Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 7

19 October 2016

Conference Moderator:
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CASE I: 16-23 (JPC 4084136).

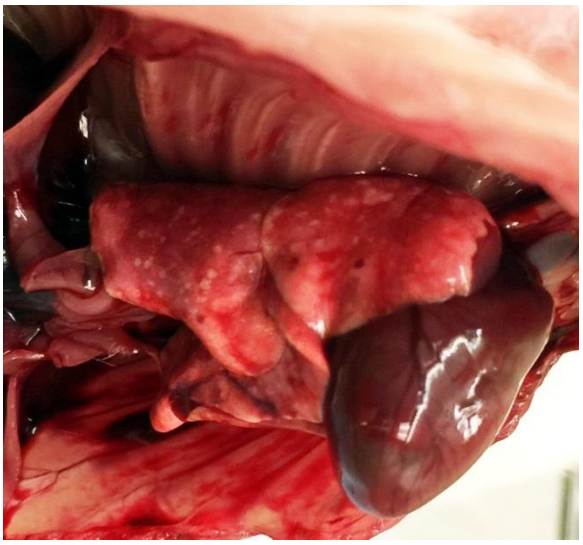
Signalment: Four-month-old, male, X-SCID rat (*Rattus norvegicus*).

History: Rats within this colony were under clinical and pathologic investigation after a positive serology result for *Pneumocystis carinii* during routine quarterly sentinel surveillance. Few rats displayed evidence of mild to moderate respiratory distress and were sacrificed for follow-up *P. carinii* PCR and lung histopathologic evaluation. Lesions in the lungs suggested another viral etiologic agent in addition *to P. carinii*, and a full pathology evaluation was performed on two more rats.

Gross Pathology: Characteristic gross findings expected for the X-SCID strain (1) were present in all rats examined and consisted of severe thymic hypoplasia, unidentifiable lymph nodes, and hypoplastic spleens. All adult rats had mild crusting of the rostral nasal turbinates and multifocal, 1-2 millimeter diameter, white-tan foci on the pleural surface and fewer within the parenchyma on cut section. Few similarly-sized red foci were also present on the pleural surface and throughout the parenchyma.

Laboratory results: Rats within this colony tested positive for *Pneumocystis carinii* via serology and PCR of nasal swabs.

After histopathology was performed, additional PCR tests were submitted for rat cytomegalovirus and mouse adenoviruses 1 & 2. An additional PCR for polyomavirus was performed with primers designed using



Lung, X-SCID rat. Adult rats in this group had severe thymic hyperplasia, and multifocal 1-2mm white-tan as well as red foci are scattered through the parenchyma. The white-tan foci are consistent with Pneumocystis carinii. (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)

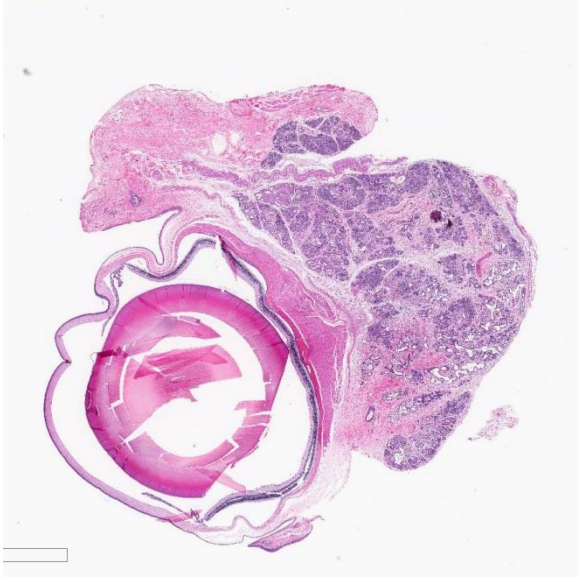
a target a region of the VP1 gene that is partially conserved among polyomaviruses, including those found in mice and hamsters; all follow-up PCR tests were negative.

Histopathologic Description: Harderian gland: There is severe atrophy and loss of the glandular acini with replacement by fibrous connective tissue. The epithelium of remaining glands is often necrotic or attenuated and infiltrated by mixed inflammatory cells composed of lympho-cytes, plasma cells, macrophages, and fewer neutrophils and mast cells. These inflammatory cells are present surrounding glands, ducts, and extend into fibrous connective tissue. There are increased numbers of intralobular and interlobular ducts which are dilated and lined by hyperplastic or attenuated epithelium and multifocally contain sloughed cells and cellular debris. Occasionally, ductal and acinar epithelial cells contain eosinophilic to amphophilic intranuclear inclusion that are up to 35µm in diameter and are sometimes surrounded by a clear halo.

Contributor's Morphologic Diagnosis: Harderian gland: Acinar atrophy, diffuse, severe, with fibrosis, lymphoplasmacytic inflammation, ductal hyperplasia, and epithelial intranuclear inclusion bodies.

Contributor's Comment: Additional histopathologic lesions, including epithelial necrosis, hyperplasia, dysplasia, and intranuclear inclusion bodies, were present in the nasal cavity, lung, salivary gland (parotid and submandibular), prostate gland, and uterus.

Immunohistochemistry of the organs listed above showed strong staining with the pan-polyomavirus marker, PPIT.[6] The virus was

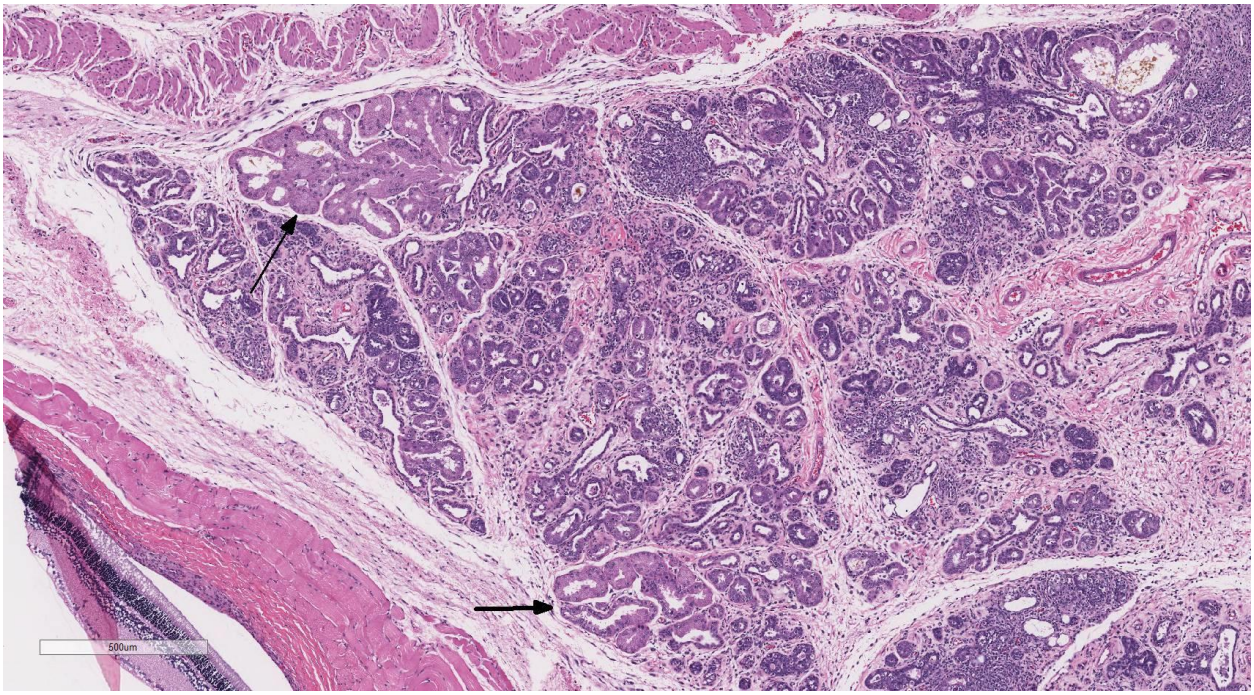


Eye, with Harderian gland, X-SCID rat. At subgross magnification there is marked basophilia of the Harderian gland with dilation of numerous glands and expansion of the interstitial connective tissue. (HE, 4X)

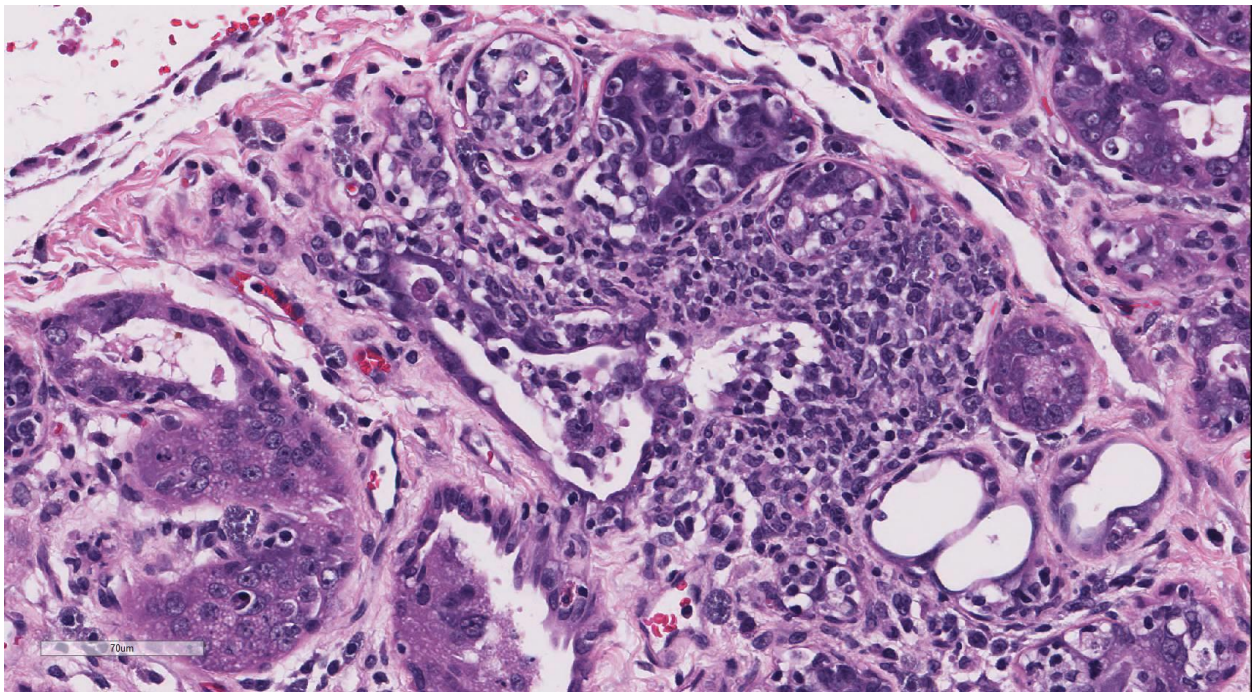
subsequently isolated from the salivary and Harderian glands of rats within this colony and sequenced. This is a novel polyomavirus phylogenetically distinct from the rat polyomavirus isolated and sequenced from feral Norway rats in 2015.[2]

Polyomaviruses (PyVs) are a family of DNA tumor viruses that are known to infect a variety of mammals, birds, and fish.[1] Most mammalian polyomaviruses cause subclinical infections with life-long persistence in their natural immune competent hosts. However, when the host immunity is compromised, the virus can reactivate and cause disease.[5] Until this discovery, five distinct PyVs have been identified in rodent hosts: murine PyV, mouse pneumotropic virus, hamster PyV, Mastomys PyV, and Rat PyV, whose full genomes are available in the GenBank database.[2]

The first polyomavirus in rats was described as a wasting disease in athymic nude rats by Ward, et al, in 1984.[7] Inclusion bodies were described in the salivary glands, Harderian glands, lungs, and nasal glands, similar to those present in this colony.[7] Also, similar to the previously described report is the fact that X-SCID rats are severely immune suppressed.[3] This strain has severely hypo-plastic lymphoid organs and markedly decreased T cells, B cells, and NK cells, making them an excellent model for xenotransplantation studies.[3] This severe immune suppression makes them particularly susceptible to viral infections like PyV. It is not yet clear where these rats were infected with the virus or whether immune competent rats can be infected, show clinical symptoms, or have histologic



Harderian gland, X-SCID rat. There are diffuse changes within the Harderian gland. Few normal acini remain (black arrows). Other acini exhibit necrosis, atrophy, and regeneration, as well as moderate lymphocytic inflammation. (HE, 46X)



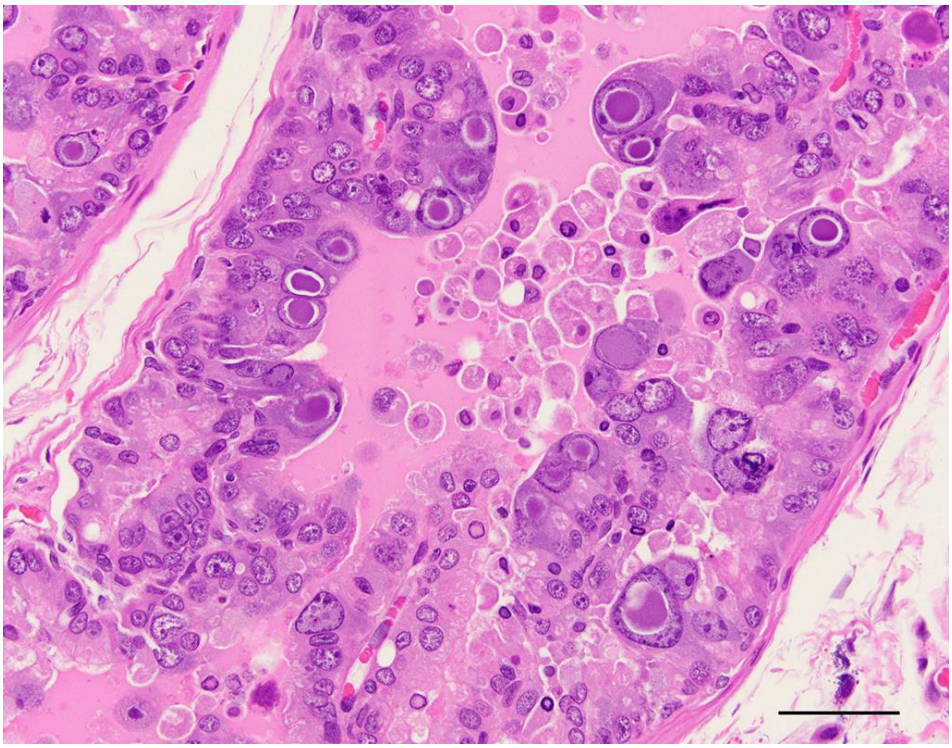
Harderian gland, X-SCID rat. Affected glands display a range of features including necrosis, attenuation of lining epithelium, and regenerative changes including piling up and mild dysplasia. Affected acini are surrounded and infiltrated by moderate numbers of lymphocytes, macrophages, and fewer neutrophils. (HE, 400X)

lesions when infected with this novel Rat PyV. A full description of pathology findings and genomic sequencing information for this novel virus is pending publication (Rigatti and Toptan, et al).

JPC Diagnosis: Harderian gland: Dacryoadenitis, necrotizing and histiocytic, chronic, diffuse, moderate to severe, with edema and occasional epithelial intranuclear inclusion bodies, X-SCID rat, *Rattus norvegicus*.

Conference Comment: The contributor provides an outstanding description and synopsis of the lesions of a novel polyomavirus (PyV) infection in an X-SCID rat. Particularly striking are the characteristic large, prominent epithelial intranuclear viral inclusions that marginate the chromatin and often enlarge the nucleus; there is variation among the slides in the number of intranuclear inclusions present.

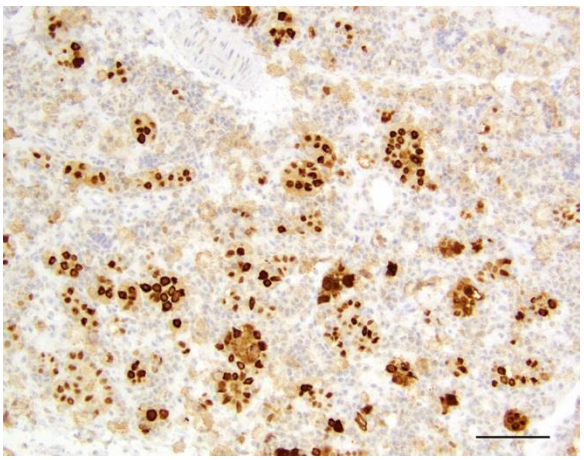
As mentioned by the contributor, the majority of mammalian PyVs cause subclinical infections with life-long persistence in immune competent natural hosts, much like herpesviruses.[4] However, when the host immunity is compromised, such as in this particular strain of rat, the virus can cause disease. Polyomaviruses are of particular research interest, and murine PyVs are used as models of



Harderian gland, X-SCID rat. Occasionally, ductal and acinar epithelial cells are karyomegalic and contain eosinophilic to amphophilic intranuclear inclusions that are up to 35µm in diameter and are sometimes surrounded by a clear halo (HE, 400X) (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)

persistent virus infection in human disease.[1,2,3,6] The most well-known human PyVs, BK virus and JC virus, are associated with severe disease in immunosuppressed human patients; and Merkel cell PyV is associated with Merkel cell carcinoma, a rare and highly aggressive neoplasm of neuroendocrine cells of the skin.[2] The virus has long been established as potentially carcinogenic, causing many different types of tumors in experimental systems, hence the name poly(many)-oma(tumor)-virus.

Conference participants noted that this case nicely demonstrates cytomegaly, karyomegaly, and glassy intranuclear inclusions characteristic of PyV infection in the Harderian gland. Many participants noted that rat cytomegalovirus infection can cause similar inclusions in the Harderian gland of rats, with large eosinophilic "owl-eye" inclusions that marginate the chromatin.[4] However, PyV inclusions in tissue have a homogenous basophilic or amphophilic appearance, which is distinct from cytomegalovirus and adenoviral inclusions.[4] Participants also discussed that sialodacryoadenitis virus, a highly contagious betacoronavirus, which can cause similar lesions in the Harderian gland of rats; however, that virus does not result in the formation of intranuclear inclusions.[4]



Harderian gland, X-SCID rat . **Acinar epithelial cells exhibit strong intracytoplasmic positivity for pan-polyomavirus marker(anti-PPIT(2), 100X)** ***(Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)***

Attendees discussed some other significant PyVs of veterinary importance, including simian virus 40 (SV40) which caused progressive multifocal leukoencephalopathy in immunosuppressed rhesus macaques; the *Mesocricetus auratus* PyV1 which induces trichoepithelioma and lymphoma in hamsters; the K virus and murine pneumotropic virus in mice; *Procyon lotor* PyV1 which causes high-grade neuroglial olfactory tumors in raccoons; Aves PyV1 that results in budgerigar fledgling disease in psittacine birds; and goose hemorrhagic PyV1, the cause of hemorrhagic nephritis and enteritis in anseriform birds.[1-7]

The conference moderator cautioned participants that, while inclusions present in the intra-orbital Harderian gland are due to viral infection, pseudoinclusions and syncytial cells in the exorbital lacrimal gland are part of its normal anatomy and should not be confused for viral cytopathic effect. In addition, participants noted numerous mast cells within the interlobular connective tissue, which is also a normal finding in rats. The moderator further observed that within the adjacent eye the retinal epithelium lacks pigment, indicating that this rat is an albino. As a result of the lack of pigmentation, albino rats are much more susceptible to retinal degeneration and cataract formation induced by ultraviolet light as compared to normally pigmented animals. Degenerative changes may also occur in the Harderian glands of rats exposed to high-intensity lights.[4]

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CASE II: 13A815 (JPC 4066315).

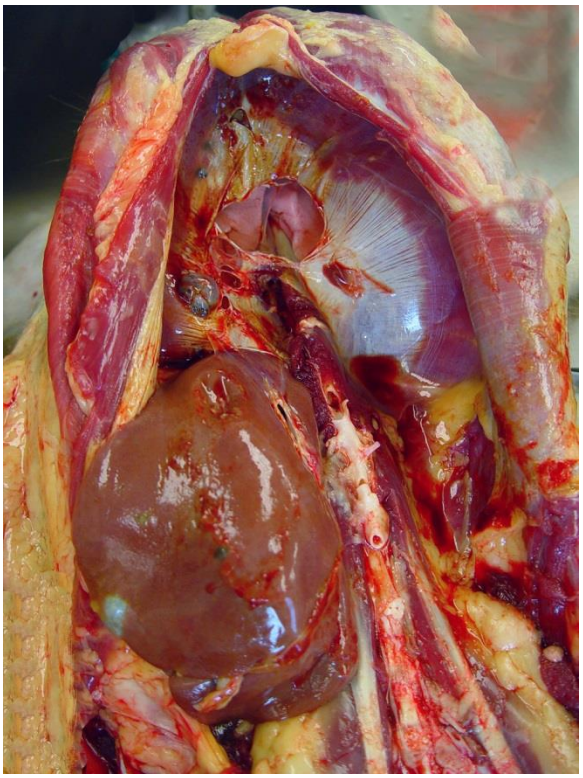
Signalment: 20-year-old female Indian rhesus macaque (*Macaca mulatta*).

History: This animal had 2 months of lethargy, large clitoris, pain/discomfort vaginal bleeding. The body weight lost 3 kg in 2 months. A large firm mass was palpable in the lower abdomen, mainly on left side. The mass was round, measuring 7 x7 cm. Ultrasound revealed large mass with multiple round cavities inside. The animal developed a moderate regenerative anemia. Although extensive care and treatment were given, euthanasia was elected due to the grave prognosis.

Gross Pathology: Presented was a thin, dehydrated aging animal. A large, firm mass was palpated at the lower abdomen. The subcutaneous tissue over the abdominal mass was dark-red and edematous. Extending from the uterus to the abdominal wall and incorporating with ovaries, ovary ducts, and serosa of the colon was a 15 -20 cm in diameter, dark-red cystic masses, which contained a large amount of dark-red fluid on the cut section. Multifocal variable-sized, dark-red cystic masses are also noted on the diaphragm, liver, and mesentery. The central tendon of the diaphragm was fragile and easily pierced by force. Other findings included severe thymic atrophy and mild, bilateral hydronephrosis.

Laboratory results: N/A

Histopathologic Description: Expanding and disrupting the diaphragm are multiple, unencapsulated masses composed of variable tortuous endometrial glands surrounded by abundant, densely cellular endometrial stroma. The endometrial glands are generally lined by simple columnar, ciliated epithelial cells. Some parts of glands are lined by flattened to pseudostratified cells. Occasionally the epithelial cells form islands or papillary projections in the lumen. The epithelial cells have indistinct cell borders, a moderate amount of eosinophilic cytoplasm, and prominent basilar nuclei. Nuclei are round to oval with finely stippled



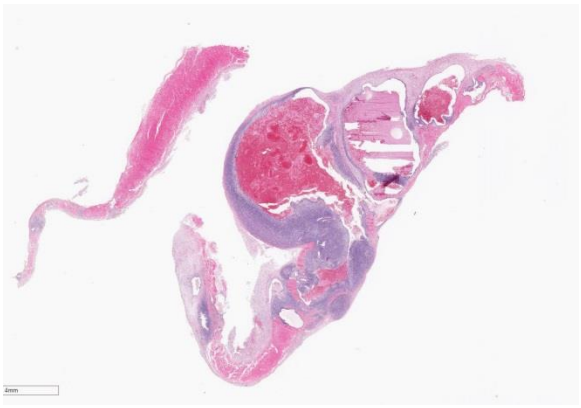
Diaphragm, rhesus macaque. Multifocal variably-sized, dark-red cystic masses are present on the diaphragm, liver and mesentery. A large defect is present in the tendinous portion of the diaphragm caused by digital pressure. (Photo courtesy of: Division of Comparative Pathology, Tulane National Primate Research Center, 18703 Three Rivers Rd., Covington, LA 70433. http://tulane.edu/tnprc/)

chromatin and 1-2 prominent nucleoli. There are large amounts of amorphous, eosinophilic material and admixed with numerous erythrocytes. The endometrial stroma is composed of spindle cells with indistinct cell borders, scant eosinophilic, fibrillar cytoplasm and an oval to elongate nucleus with finely stippled chromatin. The mitotic figures are 1-2/HPF in both glandular epithelium and stroma. Diffusely the serosa is markedly expanded by reactive fibroblasts, edema, hemorrhage, and many lymphocytes, plasma cells, and macrophages. Some macrophages contain abundant cytoplasmic brown pigment (hemosiderosis). Multifocally the muscle adjacent to the masses showed variable degeneration and necrosis.

Contributor's Morphologic Diagnosis: Diaphragm, Endometriosis.

Contributor's Comment: Endometriosis usually occurs in the pelvis and the most commonly involved organs are ovaries, uterosacral and broad ligaments, and parietal pelvic peritoneum. Diaphragmatic endometriosis is rare, often asymptomatic, and always associated with severe pelvic involvement.[4,7] Ectopic endometriosis has also been reported in umbilicus, skin, vagina, vulva, cervix, inguinal canal, upper abdominal peritoneum, liver, spleen, gastrointestinal tract, urinary system, breasts, pleural cavity, brain, eye, lymph nodes, lung and pericardium in human.[4] Pleural and lung endometriosis have been reported in an aged rhesus macaque1 and sooty mangabey (2009 conference 19, case 01), respectively.

Diaphragmatic endometriosis, like in this case, can involve entire thickness of the muscle. It is common to extend into pleural space in human, but not presented in this



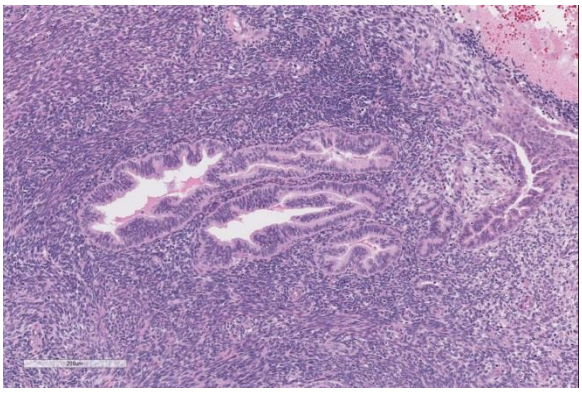
Diaphragm, rhesus macaque. The diaphragm is expanded by several cellular masses which contain cystic, blood-filled centers. (HE, 5X).

case.[4] The pathogenesis of endometriosis has been well discussed in the previous conferences (2011 conference 11, case 01 and 2009 conference 19, case 01). The primary theory of Sampson's three-fold transplantation likely applies to this case. The retrograde "regurgitation" of endometrial cells passes through the oviducts into the peritoneal cavity and proliferates in ectopic sites.[8]

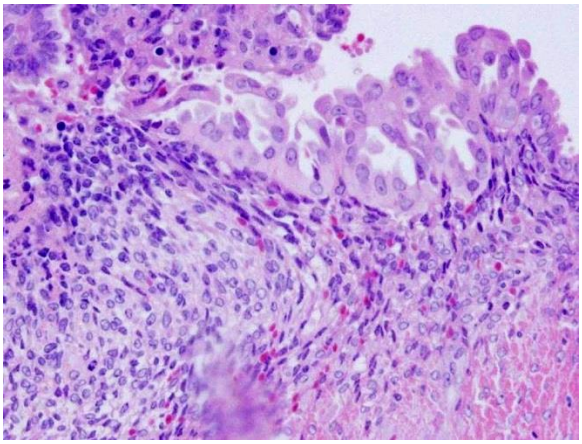
JPC Diagnosis: Diaphragm: Endometriosis, rhesus macaque, *Macaca mulatta*.

Conference Comment: Endometriosis is characterized by the presence of well-differentiated, viable, and hormone responsive endometrial glands and stroma outside the uterus.[1,4,5,7] This is one of the most common gynecologic diseases encountered in female humans as well as Old World primates.[1] There have been rare reports of endometriosis in the elephant shrew, long-tongued bat, and one case report of an endometrioma in a dog.[3,7] The most widely studied animal model for endometriosis in humans is the baboon. The prevalence of endometriosis in captive female baboons is as high as 20%. It is thought that baboons in captivity develop endometriosis at a higher rate than wild baboons because of regular menstrual cycles without intervening pregnancy in this population of animals.[7] The vast majority of endometriosis cases in Old World primates occurs within the abdominal cavity and is grossly identifiable as blood-filled "chocolate" cysts which can progress to fibrotic scars in chronic cases. Typically, endometriosis demonstrates positive immunoreactivity for pancytokeratin, vimentin, estrogen receptor, and progesterone receptor.[1]

In this case, several conference participants noted that the endometrial glands are filled with abundant hemorrhage, consistent with active menses in this animal. The endometrial tissue in endometriosis is responsive to cycling estrogen and progesterone, and therefore will undergo cycles of proliferation and degradation in response to the normal menstrual cycle.[1,7,8] Hemorrhage associated with endometriosis can be severe enough to cause anemia and can rarely rupture causing hemorrhagic



Diaphragm, rhesus macaque. The masses are composed of well-differentiated endometrial glands surrounded by dense interlacing bands of endometrial stroma. (HE, 320X)



Diaphragm, rhesus macaque. There is multifocal epithelial plaque formation and decidualization of underlying stroma (Arias-Stella reaction)(Photo courtesy of: Dr. Tim Cooper)(HE, 400X).

ascites.[1,2,7,8] Conference participants discussed various risk factors for the development of endometriosis in non-human primates. These include chronic uninterrupted estrus cycles throughout life, resulting in increased endometrial turnover compared to multiparous primates. Females older than ten years with an affected first-degree relative are also predisposed. Non-laparoscopic abdominal surgical procedures, including hysterectomy and cesarean section, are also implicated, in addition to estradiol implants.[1,5] The moderator also discussed that aged non-human primates are much more likely to develop endometriosis compared to older women due to lack of menopause in these species.[5] Unfortunately, the reproductive history of this animal was not provided.

The conference moderator also discussed the importance of distinguishing well-differentiated endometrial tissue in endometriosis from endometrioid carcinoma. In endometrioid carcinoma, glands will be irregular with little to no intervening stroma and demonstrate significant nuclear atypia. Endometrioid carcinoma has been rarely reported to develop from endometriosis.[1,4] In addition, multifocally within this section there are large areas where the endometrial epithelial cell nuclei are rounded and vesicular with abundant pale vacuolated cytoplasm, consistent with stromal decidualization and epithelial plaque reaction. Decidualization of the endometrial glands occurs under the influence of progesterone and is a response to blastocyst implantation or other trauma to the endometrial stroma. This is required for maintenance of normal pregnancy in humans and non-human primates.[2] In endometriosis, it is thought that this reaction develops secondary to elevated progesterone. Endometrial decidualization is non-neoplastic, but grossly and histologically mimics malignant neoplastic lesions such as mesothelioma and carcinomatosis. In a recent article in *Veterinary Pathology*, Atkins et al. demonstrates that the decidualized stroma stained positive for vimentin, CD10, progesterone, and estrogen consistent with reported deciduosis in humans.[2]

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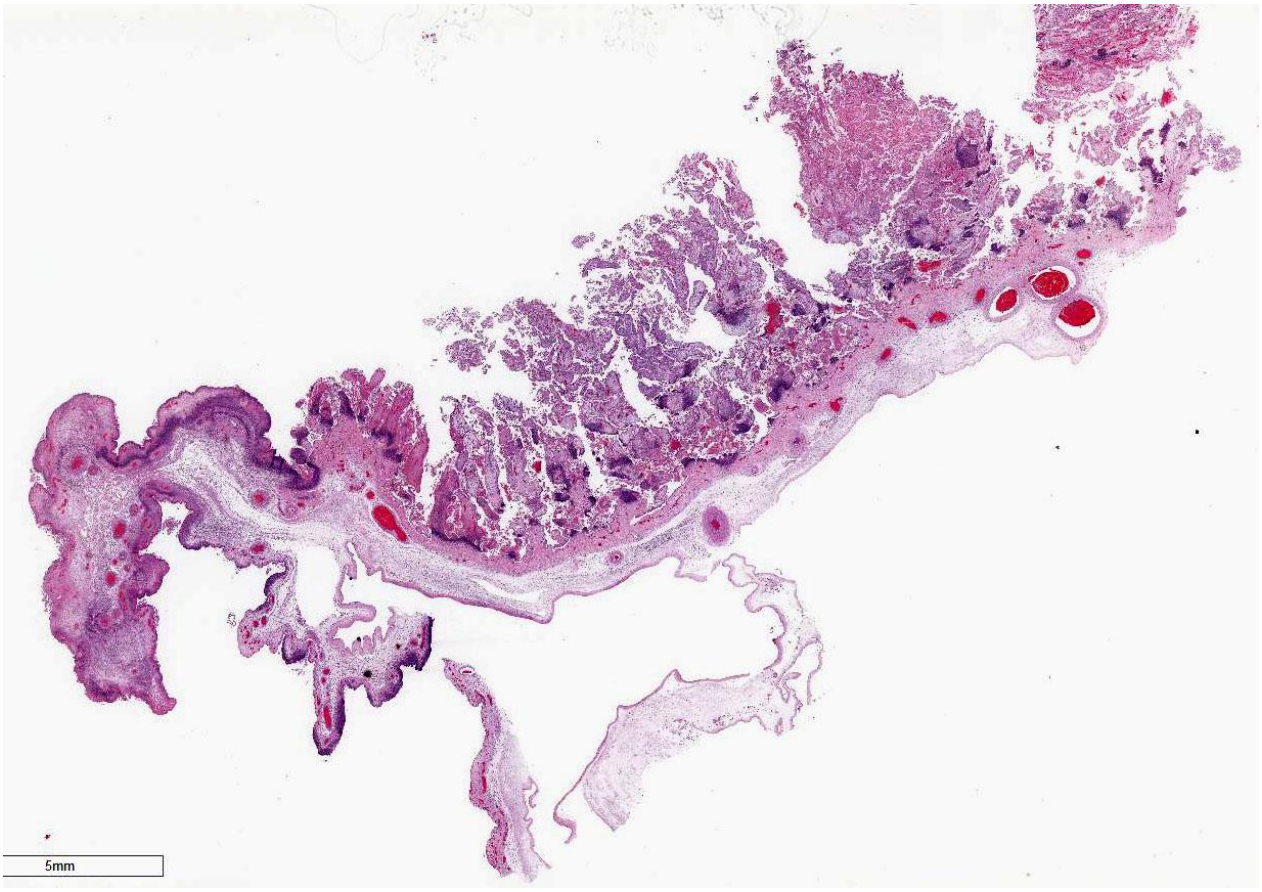
CASE III: 16-3320D (JPC 4083858).

Signalment: Age unspecified female Columbia X Rambouillet ewe (*Ovis aries*).

History: A flock of 7500 ewes were



Placenta, sheep. There is multifocal to coalescing necrosis of cotyledonary, intercotyledonary, and allantoic epithelium, often outlined by a dense band of cellular infiltrate. (HE, 4X)

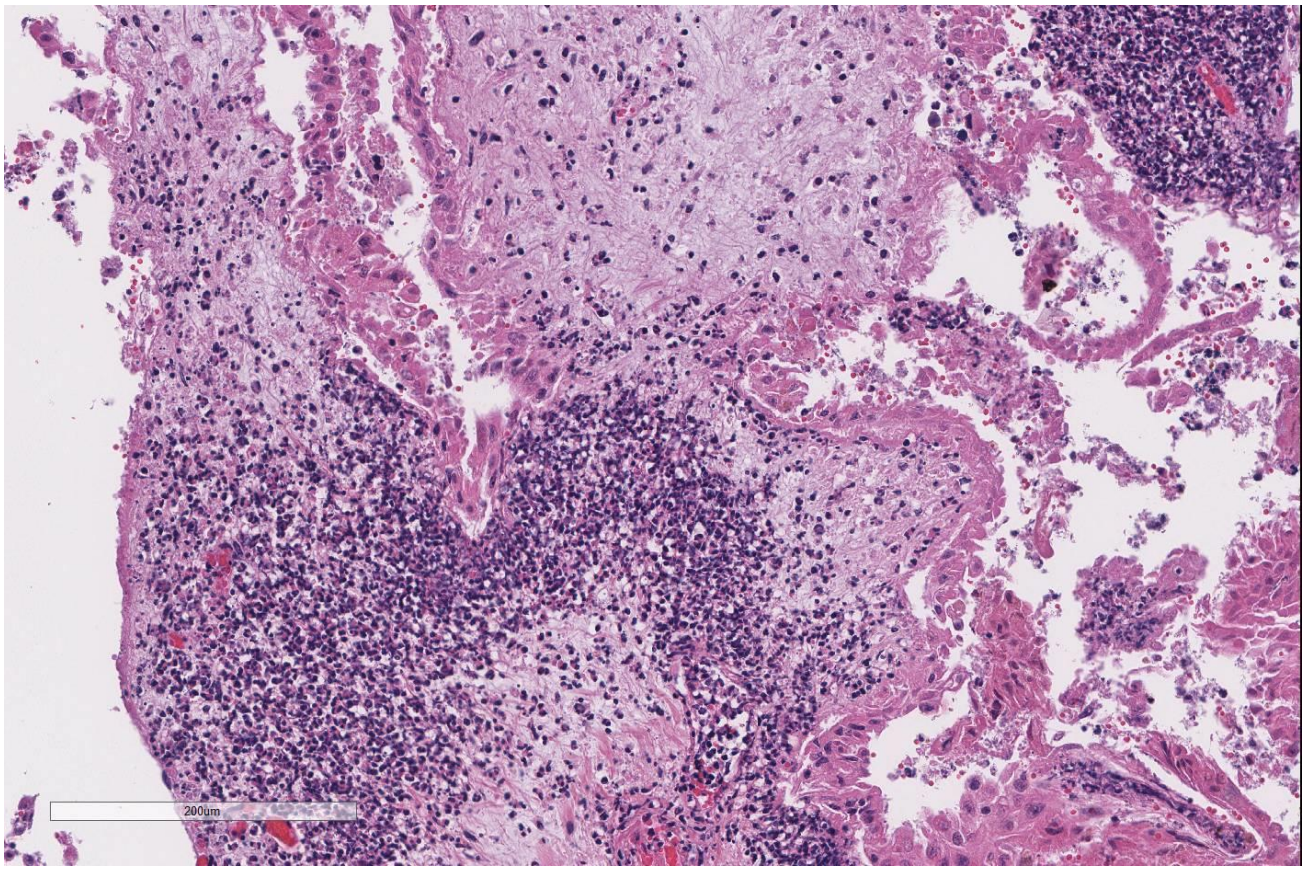
grouped in mobs of 1500. Over the course of the 2016 lambing season, 1200 ewes, ranging in age from 2 – 8 years aborted. Abortions continued even after feeding tetracycline pellets. At the end of the lambing season, older aborted ewes were culled and younger recovered ewes were mixed with ewe lambs as a vaccination strategy.

Gross Pathology: Three fetuses and placentas were markedly autolyzed and had no significant gross lesions. A fourth placenta was in good to fair post-mortem condition. That placenta had inter-cotyledonary edema and multifocal tan-grey discoloration of cotyledons.

Laboratory results: Numerous *Campylobacter jejuni* were isolated from one of four placentas. Other tests performed with negative results included FA for *Leptospira interogans*, ELISA for *Chlamydophila* sp. and PCR for pestivirus. Selenium level in one of three livers was marginal. Immunohistochemistry for *Coxiella burnetii* on fixed placenta was negative.

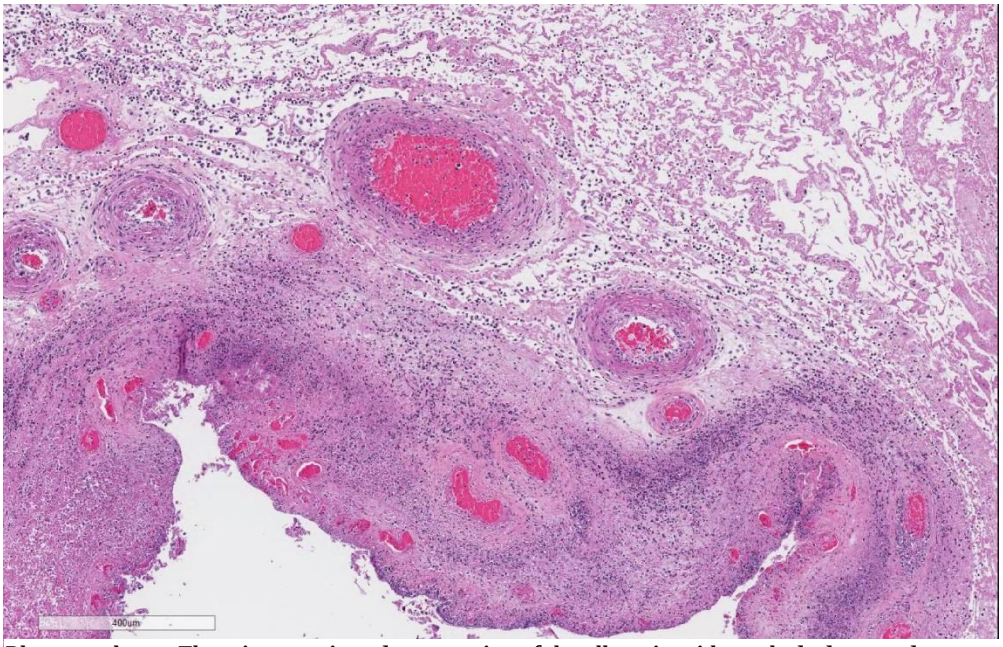
In two previous submissions from the same farm, *C. jejuni* was isolated from fetal tissue pools, stomach contents or placentas from four additional aborted fetuses.

Histopathologic Description: Both cotyledonary and intercotyledonary areas were characterized by necrosis of tropho-blastic and intercoteledonary epithelium,



Placenta, sheep. Necrotic cotyledonary vill exhibit diffuse loss of cytoplasmic staining; a dense band of degenerate neutrophils is present the necrotic villus. (HE, 4X)

with hypereosinophilic shrunken cytoplasm and karyorrhectic or karyolytic nuclei. Foci of necrosis were expanded by necrotic cellular debris and large numbers of degenerate neutrophils within the immediate underlying chorioallantoic connective tissue. Several areas of necrosis are associated with many clustered bacilli. Adjacent trophoblasts often contain small intracytoplasmic bacilli. Rare trophoblasts contain, intracytoplasmic, 2 µm diameter, basophilic material. Scattered throughout the chorioallantois, many blood vessels are surrounded by neutrophils and a few vessels are partially occluded by large aggregates of fibrin and neutrophils. Rare small vessels are lined by necrotic endothelial cells with separation of the wall by neutrophils and fibrin (fibrinoid necrosis). Diffusely the chorioallantoic connective tissue is expanded by edema and all vessels are markedly congested.



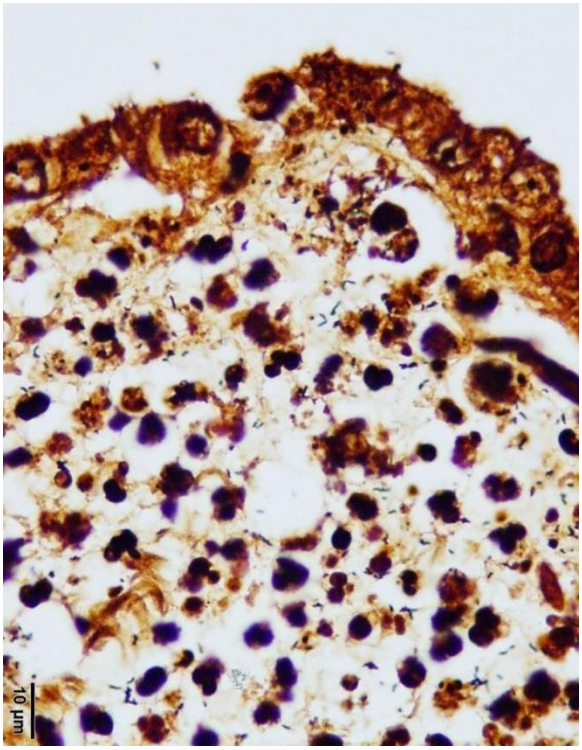
Placenta, sheep. There is necrosis and suppuration of the allantois, with marked edema and moderate necrotizing arteritis of the allantochorion. (HE, 100X)

Similar lesions were seen in placentas from the other fetuses submitted. No significant lesions were identified in any of the fetal tissues.

Contributor's Morphologic Diagnosis: Placentitis, necrosuppurative, cotyledonary and intercotyledonary, diffuse, severe, with necrotizing vasculitis and bacteria.

Contributor's Comment: *Campylobacter jejuni* is one of three species in the genus causing reproductive and enteric disease in a variety of animal species and in humans.[8] *C. fetus subsp. venerealis* primarily causes infertility and abortion in cattle, whereas *C. fetus subsp fetus* and *C. jejuni* are important causes of abortion in small ruminants and occasionally cattle. *C. jejuni* is an important cause of food-borne illness in people and has become an increasingly important cause of late term abortions in small ruminants.[4] Abortions have also been documented in humans and in dogs.[6] In sheep, infection by either *C. fetus f*etus or *C. jejuni* causes late term abortion, still birth or weak lambs. Transmission is by the oral route. Placentas are not retained and often have gross lesions of intercoteledonary edema and cotyledonary necrosis. Aborted fetuses may have characteristic gross lesions of necrotizing hepatitis and histologic lesions of suppurative bronchopneumonia (neither present in this case). Occasionally ewes become ill and die due to endometritis.[8]

Campylobacter is reportedly the most common cause of abortion in sheep and *C. jejuni* is now the most common species to cause abortion in sheep flocks.[4] Recently, a single clone of *C. jejuni* (SA) has been shown experimentally to cause abortion in sheep[9] and it is genetically similar to clones causing gastroenteritis in people.[5] In addition, most isolates of *C. jejuni* from sheep abortions in the United State (including the one in this case) are highly resistant to tetra-cyclines, the only approved drug for treating infection in sheep.[4] In contrast, isolates from the United Kingdom are susceptible to tetracyclines[9], suggesting common treatment of sheep abortions with teracyclines in this country may have led to the emergence of the resistant clone. Drug resistance and links to human enteric disease indicate that owners, veterinarians, and laboratory personnel should be cautioned about zoonotic potential when handling suspect fetal tissues.



Placenta, sheep. Villar stroma contains numerous short often branching bacilli consistent with Campylobacter. (Warthin Starry 4.0, 400X). (Photo courtesy of: Dr. Tim Cooper).

Other important differential diagnoses for placentitis in sheep and goats include *Brucella ovis*, *Brucella mellitensis*, *Toxoplasma gondii*, *Chlamydophila abortus* and *Coxiella burnetii*.[8] The most common manifestation of ovine brucellosis in the US is epididymitis in rams; abortion with placentitis is less common. *Toxoplasma gondii* should have characteristic gross and histologic lesions, with the presence of organisms within the placental lesions, but can also be ruled out by PCR. The three main differentials, in this case, are *C. jejuni*, *C. abortus* and *C. burnetii* due to the presence of intracellular bacteria on H&E. The bacteria were not positive with the Gimenez stain, making diagnosis of the two latter organisms less likely. *C. abortus* was eliminated by Ag ELISA on the placenta and *C. burnetii* by immunohistochemistry. Because of the high likelihood of encountering zoonotic agents associated with small ruminant abortions, examination of all sheep and goat abortuses, especially if accompanied by fetal membranes, should be performed in a biosafety cabinet.

JPC Diagnosis: Placenta, cotyledonary and intercodyledonary: Placentitis, necrosuppurative, diffuse, severe with necrotizing vasculitis.

Conference Comment: The contributor provides a concise summary of abortion in small ruminants caused by a *Campylobacter* spp as well as other important differential diagnoses for abortion in these animals. Despite some minor slide variability, conference participants unanimously noted the outstanding preservation and high quality of the section of placenta in this case.

Prior to discussing the case, the conference moderator spent some time reviewing placentation in ruminants. The different components of the placenta were discussed and participants identified individual layers and their orientation within the tissue section. All ruminants have cotyledonary villous epitheliochorial nondeciduate placentation. The placenta is comprised of the maternal endometrium and the fetus derived fused chorioallantoic membranes (CAM). Because ruminant placentas are nondeciduate, the maternal endometrium and fetal CAM are in contact but they do not fuse. In addition, in cotyledonary placentation, there are multiple areas where the CAM villi insert into pockets or crypts in the area of the endometrium known as the placentome, which is a combination of the fetal cotyledon and maternal caruncle. Specific to small ruminants, the caruncles have lost their epithelium, leaving five tissue layers which separate maternal and fetal blood: endothelium, connective tissue, epithelium of the CAM, and endothelium and connective tissue of the endometrium.[1]

Conference participants noted that, in this case, there are multifocal brown globular pigment present in the maternal side of the placenta in the subchorial area where hematoma and hemophagocytosis are often most prominent and a normal finding. Additionally, thrombosis within the chorionic plate, present in some slides in this case, is also a normal finding in the post-partum placenta. However, if there is similar brown staining material on the CAM, it could be indicative of meconium deposition, which is a result of pre-parturient fetal stress. Meconium staining was not seen by conference participants in this case.

Transmission of *Campylobacter* spp. often occurs via fecal-oral route most commonly through contamination of water supplies.[8] The organism is a common commensal bacterium in the intestinal tract of cattle, sheep, and swine as well as dogs, cats, and rodents. When taken in orally in susceptible animals, there is a transient bacteremia. The bacteria are then localized to the gut and bile. In pregnant ewes, the bacteria localize to the uterus via the Surface (S)-layer protein, which is thought to allow the bacteria to colonize and translocate from the uterus to the placenta and subsequent abortion in about 25% of cases.[8]

Characteristic findings of campylo-bacteriosis are edematous intercotyledonary areas and friable yellow cotyledons with necrotizing and suppurative placentitis and vasculitis most severe in chorionic villi. There will often be large dense Gram-negative bacterial emboli within chorionic capillaries, although that was not a prominent feature in this case.[8] However, numerous *Campylobacter* spiral organisms are present throughout the tissue and easily visualized on the Warthin-Starry silver stain. Many conference participants noted intra-cellular bacilli within trophoblasts on the H&E. In the fetus, there will typically be yellow hepatic foci with targetoid depressed red centers (necrotizing hepatitis) and fibrinous peritonitis.[8]

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CASE IV: 20109-13350 (JPC 4002842).

Signalment: Nine-year-old, female, Papillon (*Canis familiaris*).

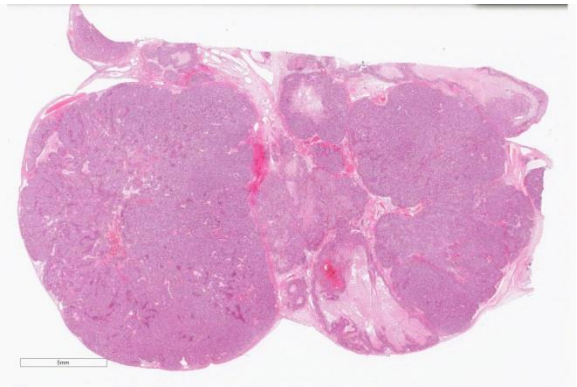
History: The dog presented for the mammary masses near the right third nipple and under the left forth nipple. As a result of the physical examination, the ovarian mass was also found. The ovaries and uterus were surgically resected with the mammary masses. The left ovarian mass, left uterus, and mammary masses were sent to our laboratory for pathological examination.

Gross Pathology: The left ovarian mass after fixed in neutral-buffered formalin was 5.2 x 4.5 x3.2 cm and was soft with milky white smooth to nodular surface. The cut surface showed white to light yellow solid area with necrosis.

Laboratory results: N/A

Histopathologic Description: The left ovarian mass consists of multiple lobules surrounded by a thin connective tissue stroma with very few interstitial glands of original ovarian structures. There are occasional multifocal to coalescing areas of necrosis in the mass. Lymphocytes and plasma cells slightly infiltrated in stroma around tumor cells. Each lobule mainly composed of solid and irregular nests of round tumor cells. Ductal structures and keratinizing epithelial cell nests were often mingled. Neoplastic round tumor cells showed a high N/C ratio and resembled to germ cells of seminoma/dysgerminoma. The

tumor cells have large round nuclei with scattered chromatin and one or a few large nucleoli. The cytoplasm is abundant with weak-eosinophilic or clear, and infrequently vacuolated. Mitotic figures are frequently seen. The nuclear figure of epithelial tumor cells are similar to that of round tumor cells.



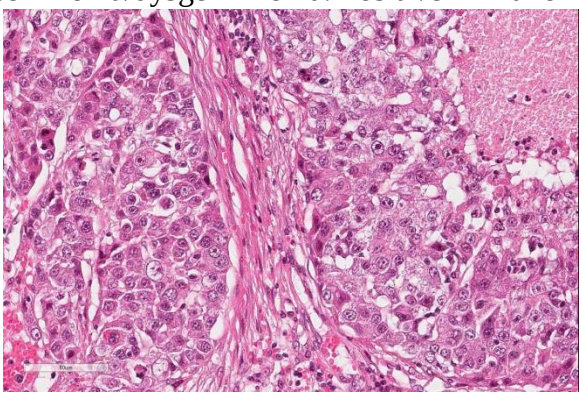
Ovary, dog. The ovary is totally effaced by an expansile multinodular neoplasm with large areas of necrosis. (HE, 4X)

Immunohistochemically, both round and epithelial tumor cells are cytoplasmic weakly positive for alpha-fetoprotein and cytoplasmic granular positive for CD30. Each tumor cell types also are positive for octamer 4 (OCT4). Cytokeratin AE1/AE3 and CAM5.2 is strongly-expressed in the cytoplasm of epithelial tumor cells and weakly positive in less than 50% of round tumor cells. However, cytokeratin 7 and 20 are negative in both tumor cells. Vimentin expression is seen in some part of round tumor cells, but is not observed in epithelial tumor cells.

Contributor's Morphologic Diagnosis: Mixed germ cell tumor in canine ovary (dysgerminoma with embryonal carcinoma).

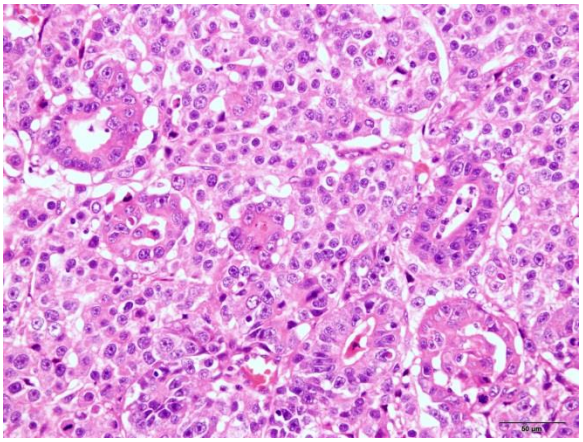
Contributor's Comment: Canine ovarian tumors are divided into sex cord-stromal (gonadostromal) tumors, germ cell tumors, epithelial tumors, and mesenchymal tumors. Epithelial tumors and sex cord-stromal (gonadostromal) tumors are the most common (80-90%). Germ cell tumors are less common, and account for 6% to 12% of canine ovarian tumors. Germ cell tumors are further classified to dysgerminoma, teratoma and embryonal carcinoma according to the WHO classification.[1,2,6,9,] In addition, yolk sac tumor and polyembryoma, chorio-carcinoma, and mixed germ cell tumor are included among ovarian germ cell tumors of human WHO classification. In canine ovarian germ cell tumors, dysgerminoma is most common and followed by teratoma.[2,9]

The present tumor is mostly composed of round tumor cells, which resemble seminoma/dysgerminoma. Positive immuno-



Ovary, dog. The neoplasm is largely composed by sheets of mildly anisokaryotic polygonal germ cells. (HE, 196X)

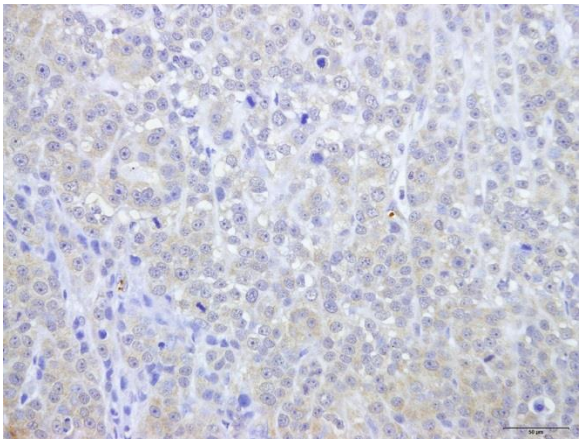
reactivitiy for OCT-4 of round tumor cells was also consistent with that of dysgerminoma, because OCT-4 is sensitive and specific immunohistochemical marker for dysgerminoma,[3] However, ductal structures and keratinizing epithelial cell nests were often mingled, and these tumor cells including round tumor cell showed positive for embryonal carcinoma markers (AFP and CD30). Embryonal carcinoma composed of undifferentiated cells of epithelial appearance with solidly cellular areas, glands, and papillary projections.[8,13,14] Areas of solid growth in embryonal carcinomas histologically resemble dysgerminomas.[8] In humans, embryonal carcinoma is a rare germ cell tumor and



Ovary, dog. Neoplastic cells arranged in ducts are scattered throughout the neoplasm. (HE, 4X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University,45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, JAPAN)

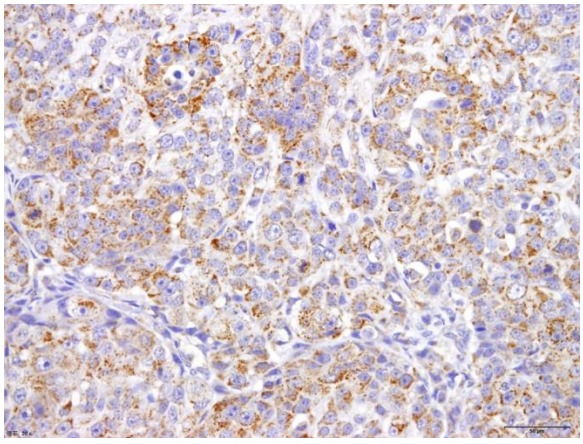
occurs as a component of mixed germ cell tumors more than pure embryonal carcinoma.[14] In animals, no pure embryonal carcinomas have been reported, but a combination (mixed) germ cell tumor with embryonal carcinoma has been reported in only two rats and a cynomolgus monkey.[11,15]

Embryonal carcinomas are immuno-histochemically distinguishable from dysgerminoma based on testing for AFP, CD30, cytokeratin AE1/AE3, CAM5.2 and cytokeratin 7, which are positive in embryonal carcinoma.[3,6,7] Thus, in the present case, immunoreactivity of tumor cells did not perfectly satisfy the diagnostic criterion for both dysgerminoma and embryonal carcinoma. In addition, round tumor cells, which resemble dysgeminoma, are only partially positive for vimentin. In contrast, epithelial tumor cells forming ducts and nests were mostly negative for vimentin. Vimentin is immunopositive in dysgerminoma, and the reactivity is higher than embryonal carcinoma.[4,8,13] We considered that the present tumor was partially differentiated from dysgerminoma, and have the characteristics of both dysgerminoma and embryonal carcinoma. Thus, the present tumor was diagnosed as dysgerminoma with embryonic differentiation (mixed germ cell tumor composed of dysgerminoma and embryonal carcinoma) rather than pure embryonal carcinoma.

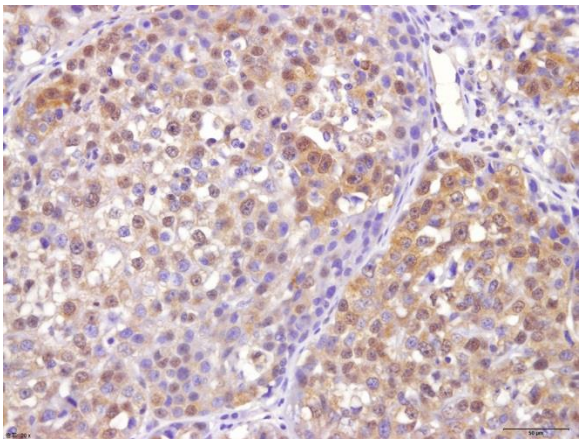


Ovary, dog. Neoplastic germ cells demonstrate weak cytoplasmic immunoreactivity for alpha-fetoprotein. (anti-alphafetoprotein, 200X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University,45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, JAPAN)

JPC Diagnosis: Ovary: Mixed germ cell tumor, papillon, *Canis familiaris*.



Ovary, dog. Neoplastic germ cells demonstrate moderate cytoplasmic immunoreactivity for octamer 4. (anti-octamer 4, 200X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University,45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, JAPAN)

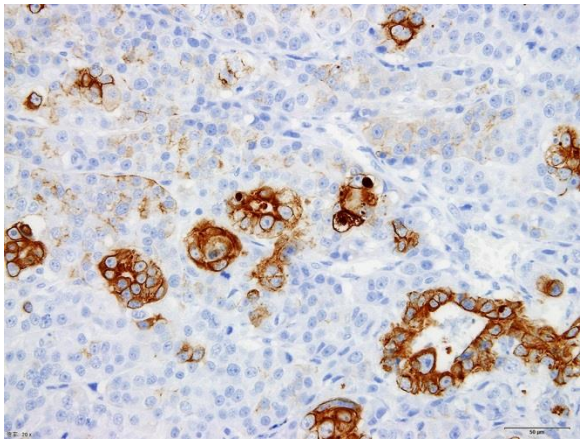


Ovary, dog. Neoplastic germ cells demonstrate moderate cytoplasmic immunoreactivity for CD30. (anti-CD30, 200X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University,45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, JAPAN)

Conference Comment: The contributor provides a challenging diagnostic case of a rare ovarian neoplasm in a dog. Due to near effacement of the normal structures of the ovary by the neoplasm, some conference participants had trouble identifying the tissue as ovary. However, at the periphery of the neoplasm in all examined sections, there is a small amount of subsurface epithelial structures and granulosa cell islands characteristic of canine ovary.

As mentioned by the contributor, tumors of the ovary are uncommon and have been described in many species. They typically originate from three distinct embryologic cell types: epithelial tumors of Mullerian origin (adenoma or carcinoma), sex cord stromal tumors (granulosa cell tumor and thecoma), and germ cell tumors (dysgerminoma, teratoma, yolk sac tumors). Mixed germ cell tumors are a combination of germ cells and sex cord stromal cells. In male dogs, mixed germ cell tumors are the fourth most common primary testicular neoplasm and are typically characterized by a combination of seminoma and Sertoli cell tumor, with the tubular structures of Sertoli cell tumors containing neoplastic germ cells.[5,10,12] They are extremely rare in the ovary with reported cases in a Labrador retriever and a cynomolgus monkey.[10,11,15] Among canine ovarian tumors, granulosa cell tumors and epithelial tumors are by far the most common.[12] In this case, the contributors posit that this is a dysgerminoma mixed with an embryonal carcinoma, favoring the diagnosis of a mixed germ cell tumor.

This interesting case stimulated discussion among conference participants. Some favored the diagnosis of mixed germ cell tumor and others favored a sex cord stromal tumor, dysgerminoma, or a collision tumor. We reviewed this case in consultation with physician genitourinary pathologists at the Joint Pathology Center, who agreed with the contributor and the majority of conference participants, that there are foci suggesting a yolk sac tumor (5%), embyronal carcinoma (~25%) and predominantly dysgerminoma (70%), thus favoring the diagnosis of a mixed germ cell tumor. This case was also studied in consultation with Dr. Robert Foster, a board certified veterinary pathologist and



Ovary, dog. Neoplastic cells which form ductal structures exhibit strong cytoplasmic immunoreactivity for AE1/AE3 cytokeratins. (anti-cytokeratin, 200X) (Photo courtesy of: Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University,45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, JAPAN)

recognized expert with extensive experience in the area of veterinary reproductive pathology. He offers a dissenting view that the highly anaplastic cells may not be germ cells and instead favors the diagnosis of a poorly differentiated ovarian sex cord stromal tumor. He also notes that immunohistochemistry in ovarian tumors can be problematic in domestic species.

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