoides subsp. capri* (Mmc), *M. capricolum subsp. capricolum* (Mcc), and *M. putrefaciens* (Mp) are also involved.[12] Oral, respiratory and mammary (milking parlor) are the main routes of transmission within the herd.[1,7,15] Clinical signs and lesions are highly variable and affect mainly postpartum and lactating adult females.[14] CA syndrome is characterized by mastitis, polyarthritis, keratoconjunctivitis, pneumonia, and septicemia,[6] all of which were observed in our case, with the exception of pneumonia.

Depending on the infection route, the primary site of colonization is the respiratory tract, small intestine, and/or alveoli of mammary glands.[1,7,15] Subsequently, Ma is disseminated through the circulation to different organs.[13] Microscopically, acute lesions are characterized by a neutrophilic infiltrate with foci of necrosis. Chronic stages develop into an inflammatory infiltrate composed mainly of macrophages, lymphocytes, and plasma cells, together with extensive fibrosis.[4]

In this case, chronic mastitis, polyarthritis, and keratoconjunctivitis, all characterized by a lymphoplasmacytic and histiocytic infiltrate and severe fibrosis, were observed. Other necropsied goats from the same farm showed acute lesions composed of a severe neutrophilic infiltrate and foci of necrosis in previously mentioned organs. This difference in lesion chronicity among different goats suggests that Ma was chronically present in the herd; however, the outbreak continued actively producing severe and acute cases.

In the typical acute forms, clinical signs and gross and microscopic lesions can facilitate the diagnosis of mycoplasmosis.[12] However, to confirm the etiological agent and differentiate among *Mycoplasma* spp., it is necessary to perform molecular techniques such as PCR.[2,5,9,11] Relevant samples are joint fluid, eye swabs, or other affected organs seen at necropsy.[10] Bulk tank milk samples can in

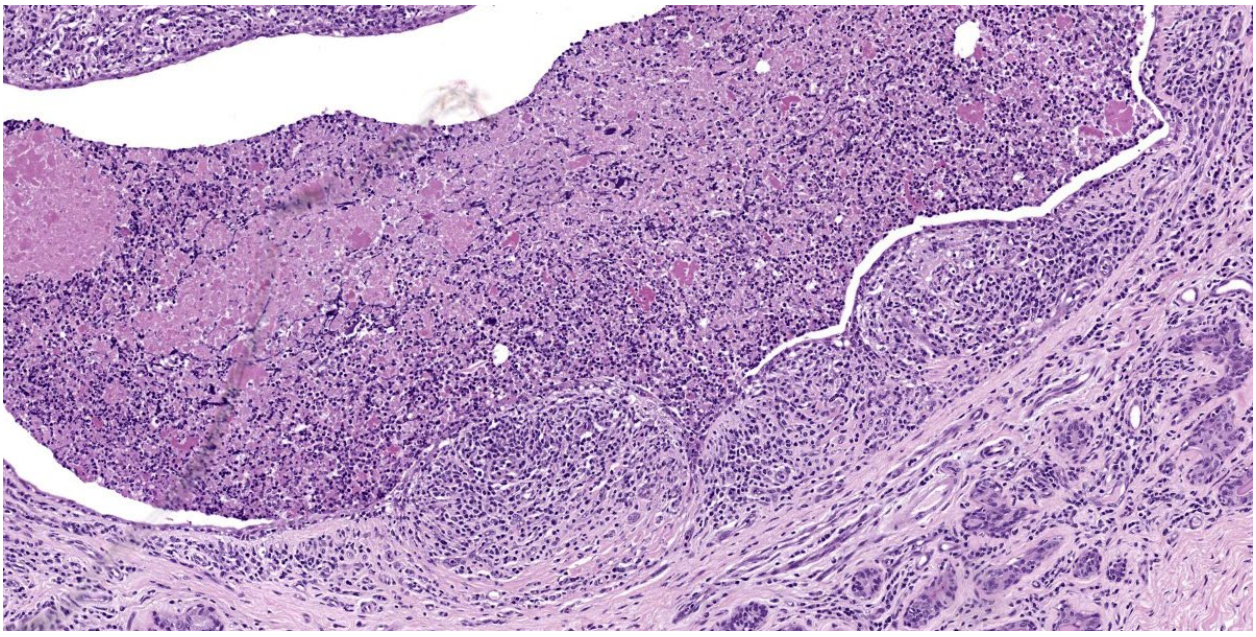

Figure 2-3. Mammary gland, goat. Macrophages and fewer lymphocytes and plasma cells accumulate beneath the segmentally ulcerated epithelium lining ectatic lactiferous ducts.

crease the detection sensitivity on a farm.[12] Once the outbreak was detected, serology by indirect ELISA and PCRs of *Mycoplasma agalactiae* of all goats were performed and the positive ones were sacrificed.

Contributing Institution:
Department of Animal Pathology
Universidad de Zaragoza, Spain
http://patologiaanimal.unizar.es/

JPC Diagnosis:
Mammary gland: Mastitis, necrotizing and lymphohistiocytic, chronic, diffuse, marked, with acinar atrophy.

JPC Comment:
Contagious agalactia (CA) is among the most important diseases of small ruminants and is characterized primarily by mammary, joint, and ocular signs, with a drop in milk production followed by increased general morbidity and mortality.[3,8] The disease was first reported in Italy in 1816, where it was named "mal di sito," or "disease of the place," due to its ability to contaminate and persist in environments and then infect newly-introduced animals.[8]

Grossly, CA mammary disease may be unilateral or bilateral and is heralded by hot, swollen, and painful mammary glands and enlarged mammary lymph nodes. Infection spreads to the joints, with arthritis characterized by the accumulation of synovial fluid in the carpal or tarsal joints, frequently with concurrent keratoconjunctivitis.[3] Other clinical manifestations of CA exist, most notably pneumonia and occasionally abortion, and clinical syndromes may appear in isolation or in various combinations, making it difficult to differentiate CA from other infectious scourges of sheep and goats.

The differential list for CA signs in sheep and goats is long. For mastitis, *Staphylococcus, Streptococcus*, and *Mannheimia* spp. should be ruled out, while caprine arthritis encephalitis virus is a prime differential for caprine

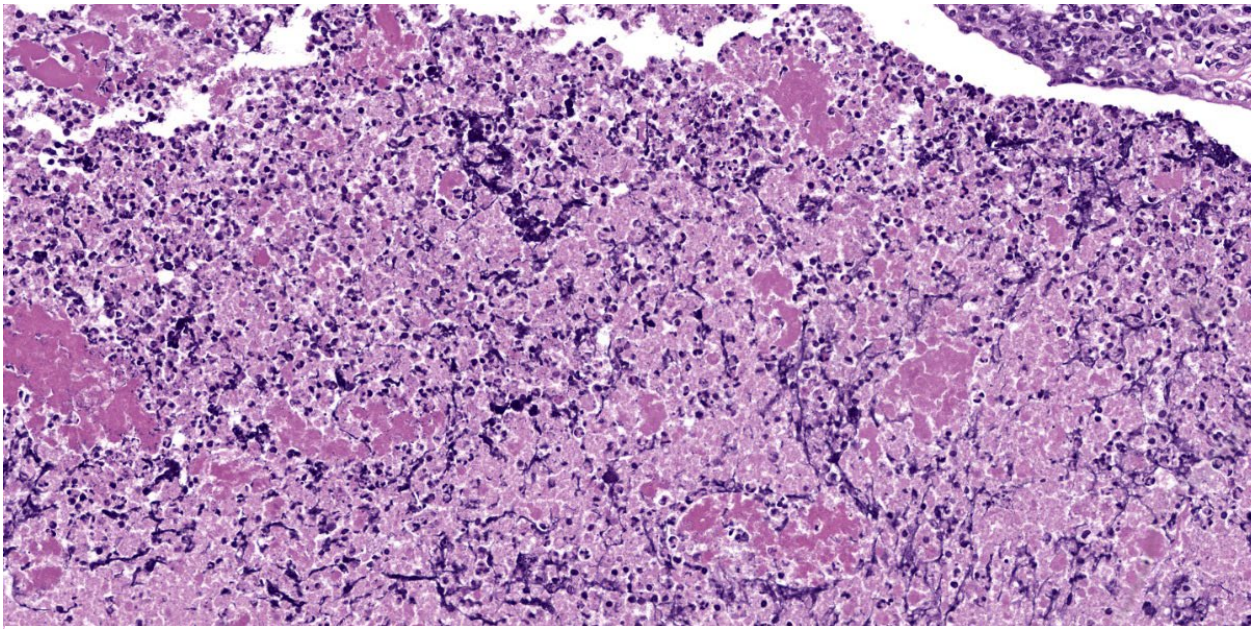

Figure 2-4. Mammary gland, goat. Lactiferous ducts contain a mix of viable and necrotic neutrophils, coagulated secretory product, basophilic homogenous aggregates of nuclear debris, and eosinophilic cell debris. (HE, 363X)

arthritis.[3,8] The *Pasteurellacae,* along with visna-maedi and peste de petits ruminants viruses, as well as other *Mycoplasma* spp. such as *M. capricolum* subsp. *capripneumoniae* and *M. ovipneumoniae* should be excluded as causes for respiratory diseases.[8] Differentiating among these agents often requires extensive testing, and, as the contributor notes, milk, joint fluid, and eye swabs from several different animals in an infected herd are the recommended diagnostic samples.[8]

Histologically, CA mastitis is initially characterized by a mononuclear interstitial infiltrate composed primarily of macrophages, though with time, the prominent inflammatory cell becomes CD8+ T lymphocytes.[3] Lymphoid follicles are often present just beneath the epithelium, as nicely illustrated in this case.

All participants agreed that this mammary gland, even from subgross, looks "baaad". Participants appreciated the remarkable ductal ectasia that is a notable histologic feature of this slide. Participants also questioned that nature of the deep purple material present within the ducts, leading to a tangential but informative discussion of oat cells. Oat cells have a similar histologic appearance to the streaming purple material evident in section; however, oat cells are associated with agents that produce a leukotoxin that debilitates the affected cells. These cells remain intact, but their nuclear and cytoplasmic material stream out, particularly when sectioned, producing the characteristic histiologic oat cell appearance. The intraductal material observed here occurs with massive cell death and the resultant commingling of nuclear and cytoplasmic material that adheres together in the necrotic milieu.

Back on task, participants noted the lymphoid nodes which originally made them consider a viral etiology such as arthritis encephalitis virus as a possible cause. While these lymphoid nodules are a reported histologic feature of AC, the moderator noted that mixed mammary gland infections aren't particularly uncommon, and the presence of one agent doesn't necessarily preclude the presence of another. Dr. Bender concluded discussion of this case with a review of the various histologic changes associated with mammary development and lactation, noting that evaluating reproductive tissue is inherently challenging as the tissues dynamically respond to the ebb and flow of hormonal tides.

Conference participants felt that necrosis was a significant histologic feature of the examined slide and wanted to include it in the morphologic diagnosis. There was a brief discussion about including fibrosis, however most participants felt that chronicity implies fibrosis and it was omitted in the interest of brevity.

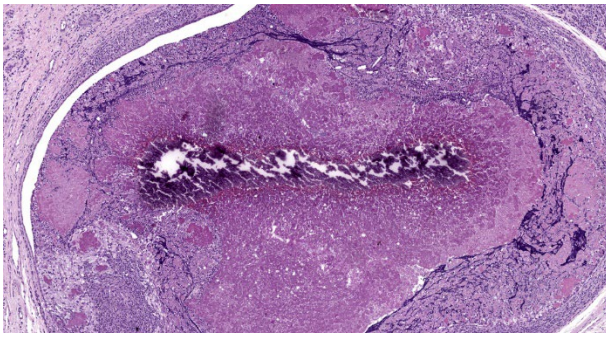

Figure 2-5. Mammary gland, goat. The debris within lactiferous ducts occasionally contains crystalline mineral. (HE, 363X)

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CASE III:

Signalment:
9-year-old female breed unspecified rabbit (*Oryctolagus cuniculus*).

History:
The owner noted bloody urine for a few weeks and hemorrhagic urine was found on urinalysis. The clinician suspected uterine adenocarcinoma based on clinical findings, so the patient was spayed.

Gross Pathology:
The uterus contained an 8cm mass.

Laboratory Results:
Hematuria, otherwise no significant findings.

Microscopic Description:
Uterus: Multifocally and transmurally infiltrating and effacing the uterine wall is an unencapsulated, densely cellular, poorly demarcated neoplasm composed of epithelial cells arranged in tubules, acini, and solidly cellular areas on a variably dense collagenous to myxomatous stroma. Neoplastic cells have indistinct cell borders, a small amount of eosinophilic cytoplasm, and a round to oval nucleus with finely stippled chromatin and indistinct nucleoli. Anisocytosis and anisokaryosis are mild, and there are less than 1 mitotic figures per 2.37mm.^{2} Neoplastic tubules are ectatic with fronds of neoplastic epithelial cells bulging into the lumina, and lumina are often filled with eosinophilic proteinaceous and/or mucinous exudate admixed with variable amounts of necrotic cellular debris. There is abundant predominantly coagulative necrosis at the center of the neoplastic mass characterized by loss of differential staining with retention of architecture. The non-neoplastic uterine mucosal epitheli

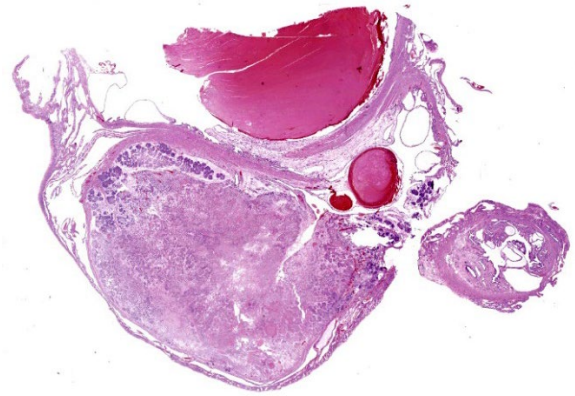

Figure 3-1. Uterus, rabbit. Multiple sections of uterus are submitted for examination. (HE, 4X)

um is multifocally hyperplastic, and uterine glands are multifocally dilated, forming ectatic cysts up to 2 mm in diameter lined by attenuated epithelium (cystic endometrial hyperplasia). Arising from the endometrium and projecting into the uterine lumen, there are several cross sections of markedly dilated, endothelial-lined veins (endometrial venous aneurysm) measuring up to 5 mm in diameter. These veins are subtotally occluded by large fibrin thrombi that often contain well-defined lines of Zahn, low numbers of enmeshed erythrocytes and leukocytes, and a rare dusting of mineral in areas of coagulative necrosis.

Contributor's Morphologic Diagnosis:

1. Uterus: Uterine adenocarcinoma with vascular invasion.
2. Uterus: Endometrial venous aneurysm with thrombosis and rare mixed bacteria.
3. Uterus: Cystic endometrial hyperplasia, multifocal, moderate.

Contributor's Comment:

Uterine adenocarcinoma is often considered the most common spontaneous neoplasm of domestic rabbits, although a recent large-scale study found that trichoblastomas and mammary tumors may surpass uterine adenocarcinomas in frequency of diagnosis.[1-3,6] The incidence of uterine adenocarcinoma increases with age, affecting approximately 80% of 5-6 year old does; most animals in research facilities and commercial rabbitries are relatively young, which explains why this tumor is infrequently seen in this in these facilities.[1,3] Uterine adenocarcinomas may be multicentric within the uterus, and may involve both uterine horns.[1,3] Metastasis to the lung is most common; metastasis to the liver and intra-abdominal carcinomatosis are also commonly reported.[1,3] Intratumoral necrosis is common.[1,3] Uterine adenocarcinomas are often concurrently diagnosed with endometrial hyperplasia.[2,3,6,10] Additionally, one study found that, of 84 uterine adenocarcinomas, 10 had concurrent uterine leiomyoma or leiomyosarcoma.[2]

Endometrial hyperplasia follows second to uterine adenocarcinoma in frequency of diagnosis in rabbit uteri and is the most commonly diagnosed non-neoplastic uterine lesion in rabbits.[1,3,6] The incidence increases with age, although it has been reported in rabbits less than 1 year of age.[3,6,10] It is often cystic and is often diffuse.[2,3] Endometrial hyperplasia is controversially considered to be a pre-neoplastic lesion by some, and is considered hormonally induced.[1,2,3]

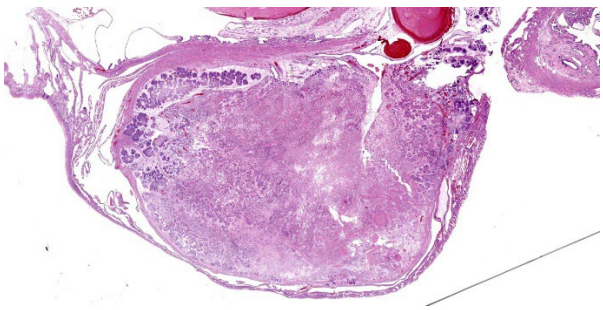

Figure 3-2. Uterus, rabbit. A polypoid neoplasm composed of glands on an edematous fibrous stroma arises from the endometrium and fills the uterine lumen. (HE, 4X)

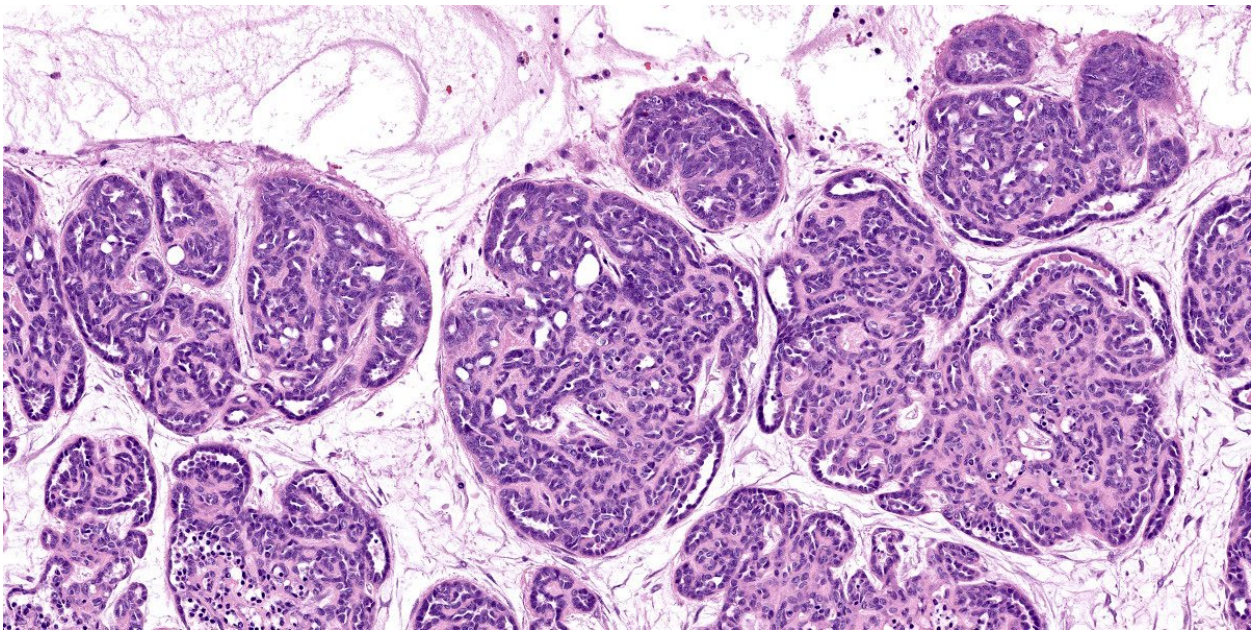

Figure 3-3. Uterus, rabbit. The neoplasm is composed of tortuous glands on an edematous stroma. (HE, 161X)

Endometrial venous aneurysms were first reported in 1992 in rabbits and have yet to be reported in any species other than lagomorphs.[4] They have been reported in New Zealand White rabbits,[4] unspecified breeds of pet rabbits,[3,5] and a Holland lop rabbit.[7] They consist of venous varices that project from the endometrium into the uterine lumen. They have been reported in nongravid, multiparous does, but no predisposing factors have been identified.[1,3] They may cause uterine distension with subsequent abdominal enlargement.[1,4,7] The venous aneurysms may rupture, resulting in periodic bleeding into the uterine lumen (hemometra) with subsequent urogenital bleeding/hematuria, and clotted blood is often noted within the uterine lumen at necropsy.[1,2,4,7] In a recent large-scale study of genital tract pathology in female pet rabbits, Bertram and colleagues identified endometrial venous aneurysms in 1.6% (n=14) of all post-mortem examinations of entire female rabbits and in 3.3% (n=5) of all uterine biopsy samples, with a median age of 32 months.[3] In post-mortem examinations in this study, the endometrial venous aneurysms were considered incidental in 4 of the 14 rabbits with this diagnosis.[3] In another large-scale study of uterine lesions in 1,928 rabbits, endometrial venous aneurysms were reported in 8 rabbits, none of which had clinical signs.[9] Ovariohysterectomy is considered curative and is recommended due to the risk of hemorrhage.[5,7]

All three of these uterine lesions (uterine adenocarcinoma, cystic endometrial hyperplasia, and endometrial venous aneurysm) can result in hematuria; all three of these lesions are present in this case, and hematuria was clinically noted and confirmed via urinalysis in this case.[1] Hematuria and/or serosanguinous vaginal discharge are clinical signs that should raise suspicion of uterine disease.[6] Other potential causes of hematuria in rabbits include uterine polyps, cystitis, urinary bladder polyps or tumors, pyelonephritis, and renal infarction.[1] Of note, differentials for hematuria in rabbits include pigmented urine due to crystals, porphyrin, or bilirubin; a uri-

nalysis is required to differentiate between these causes.[1]

Contributing Institution:
Tri-Service Research Laboratory
https://www.afrl.af.mil/

JPC Diagnosis:
1. Uterus: Uterine adenocarcinoma.
2. Uterus: Endometrial venous aneurysm.
3. Uterus: Cystic endometrial hyperplasia, diffuse, severe.

JPC Comment:
This dazzling slide, a lagomorph uterine lesion party pack, delights from subgross and closer inspection in equal measure, and the contributor provides excellent summaries of the component lesions. The endometrial venous aneurysms are particularly striking and, of the trio of uterine conditions vying for attention, the most uncommon.

Aneurysms occur when the quality or quantity of the connective tissue within the vascular wall is compromised.[7] In humans, aneurysm formation has been associated with conditions such as defective synthesis of elastin or collagens I and III, vitamin C deficiency, atherosclerosis, systemic hypertension, pregnancy, or trauma.[7] In the few reported cases of endometrial venous aneurysm in rabbits, no predisposing factors have been identified, leading to the classification of this condition in rabbits, for now, as congenital.[7]

The primary clinical signs associated with endometrial venous aneurysms are a palpably enlarged uterus and, as the contributor notes, hematuria. These symptoms are not specific and further diagnostics are typically required. In reported cases, hematologic parameters have shown little diagnostic value, though the

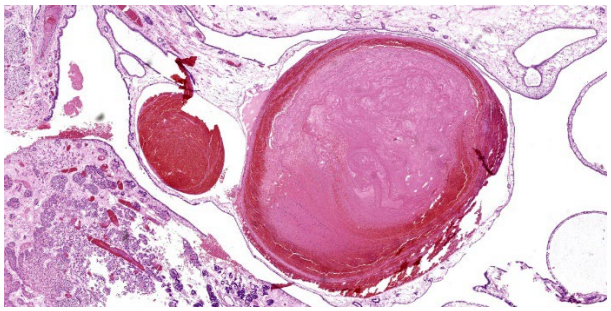

Figure 3-4. Uterus, rabbit. Multiple cross sections of a large thin-walled vein within the endometrium (endometrial venous aneurysm) protrude into the uterine lumen. (HE, 26X)

degree of anemia may provide clues to chronicity and the amount of blood loss suffered in cases of severe aneurysm rupture.[7] Plasma chemistry changes may include hyperglycemia and high creatine kinase, likely due to handling stress rather than any derangement caused by the endometrial aneurysm.[7]

Histologically, the weakened vessel wall can lead to massive dilation of the vein which, as in this case, can be rather obtrusive and, if we're being honest, a little gaudy. The focally dilated vein maintains a complete endothelial lining of attenuated cells that expands the surrounding endometrial tissue. The attenuated wall may exhibit loss of smooth muscle cells and replacement with fibrous connective tissue components such as collagen or fibroblasts.[7] Within the vein, blood and thrombi are usually present, with thrombi characterized by varying amounts of organization, inflammatory cells, and hemosiderin resulting from erythrocyte degradation.[7]

Due to the risk of sudden, severe hemorrhage, venous aneurysms carry a poor to grave prognosis unless ovariohysterectomy is performed.[7] Additional sequelae include thromboembolism and recurrent bouts of excessive bleeding. Prognosis is good with ovariohysterectomy; however, it is still un-

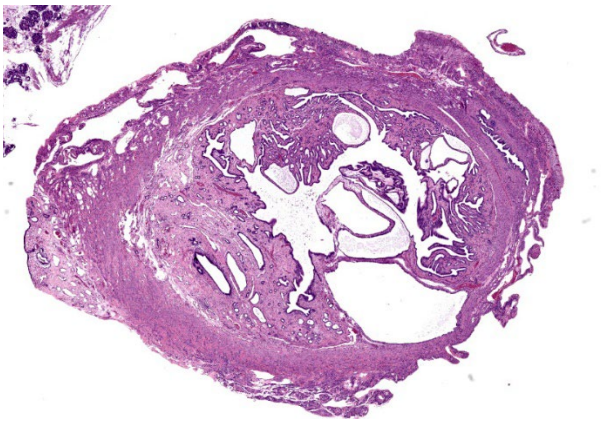

Figure 3-5. Uterus, rabbit. Another cross section of the uterine body demonstrates marked cystic endometrial hyperplasia of the endometrium, with glands occasionally deep in the smooth muscle wall (adenomyosis). (HE, 14X)

known whether this condition is truly congenital and, if so, whether endometrial venous aneurysm indicates a greater risk of aneurysm in other anatomic locations.[7] While there is currently no evidence of an association with a generally heightened risk of aneurysm, more research is needed to determine the prevalence and underlying pathogenesis of this uncommon, striking condition.

Discussion of this case focused generally around the difficult in distinguishing endometrial hyperplasia from neoplasm, and the general consensus was that these two entities likely exist on a neoplasm, though evidence is currently lacking. The moderator discussed a long list of causes for hematuria in rabbits before dropping the inconvenient fact that rabbit urine can normally be red under certain conditions, so be careful about hopping to conclusions. Physiologically normal, red urine should be relatively clear, while true hematuria will have the characteristic opacity of blood.

Discussion of the morphologic diagnosis was similarly straightforward; however, conference participants were unable to identify the vascular invasion or the bacteria noted by the contributor on the section examined at conference and these features were consequently omitted from the diagnoses.

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CASE IV:

Signalment:
Age and breed unspecified ram (*Ovis aries*).

History:
During mating, the animal would start shaking and mating could not continue. Since this happened several times, the animal was euthanized and submitted for necropsy.

Gross Pathology:
The subcutaneous tissue of the ventral part of the abdomen, preputium, and penis was affected by severe suppurative inflammation in the form of large encapsulated abscess.

Laboratory Results:
Bacteriological culture examination showed an increase in the mixed bacterial flora in which *Corynebacterium* spp. predominated. Species identification was done by the MALDI-TOF method which confirmed that the species belonged to *Corynebacterium xerosis*.

Microscopic Description:
Penis (glans): The most outer layer (penile mucosa) is replaced with amorphous eosinophilic material admixed with cellular debris mainly composed of degenerate neutrophils (necrosuppurative inflammation). There are

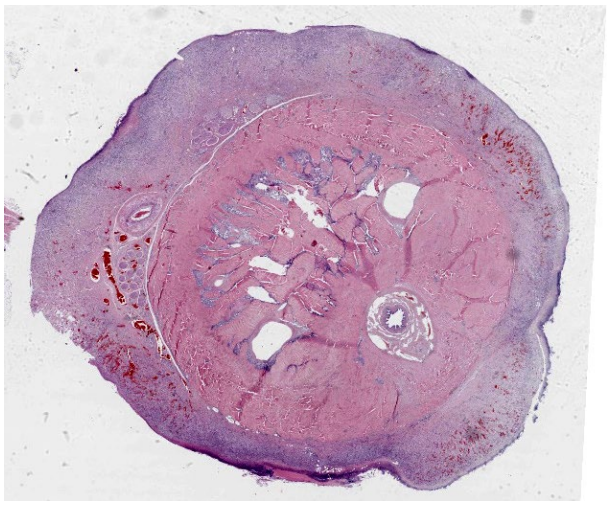

Figure 4-1. Penis, sheep. One section of penis is submitted for examination. The mucosa is ulcerated and the lamina propria is markedly expanded. (HE, 5X).

multifocal colonies of coccobacillary bacteria on the surface. The lamina propria is diffusely infiltrated with larger numbers of lymphocytes, plasma cells, macrophages, and low numbers of neutrophils. The amount of inflammatory infiltrate decreases toward the internal parts of the penis (corpora cavernosa, corpus spongiosum, and urethra), which do not show pathological changes.

Contributor's Morphologic Diagnosis:
Penis (glans): Balanitis, necrosuppurative, subacute to chronic, focally extensive, severe with intralesional coccobacillary bacteria.

Contributor's Comment:
Rams can develop a range of lesions of the penis and prepuce and inflammation is common pathology. Inflammation of the penis is *phallitis*, that of the head (glans) of the penis is *balanitis*, and inflammation of the prepuce is *posthitis*. It can be of viral and bacterial etiology, but foreign materials can also cause inflammation.[3] Although in our case the bacteriological examination showed an increase in mixed bacterial flora with a predominance

of *Corynebacterium* spp., we believe that the isolated *Corynebacterium xerosis* (*C. xerosis*) played a role in the pathogenesis of this condition.

The genus *Corynebacterium*, which currently has more than 110 validated species, is highly diversified. It includes species that are of medical, veterinary, or biotechnological relevance.[1,7,9] The most common cause of cutaneous abscesses and caseous lymphadenitis (CLA) in sheep is *Corynebacterium pseudotuberculosis*. Caseous lymphadenitis in sheep almost always follows a wound infection, usually a shearing wound.[10] The sequence of events in progressive CLA is infection of a superficial wound, spread of infection to the local lymph nodes, which suppurate, and then lymphogenous and hematogenous extension to produce abscesses in internal organs. The progression is slow and may reach the bloodstream only in older animals, whereas in young animals the disease tends to be confined to the superficial lymph nodes, most commonly the precapsular and precrural.[10]

Corynebacterium xerosis is a commensal organism normally present in the skin and mucous membranes of humans.[2] It is considered an unusual pathogen, but it is able to cause endocarditis, skin infections, and other illnesess like maternal ventriculoperitoneal shunt infection, pneumonia, ear and brain abscess, and vertebral osteomyelitis.[1,2,5,12] *Corynebacterium xerosis* grows in colonies of 0.2–1.0 mm in diameter that are brown-yellowish in colour, slightly dry in appearance, and non-haemolytic when cultured in blood agar. Microscopically, *C. xerosis* appears as irregularly stained, pleomorphic gram-positive rods presenting club-like ends.[4]

Infections with *Corynebacterium xerosis* in animals are rarely described. It has been isolated in pure culture from normally sterile organs of animal clinical specimens. *C. xerosis* was isolated from the joint of a pig suffering from a subcutaneous abscess and from a goat liver suspected to have paratuberculosis.[11,12] The case for *C. xerosis* producing a clinical cutaneous abscess in sheep was reported in Mexico in 2016 and *C. xerosis* strain GS1 was isolated from a liver lesion with caseous nodules from a yak.[4,12]

Unlike *C. pseudotuberculosis* infection, the virulence factor that could contribute to the development of abscesses as a pathogenic toxin has not been reported in *C. xerosis*. The main factor of virulence and pathogenicity in *C. pseudotuberculosis* is the exotoxin phospholipase D, which is a permeability factor that promotes hydrolysis of the ester bonds in sphingomyelin in mammalian cell membranes, possibly contributing to the spread of bacteria from the initial site of infection to secondary sites through the lymphatic system to regional ganglia.[6] This highlights the importance of carrying out further research regarding the mechanisms of infection present in this *Corynebacterium* species.[4]

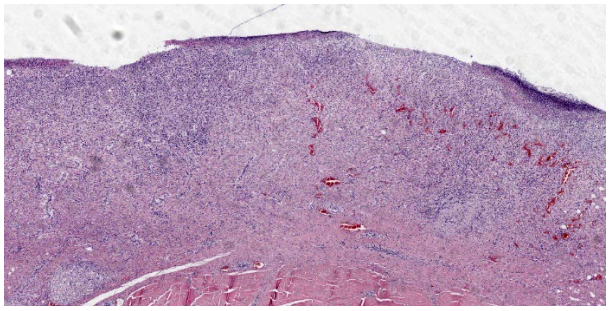

Figure 4-2. Penis, sheep. The submucosa and tunica dartos are effaced by densely cellular granulation tissue; the overlying mucosa is ulcerated. (HE, 30X)

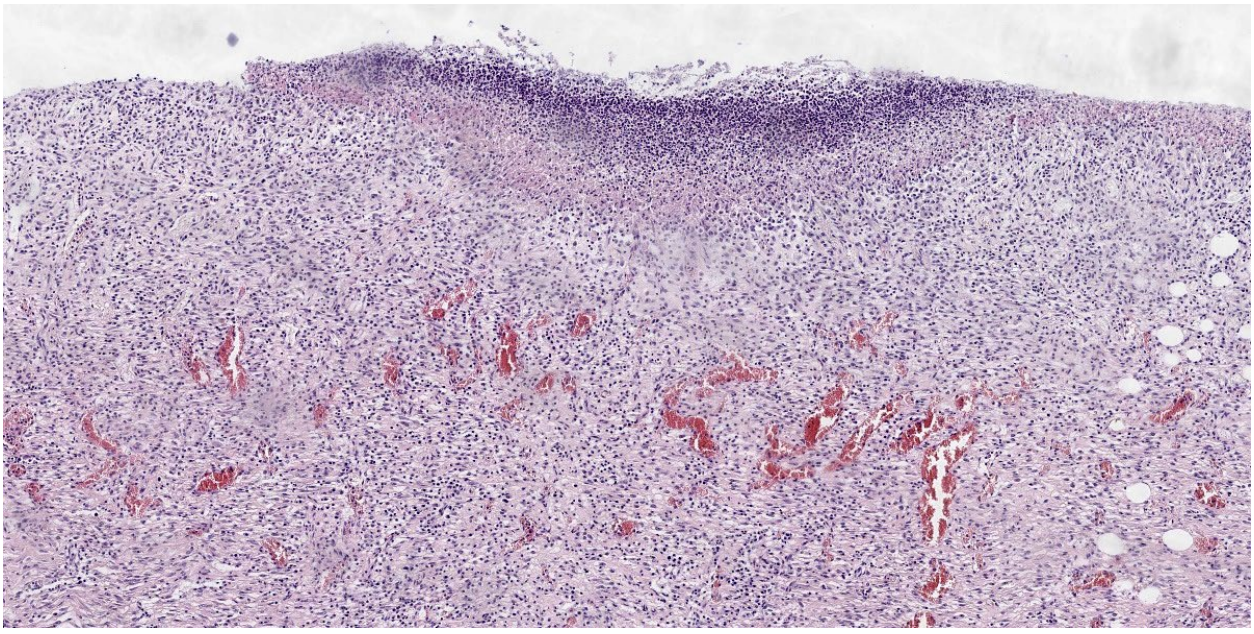

Figure 4-3. Penis, sheep. Higher magnification of the submucosal granulation tissue and ulcerated debris-laden surface. (HE, 38X)

In the presented case, the source of the infection remains unknown. Hernandez-Leon et al. discussed the possible epidemiology of *C. xerosis* in a sheep herd and hypothesized that a possible source of this bacterium could be pigs.[4] The ovine production system where the sample was obtained was previously used for swine, a species where *C. xerosis* has been reported as a common pathogen. Additionally, sharp objects were widespread all over the facilities, which increased the chance of injury to the animals, opening a portal for microorganisms including *C. xerosis*.[4,8,11] All these data indicate that *C. xerosis* has a certain clinical significance in veterinary medicine and that this bacterium should be considered as a possible cause of subcutaneous abscesses but also suppurative inflammation in other organs.

Contributing Institution:
Department of Veterinary Pathology
Faculty of Veterinary Medicine
University of Zagreb, Croatia
http://www.vef.unizg.hr/

JPC Diagnosis:
Penis: Balanitis, ulcerative, chronic, circumferential, severe, with granulation tissue and mixed colonies of bacteria.

JPC Comment:
As the contributor notes, *Cornynebacterium xerosis* is uncommonly isolated from veterinary patients and much of what is understood about this pathogen is extrapolated from human infections. Despite the novelty of the pathogen, however, this case presents a fairly straight-forward case of balanitis, which is typically accompanied by an inflammatory infiltrate composed of lymphocytes, plasma cells, and macrophages.[3] Lymphocytes and plasma cells, and occasionally lymphoid fol-

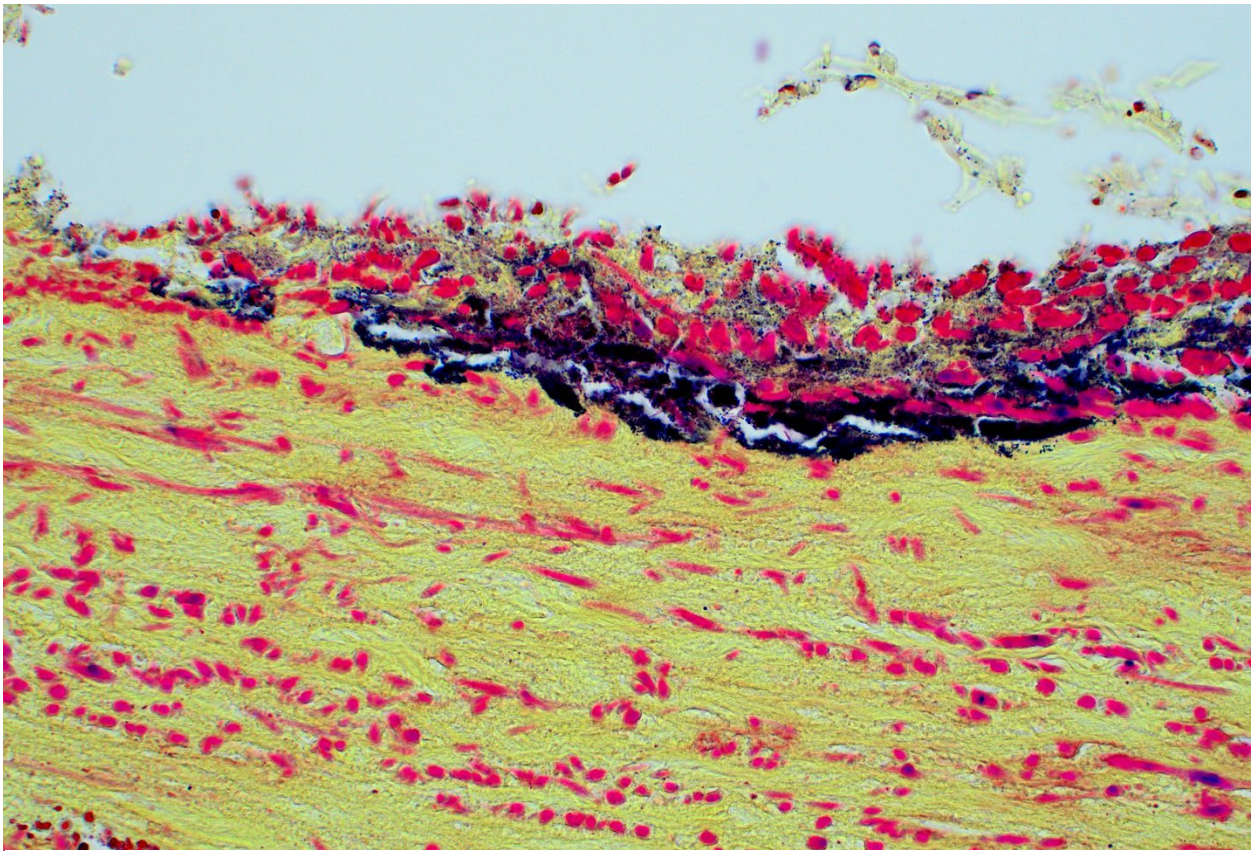

Figure 4-4. Penis, sheep. The necrotic coagulum contains mixed bacterial colonies. (Brown and Hopps, 400X).

licles, are present within the normal preputial mucosa, and immunoglobulins derived from these plasma cells are present in preputial washes.[3]

In most cases of balanitis or balanoposthitis, ascribing pathogenicity to organisms isolated from a penile lesion is fraught with difficulty, as normal preputial bacterial flora may include a variety of nonpathogenic and potentially pathogenic bacteria such as *Corynebacterium renale*, fungi, mycoplasmas, ureaplasmas, chlamydial species, and a variety of protozoa.[3] Attempting to determine which, if any, of these resident organisms is responsible for particular lesions has lead to conflicting research and a morass of speculation in most cases.

Dorper sheep and Leicester rams have a distinct, severe form of ulcerative balanitis which can also cause vulvovaginitis in ewes with whom they are mated.[3] In the male, the condition is characterized by large, deep ulcers that occur on the ventral surface of the penis and typically progress to excessive granulation tissue and hemorrhage.[3] Chronically, adhesions form between the penis and the prepuce as a result of necrotic and purulent material covering the head of the penis. Mated ewes develop shallow ulcers on the labia and posterior vagina. The etiologic agent responsible for this condition remains unknown.[3]

Rams and wethers may also develop "ulcerative posthitis of wethers," commonly known as pizzle rot. The condition, which is unfortunately common, develops in the presence of urea-hydrolyzing bacteria, such as *Corynebacterium renale*, in animals that excrete urea-rich urine.[3] Lesions progress from small areas of epithelial necrosis around the preputial orifice to more extensive geographic ulceration and granulation tissue that results in occlusion of the preputial orifice. The occlusion leads to the accumulation of urine and pus and, ultimately, to extensive internal ulceration of the prepuce, the urethral process, and the head of the penis.[3]

Balanitis occurs in all domestic species and is caused by a variety of familiar infectious agents, including bovine herpesvirus I in bulls; canid herpesvirus 1 and leishmaniasis in dogs; and Equid herpesvirus 1, *Trypanosoma equiperdum* (the causative agent of dourine), cutaneous habronemiasis, pythiosis, and cutaneous halicephalobiasis in horses.[3]

Conference participants began discussion of this case by noting the large colonies of bacteria, noting that these large colonies are a possible way to begin narrowing down the list of possible etiologic agents to one of the YAACSS agents (*Yersinia, Actinomyces, Actinobacillus, Corynebacterium, Streptococcus,* and *Staphylococcus*). The moderator cautioned participants not to read too much into the large colonies, as virtually any agent can form large colonies given enough time.

The agent isolated in this case is, of course, a member of YAACSS, albeit a somewhat enigmatic one, and discussion of this case largely centered on whether *C. xerosis* was the actual cause of the histologic lesions or just an opportunist. The question is largely rhetorical, and Dr. Bender used the discussion as a pivot to other *Corynebacterium* spp. of veterinary importance, including *Corynebacterium renale* and *Corynebacterium pseudotuberculosis*.

Discussion of the morphologic diagnosis centered largely on how to capture the unique appearance of this lesion and how to describe the bacteria. Participants felt the primary lesion was ulceration and that the granulation tissue was abundant enough to merit specific mention. Participants also felt that the bacterial population consisted of both cocci and bacilli and preferred to describe the bacterial population as mixed.

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