



WEDNESDAY SLIDE CONFERENCE 2017-2018

Conference 25

9 May 2018

CASE I: 17B-0101 (JPC 4102436).

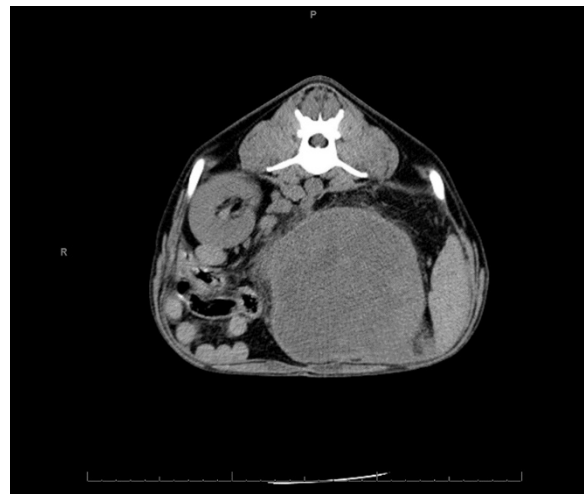
Signalment: 9-year-old, neutered male, mixed breed (*Canis familiaris*), canine.

History: The dog had an approximately 3 week history of hematuria which improved but did not resolve with antibiotics. Abdominal ultrasound and CT scan revealed a mass lesion involving the left kidney. Cytologic evaluation of the mass lesion revealed spindle cells of uncertain origin (reactive vs. neoplastic). The left adrenal gland was not visualized via imaging or during surgery. Surgical findings reported white streaking along the mesentery suggestive of lymphatic invasion.

Gross Pathology: Received is a 20 x 11 x 10 cm multilobulated mass lesion with multifocally adhered omentum. The kidney itself is not apparent until sectioning. On cut section, the mass lesion is soft, multilobular, red and tan with a thin rim of remnant kidney representing approximately 10% of the submitted tissue. The adrenal gland is not apparent.

Laboratory results: None provided.

Microscopic Description: Left kidney: Where anatomic borders are apparent, the leading edge of the mass lesion is necrotic and thus, poorly demarcated. Regions of the border are composed of fibrous connective tissue which could either represent tumor capsule or could represent reactive fibrosis of the kidney.



Presentation, dog. Abdominal computed tomography image of mass in region of left kidney. (Photo courtesy of: University of Wisconsin Madison, School of Veterinary Medicine, Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706)

The mass lesion is composed of multiple lobules of spindle cells usually arranged in streams, but occasionally forming tubules, acini, and rosettes all of which have similar cellular morphology. The fibrovascular stroma is fine. Neoplastic cells have indistinct cell borders, moderate amounts of eosinophilic cytoplasm, and round to oval nuclei with coarsely stippled chromatin. Mitotic figures average 8 in 10 high powered fields examined. Anisocytosis and anisokaryosis are moderate. Multifocally there is abundant tumor necrosis, variably coagulative and lytic, with multifocal hemorrhage. The tumor is separated from the surgical margin by a single layer of spindle cells. There is no evidence of vascular invasion. The adrenal gland is not histologically apparent.

Contributor's Morphologic Diagnosis:
Left kidney: Nephroblastoma

Contributor's Comment: Nephroblastoma is the most common primary renal tumor of swine, chickens and fish, the fifth most common primary renal tumor in dogs, and the fourth most common primary renal tumor in cats.⁷ A tumor with similar morphology also

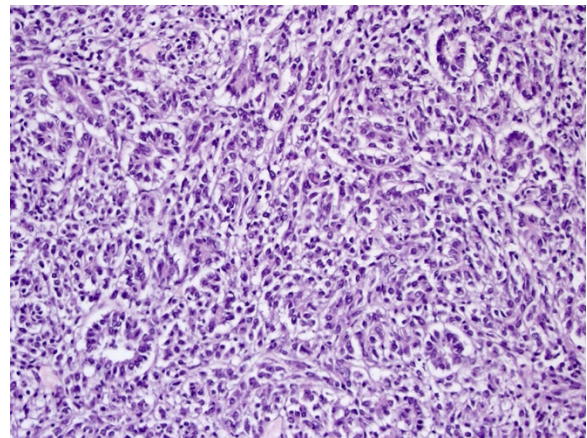


Kidney, dog. Left kidney with adhered omentum. (Photo courtesy of: University of Wisconsin Madison, School of Veterinary Medicine, Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706)

occurs in the thoracolumbar spinal column of young dogs.⁷

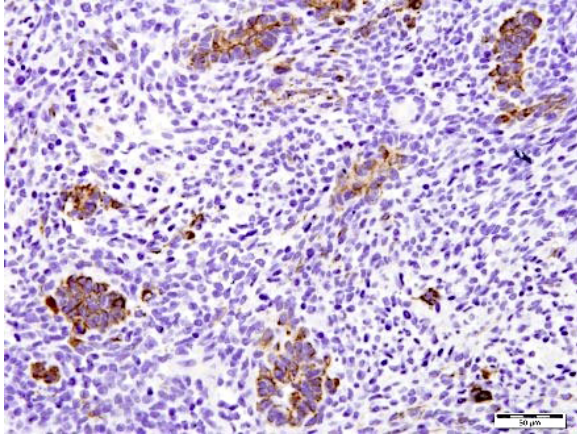
The age distribution in veterinary species follows that in people where it is an embryonal, childhood tumor. In this case and other reported cases in dogs, the tumor is discovered in adulthood but is suspected to either be present from a young age or to arise from remnant nephrogenic rests later in life.^{2,7}

Despite Dr. Meuten's commentary that "searching sufficient sections will eventually identify a few [glomeruloid structures] that



Kidney, dog. The neoplasm is primarily composed of spindled cells, throughout which primitive tubules are scattered. (HE, 400X)

are worthy of the diagnosis or a photograph," numerous DVM and MD pathologists at our institution were unable to do so. Because this tumor did not have classic features of primitive glomeruli, the slides were reviewed with local collaborating MD pathologists with expertise in embryonic renal tumors. Positive immunohistochemical staining for Wilms' tumor 1 (WT1) confirmed the diagnosis of nephroblastoma and ruled out their differential diagnosis of synovial cell sarcoma which, in humans, can be a primary mesenchymal renal tumor diagnosed via



Kidney, dog. Tubular structures show cytoplasmic immunoreactivity for cytokeratin. (anti-cytokeratin, 400X)
 (Photo courtesy of: University of Wisconsin Madison, School of Veterinary Medicine, Pathobiological Sciences, 2015 Linden Dr., Madison, WI 53706)

morphology together with specific chromosomal translocations.⁷

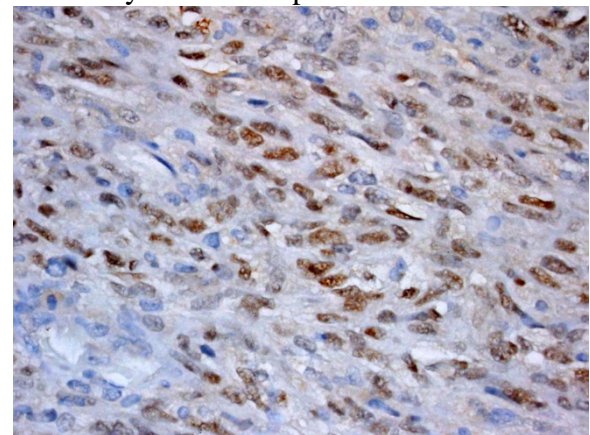
Metastatic risk is considered high in dogs^{4,7}, but primary source data for this assertion is difficult to confirm, and sample sizes are usually small in published studies. In a 2006 retrospective paper¹ detailing clinical outcome of dogs with primary renal neoplasia, only 5 of 82 dogs had the diagnosis of nephroblastoma. Of those, one reported metastatic disease at the time of diagnosis and three of four from which follow-up was available had metastatic disease at the time of death. The spindle cell variant is also reported to be of higher metastatic potential⁴ also without a reference to primary source data. The tumor in the submitted case progressed quickly both locally and distantly in spleen and abdominal lymph nodes within 6 weeks of resection. The blastemal predominant morphology of this tumor has been reported in at least two other single case reports in dogs^{9,10}, the former representing a benign variant.

Cianciolo and Mohr report tumors with more differentiated tubular and glomerular structures have a better prognosis than those

with anaplasia and sarcomatous features² although the primary source for this statement is unclear. Diagnosis in this case was challenging in that the characteristic feature of glomeruloid structures were not apparent.

JPC Diagnosis: Kidney: Nephroblastoma, mixed breed (*Canis familiaris*), canine.

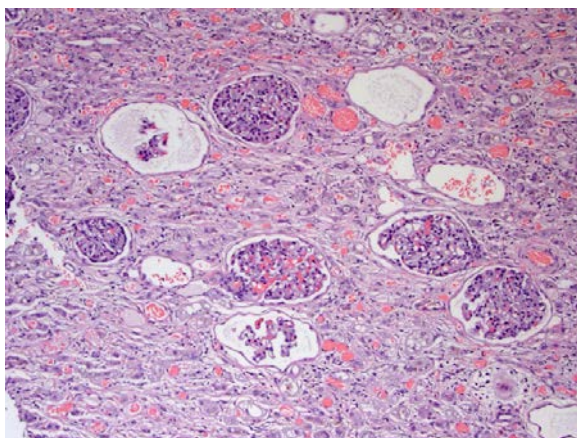
Conference Comment: Nephroblastomas are a generally disorganized mixture of three components: epithelial, mesenchymal, and blastemal which arise from the metanephrogenic renal blastema. In different tumors and even different areas within the same tumor one or more of those components can predominate. In humans, the causative factor is mutation of the tumor suppressor gene Wilm's tumor-1 (WT-1) which is located on chromosome 11p13. A genetic cause in animals has not yet been identified. Grossly, nephroblastomas are most often unilateral and isolated to one pole of the kidney, encapsulated, multilobulated, white to tan, and firm with spongy/cystic areas and foci of necrosis and hemorrhage. Microscopically, the epithelial component often forms abortive tubules or primitive glomeruli within tubules surrounded by the mesenchymal components which is



Kidney, dog. Spindle cells are diffusely and strongly positive for WT-1. (anti-WT1, 400X)

organized in long interlacing streams and can differentiate into muscle, fibrous, adipose, cartilage or bone tissues. The blastemal component is often found in clumps between the epithelial and mesenchymal components and are small cells with high nuclear to cytoplasmic ratios and round, hyperchromatic nuclei.^{2,7}

As mentioned above, it has been described that tumors with mesenchymal differentiation often exhibit frequent metastasis.⁴ There has been recent publication of a blastemal-predominant canine renal nephroblastoma that had gingival metastasis which suggests that all types of nephroblastomas have metastatic potential. In dogs, a common presentation occurs not in the kidney, but in the thoracolumbar spinal cord, arising from remnants of metapnephric rests located between the dura mater and spinal cord. This condition is most commonly observed in young German Shepherd Dogs and is also called ectopic nephroblastoma. Additionally, there have been rare reports of multifocal spinal cord involvement which may represent metastasis or multifocal generation of the tumor.^{3,11} Traditional renal nephroblastomas



The adjacent neoplasm exhibits marked tubular atrophy, interstitial expansion by mature collagen, and cystic changes to glomeruli. (HE, 200X)

have been identified in all species but occur with greatest frequency in chickens and pigs.² Where in chickens, they are often associated with avian leukosis virus.⁸ In fish (Japanese eel and Rainbow trout) and rats (Sprague-Dawley subline) nephroblastomas are induced via carcinogens.^{5,6}

Contributing Institution:

University of Wisconsin Madison
School of Veterinary Medicine
Pathobiological Sciences

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Presentation, abortus, rhesus macaque. The crown of the head of the fetus was deformed and there is abundant subcutaneous edema and hemorrhage. (Photo courtesy of: Oregon National Primate Research Center, Oregon Health and Science University, Division of Comparative Medicine, Pathology Services Unit, 505 NW 185th Avenue, Beaverton, OR, 97006)

CASE II: 16A351 (JPC 4101577).

Signalment: Full-term stillborn male rhesus macaque (*Macaca mulatta*), non-human primate.

History: A 4-year-old primiparous, outdoor-housed female rhesus macaque from a large breeding colony presented acutely for clinical signs of dystocia. Ultrasonography revealed an absent fetal heartbeat consistent with *in utero* fetal demise. A caesarean section was subsequently performed; the dam recovered uneventfully. A full-term stillborn male rhesus macaque was submitted for necropsy along with the bidiscoid placenta.

Gross Pathology: Grossly, the crown of the head of the fetus was deformed and exhibited abundant subcutaneous edema and hemorrhage. The face demonstrated moderate edema and congestion. The lungs were diffusely deep red and atelectatic, with scattered 1- 2mm white foci. Gross examination of the primary and secondary placental discs revealed few opaque tan-white foci on the fetal surface with slight thickening of the fetal membranes. Multifocally, there were marginal regions of pallor and infarction on both placental discs.

Laboratory results: 4+ beta-hemolytic *Streptococcus* sp. was isolated from the placenta and stomach, with 2+ beta-hemolytic *Streptococcus* sp. cultured from the pleura

Microscopic Description: Two sections of placenta (primary disc) with attached fetal membranes are examined.

Diffusely expanding and infiltrating the subchorionic trophoblast layer is a nearly continuous, thick band of karyorrhectic neutrophils admixed with eosinophilic cellular debris, and few clusters of homogenous eosinophilic material (fibrin). Trophoblasts are vacuolated and necrotic. The chorionic plate is expanded by edema and contains fewer numbers of neutrophils which are often perivascular. The amniotic epithelium is sloughed and replaced by necrotic cellular debris interspersed with wispy strands of eosinophilic material. High numbers of cocci are found at the interface of the amnion and chorion, extending into the subjacent chorionic plate. Chorionic (fetal) blood vessels are infiltrated by karyorrhectic neutrophils and vessel walls are smudgy and



Placenta, rhesus macaque. The primary and secondary placental discs demonstrate few gray-white foci on the fetal surface with mild thickening of the fetal membranes. (Photo courtesy of: Oregon National Primate Research Center, Oregon Health and Science University, Division of Comparative Medicine, Pathology Services Unit, 505 NW 185th Avenue, Beaverton, OR, 97006)

hyper eosinophilic (fibrinoid necrosis). Few large- and medium-size vessels contain luminal fibrin thrombi. The fetal membranes are diffusely necrotic and multifocally expanded by high numbers of karyorrhectic neutrophils and eosinophilic cellular debris. The maternal basal plate and decidua contain lower numbers of degenerate neutrophils which occasionally surround maternal blood vessels. Numerous fibrin coagula are also present within the basal plate. There is moderate, multifocally extensive extravasation of red blood cells (acute hemorrhage) along the maternal decidua on both tissue sections.

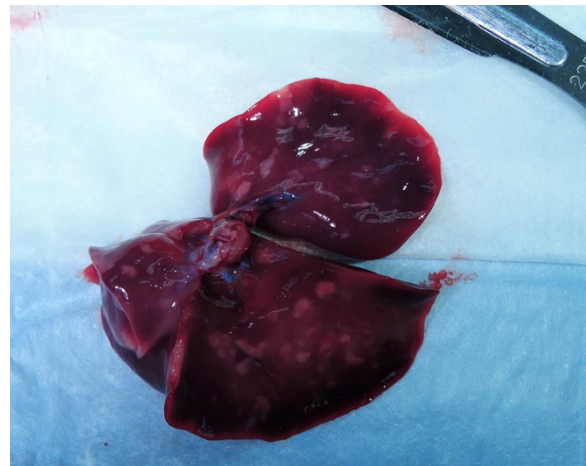
Contributor’s Morphologic Diagnosis:

Placenta, fetal membranes: Chorioamnionitis, neutrophilic, necrotizing, acute, diffuse, severe, with subchorionic microabscesses, chorionic necrotizing vasculitis, amniotic epithelial necrosis, mild neutrophilic deciduitis, decidual vasculitis, and gram-positive cocci, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Other morphologic diagnoses:

- Umbilical cord: Funisitis, neutrophilic, necrotizing, marked, multifocal with segmental necrotizing umbilical arteritis.
- Lung: Bronchopneumonia, pyogranulomatous, subacute, moderate, diffuse with peribronchial hemorrhage, high numbers of intralesional cocci, intra-alveolar squamous cells, mucin, and amorphous debris.

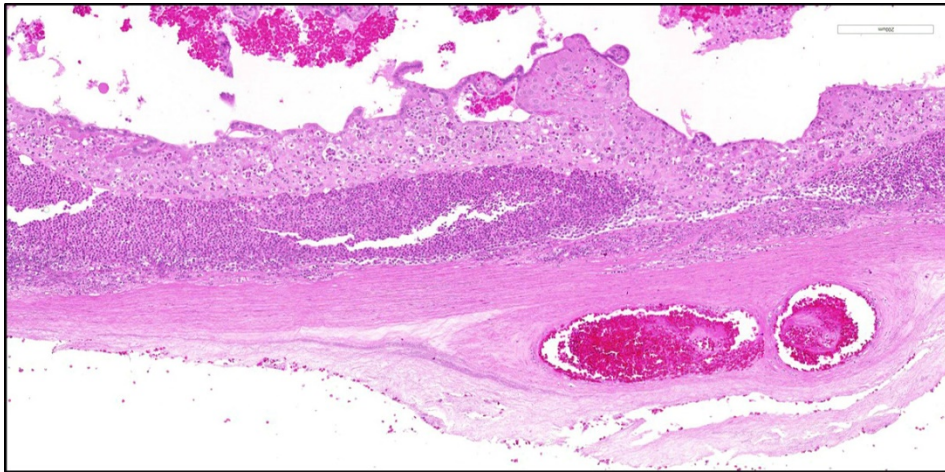
Contributor’s Comment: This is a case of acute streptococcal chorioamnionitis with fetal demise. Microbial culture of the placenta yielded 4+ beta-hemolytic *Streptococcus* sp. Gram staining of placenta sections confirmed numerous gram-positive cocci within the fetal membranes. The umbilical cord and umbilical vessels also contained neutrophilic infiltrates and exhibited necrotic changes to umbilical vessel walls. The character and distribution of inflammation within the fetal membranes in this case, is consistent with acute chorioamnionitis (ACA) due to ascending



Lungs, rhesus macaque abortus: Numerous 2mm white foci are scattered throughout all lung lobes. (Photo courtesy of: Oregon National Primate Research Center, Oregon Health and Science University, Division of Comparative Medicine, Pathology Services Unit, 505 NW 185th Avenue, Beaverton, OR, 97006)

bacterial infection from the dam's lower genital tract.

In humans and non-human primates, ACA consists of a fetal and maternal acute inflammatory response characterized by diffuse neutrophilic infiltration variably involving the fetal membranes, placenta, and umbilical cord. The initial inflammatory response is maternal, with neutrophils exiting the placental intervillous space and decidual vessels with infiltration of the chorionic



Placenta, rhesus: There is a thick band of degenerate neutrophils and cellular debris lines the deep aspect of the chorioamniotic plate. (HE, 40X)

plate, later migrating through the fetal membranes and eventually into the amniotic fluid.^{1,2} As the maternal inflammatory response progresses, neutrophils become karyorrhectic and apoptotic. In contrast, the fetal inflammatory response varies depending on the gestational age, but may be manifested as chorionic vasculitis, umbilical arteritis and phlebitis, and funisitis as neutrophils transmigrate across the umbilical vessels.

Most acute chorioamnionitis cases in humans and non-human primates are considered to have an infectious etiology. Ascending microbial invasion from the lower genital tract is the most common route of intrauterine and subsequent intra-amniotic infection.^{1,5}

Following infection of the amniotic cavity, the fetus may aspirate infected amniotic fluid leading to fetal pneumonia, sepsis, and death in-utero. Infectious pathogens within amniotic fluid may also be swallowed into the gastrointestinal tract or may gain access through the middle ear and tympanic membrane. Commonly isolated microorganisms in human placentas with a histologic diagnosis of ACA include vaginal commensals such as streptococci, staphylococci, *Ureaplasma* sp., and *Mycoplasma*

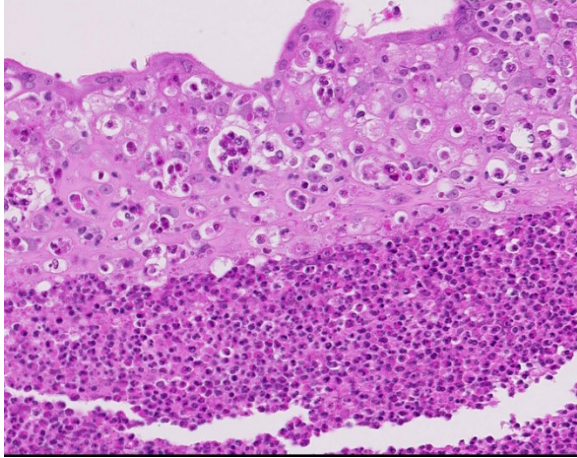
hominis, as well enteric bacteria and anaerobes including *Bacteroides* sp., *E.coli*, and

Fusobacterium sp..^{1,5,7} In addition to

commensal organisms from the lower reproductive tract, *Listeria monocytogenes*, a sporadic cause of abortion and stillbirth in captive macaques,

may also produce a neutrophilic and necrotizing placentitis characterized by intervillitis and villitis. *L. monocytogenes* undergoes hematogenous spread, gaining access to the placenta through the maternal circulation and intervillous space and eventually invading the villi and fetal circulation.

Acute chorioamnionitis also commonly and indirectly contributes to fetal morbidity and mortality by precipitating preterm labor and birth. Phospholipase production by invading bacteria and neutrophils stimulates the synthesis of prostaglandins, which induce uterine contractions and cervical dilation.^{6,7} Increased matrix metalloproteinases (MMP) may contribute to degradation of the



Placenta, rhesus: Trophoblasts superficial to the band of neutrophils are degenerate and/or necrotic; many neutrophils appear to have phagocytosed degenerate trophoblasts. (HE, 320X)

extracellular matrix of the fetal membranes, potentially resulting in premature membrane rupture. Up to one-third of preterm deliveries in women are attributed to ACA.⁷

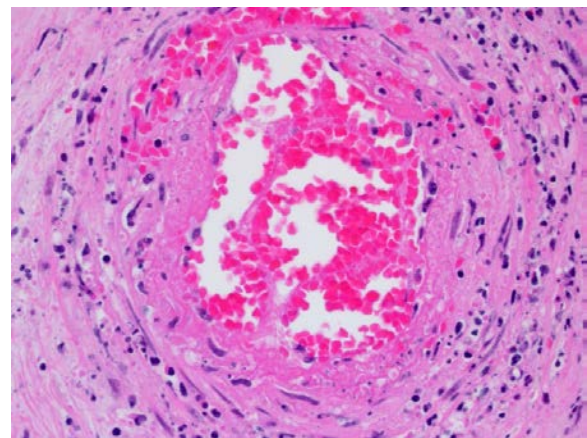
More frequently, acute chorioamnionitis remains subclinical in the mother with occasional clinical manifestations including pyrexia, leukocytosis, purulent amniotic fluid or vaginal discharge, and uterine tenderness. Maternal sepsis is infrequent and the primary clinical impacts of ACA are the adverse effects on the fetus.⁵

Histologic changes in the present case mirror many of the classic features of ACA found in human placentas. The degree of neutrophil karyorrhexis and amniotic epithelial necrosis coupled with necrotizing funisitis on sections of umbilicus (not included) are indicative of later-stage and more severe infection.¹ Beta-hemolytic *Streptococcus* sp. was isolated from the stomach and pleura of this full-term stillborn rhesus macaque, consistent with ingestion and aspiration of contaminated amniotic fluid. Gram staining of lung sections confirmed high numbers of gram-positive cocci within the alveoli and small airways. Microscopic evaluation of fetal lung

tissue showed a pyogranulomatous bronchopneumonia with increased numbers of squamous cells, mucin, and debris. Acute aspiration of meconium was also considered as an etiology for the bronchopneumonia, however, meconium pigment was not appreciated microscopically on lung sections.

Infections due to beta-hemolytic *Streptococcus* sp. in nonhuman primates, including the present case, are considered largely opportunistic and depend on host factors such as age, pregnancy and immune system status, as well as environment and social stress.³ The most pathogenic streptococcal groups in macaques are beta-hemolytic groups A and B as well as alpha-hemolytic *Streptococcus pneumoniae*, which lacks the Lancefield antigen and serogroup designation. In addition to reproductive loss, streptococcal infections in macaques have also been reported to cause suppurative pneumonia, rhinitis, conjunctivitis, meningoencephalitis, polyarthritis, sepsis, and polyserositis.

JPC Diagnosis: Placental disc and chorioamnionic membrane: Chorioamnionitis and deciduitis, neutrophilic and

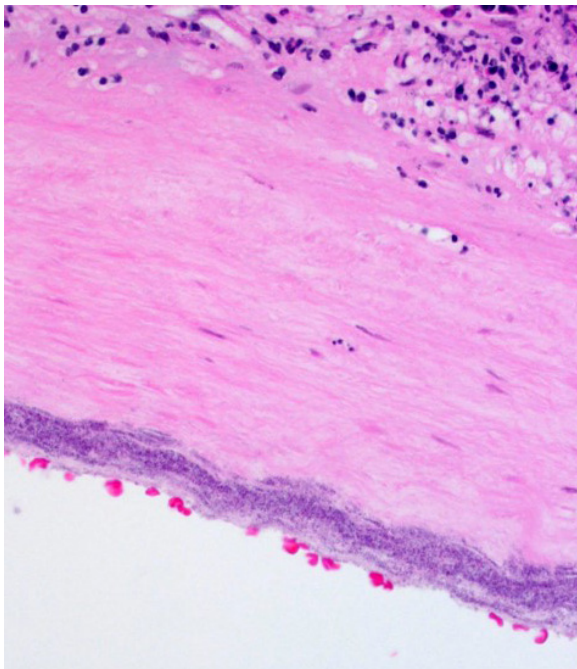


Placenta, rhesus: Chorionic vessels include arterioles, are necrotic, with abundant extruded protein hemorrhage, and cellular debris within their walls. (HE, 320X)

necrotizing, subacute, diffuse, severe, with necrotizing vasculitis, loss of amniotic epithelium and numerous cocci.

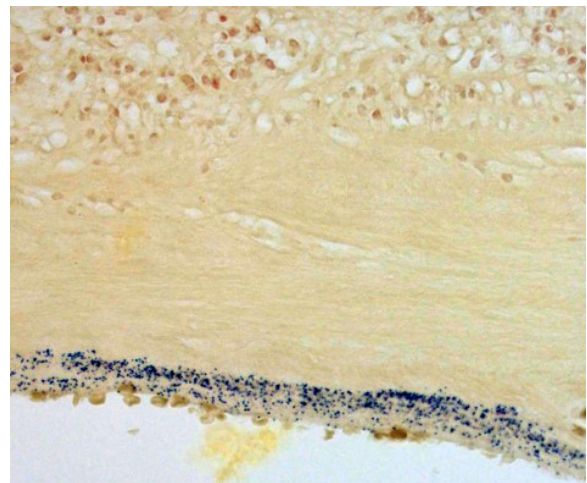
Conference Comment: The contributor has done an outstanding job in describing this case.

Streptococci are gram-positive, catalase-negative bacteria which are arranged in pairs or chains and exhibit varying degrees of hemolysis on blood agar which has been used to group the different species. α -Hemolytic streptococci produce a green zone around the colonies from hydrogen peroxide oxidation of hemoglobin to methemoglobin but do not lyse erythrocytes. β -Hemolytic streptococci tend to be the most pathogenic types and cause acute lysis of red blood cells with hemolytic zones around colonies on blood agar. Finally, γ -streptococci are non-hemolytic and non-pathogenic. Another method to divide the over 98 recognized species of *Streptococcus* is using a precipitin test based on extractable carbohydrate



Placenta, rhesus macaque: A band of streptococci lines the deep chorionic plate superficial to the chorioamniotic membrane. (HE, 400X)

antigens called Lancefield groups which are designated A through V (excluding I and J). These groups are further subdivided based on protein antigens, M, R, and T. This is of some importance clinically, as antibodies to Lancefield groups are not protective but antibodies to M, R, or T are protective. M protein, in particular, is an important virulence determinant because it allows the bacterium to evade phagocytosis. For example, the SeM protein of *Streptococcus equi* ssp. *equi* prevents phagocytosis by



Placenta, rhesus macaque: Gram stain of streptococci in the deep chorionic plate. (Brown-Hopps, 400X)

binding host immunoglobulin and coating the bacterium, masking sites for complement activation and collectin/ficolin binding. Another protein, the FbsA protein is a fibrinogen-binding protein which aids in virulence of *Streptococcus agalactiae*. Other streptococci have analogous proteins, like the FOG protein in group G *Streptococcus dysgalactiae* ssp. *equisimilis*, and the Szp protein of *S. equi* ssp. *zooepidemicus*. Most species of virulent streptococci have a capsule which is either composed of hyaluronic acid (groups A and C) or polysaccharide (groups B, E, and G). The gram-positive cell wall of streptococci contain lipoteichoic acids and peptidoglycan which interact with host macrophages and

induce release of proinflammatory cytokines.⁹

Particularly virulent streptococcal species possess pyrogenic toxin superantigens. Superantigens simultaneously bind with major histocompatibility complex class II molecules and T cell receptor molecules on the V- β region resulting in massive activation of antigen-presenting cells and T-cells which proceed to produce overwhelming amounts of cytokines. This so-called “cytokine storm” has detrimental systemic effects and often results in the death of the animal.⁹

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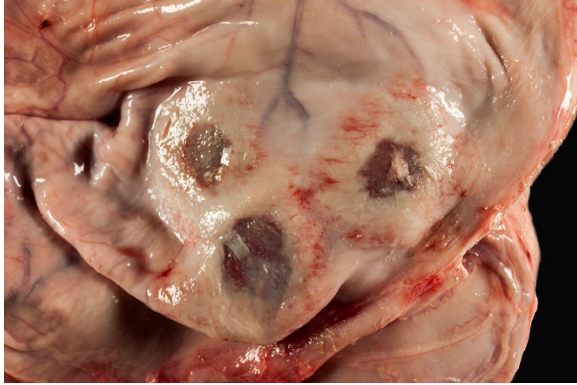
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CASE III: CASE 2 51304 (JPC 4068392).

Signalment: 3-week-old, female, Jersey (*Bos taurus*), bovine.

History: The calf had a reported history of a rotavirus scour and was treated with antibiotics and supportive therapy that consisted of tube-feeding milk by the owner.

Gross Pathology: The calf is in poor body condition and in a fresh state of preservation. The abomasum contains multiple 1-2mm brown/red foci on the mucosal surface and the luminal contents are green-yellow and watery. Several discrete red foci (10-15cm diameter) with fibrin tags are visible on the serosal surface of the rumen. These lesions are transmural and correspond with friable, brown and tan plaques surrounded by a



Rumen, calf. Several discrete red foci (10-15cm diameter) with fibrin tags are visible on the serosal surface of the rumen (Photo courtesy of: Massey University, Institute of Veterinary, Animal and Biomedical Sciences, Private Bag 11 222, Palmerston North 4442, New Zealand, <http://ivabs.massey.ac.nz> <http://vet-school.massey.ac.nz>)

hyperemic border on the mucosal surface. Approximately 70% of the intervening ruminal mucosa is grey and mildly thickened. The rumen is filled with milky fluid and clots and has a pH of 5. Multiple serosal segments of the small and large intestine are reddened.

Laboratory results: None provided.

Microscopic Description: Rumen: focally and transmurally, the rumen wall is markedly expanded by edema, fibrin and eosinophilic karyorrhectic debris intermixed with numerous inflammatory cells that consist predominantly of intact and degenerate neutrophils, macrophages and smaller numbers of lymphocytes and plasma cells. Multifocally and affecting the serosa and submucosa adjacent the necrotic lesion, there are large numbers of mesenchymal cells admixed with thin strands of collagen. Multiple submucosal blood vessels contain fibrin thrombi and are hyalinized and disrupted by moderate numbers of neutrophils and fungal hyphae that are 5-20um wide, pauci-septate, have thin, non-parallel walls, exhibit irregular, non-dichotomous or right-angled branching and occasionally contain focal bulbous

dilatations. Moderate numbers of fungal hyphae are present within the mucosa, submucosa and extending to the serosa. Multifocally, there is mild to moderate hyperkeratosis, erosion and ulceration of the squamous epithelium and there are numerous intraepithelial aggregates of neutrophils (microabscesses). Multifocally between keratin lamellae there are small numbers of basophilic, 2-4um diameter, round to oval and occasionally pseudoseptate fungal organisms. Multifocally there are large numbers of purple bacterial colonies within the superficial mucosa.

Contributor's Morphologic Diagnosis:

Rumen: severe, chronic, focally extensive, necrotizing rumenitis with vasculitis and thrombosis and intralesional fungal hyphae morphologically consistent with zygomycetes. Moderate, chronic, locally extensive ruminal hyperkeratosis with intralesional fungal organisms morphologically consistent with *Candida*.
(Mycotic rumenitis)

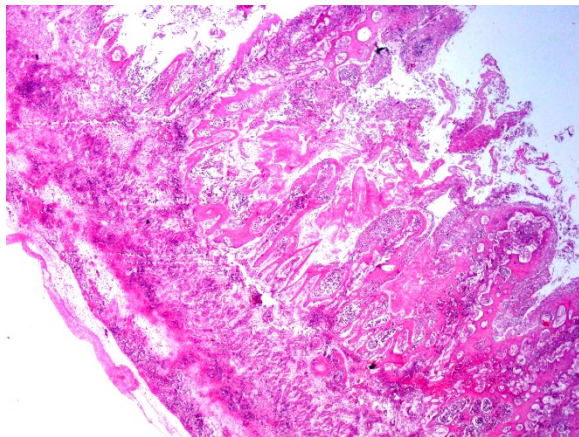
Contributor's Comment: Opportunistic fungal organisms such as the zygomycetes of the genera *Mucor*, *Absidia* and *Rhizopus*,



Rumen, calf. Mucosal view of the transmural lesions in the ruminal wall. (Photo courtesy of: Massey University, Institute of Veterinary, Animal and Biomedical Sciences, Private Bag 11 222, Palmerston North 4442, New Zealand, <http://ivabs.massey.ac.nz> <http://vet-school.massey.ac.nz>)

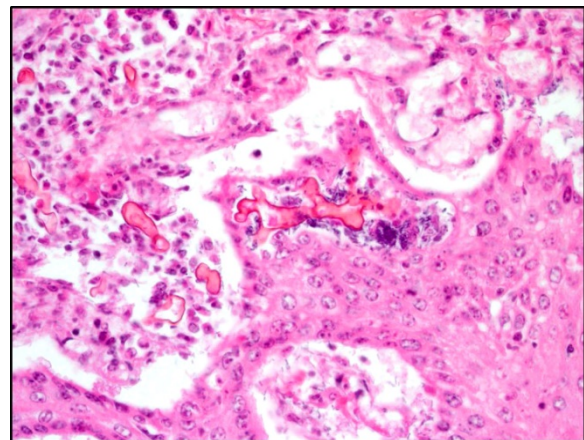
Aspergillus, and occasionally *Candida* can cause mycotic rumenitis in calves. The two fungi involved in this case, the zygomycetes and *Candida* require predisposing circumstances such as stress, sepsis, long-term administration of antibiotics or ruminal acidosis to cause disease.^{2,9} The zygomycetes are saprophytic and ubiquitous organisms found in the environment, and are common gastrointestinal inhabitants of animals. They are angioinvasive and gain access to the vascular system through injury to mucosal barriers. The yeast *Candida*, in which *C. albicans* is most often implicated in animal disease, is a common inhabitant of the gastrointestinal tract, genital mucosa and upper respiratory tract of mammals. *Candida* exists as a yeast form but can undergo a phenotypic switch to the invasive, filamentous form when there are perturbations to the mucosa or physiological state of an animal.¹¹ Neither organism should cause disease in an immunocompetent animal.

The incidence of mycotic infections in the forestomach of calves diagnosed at necropsy is relatively low. The incidence rate of alimentary mycosis in calves less than 6-



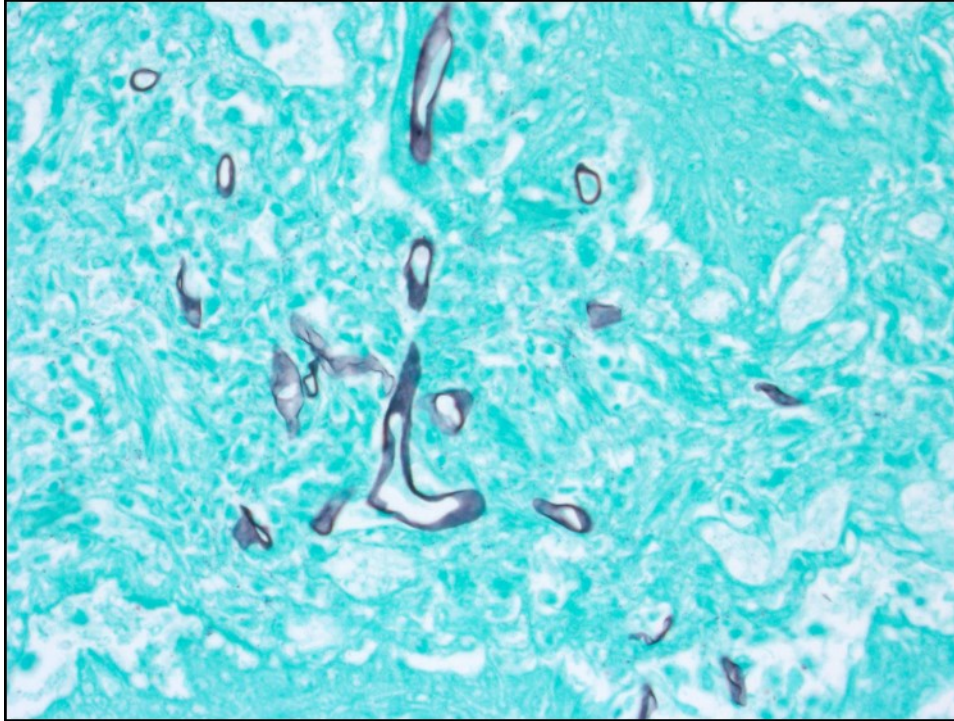
Rumen, calf. There is diffuse transmural necrosis of the ruminal wall with sloughing of the mucosa, extensive infiltration of neutrophils, and widespread necrotizing vasculitis. (HE, 40X)

months-old presented to necropsy over a 10-year period is 3%.⁴ The most common organisms isolated are the zygomycetes, followed closely by *Aspergillus*. Co-infection, where both organisms are identified, is also reported.^{4,8} Isolation of *Candida* is uncommon; however, it is reported to occur in co-infections in adjacent, non-inflamed regions of the mucosa.⁸ The most common risk factor for mycotic infection of the forestomachs in these calves is diarrhea that is treated with antibiotics.



Rumen, calf. Numerous 8-15 um fungal hyphae with non-parallel wall and non-dichotomous branching are present throughout the necrotic mucosa as well as within the walls of vessels. (HE, 400X)

The pH of the ruminal fluid in this calf was 5, which is below normal physiological levels in a milk-fed calf (6.4-7.0).⁵ Ruminal acidosis in pre-ruminant calves is primarily caused by esophageal groove dysfunction that results in the spillage and subsequent stagnation and putrefaction of milk in the rumen and reticulum.⁶ Risk factors associated with esophageal groove dysfunction include the feeding of milk from a pail or tube, and neonatal diarrhea.⁵ Ruminal acidosis causes mild mucosal inflammation, and coupled with the other precipitating factors in this case, such as the antibiotic use and stress, precipitated a high-



Rumen, calf. A silver stain of the fungal hyphae present in the section. (Gomori methenamine silver, 400X)

risk environment for opportunistic fungal infection.

JPC Diagnosis: Rumen: Rumenitis, necrotizing, focally extensive, severe with necrotizing vasculitis, widespread vascular thrombosis, and numerous fungal hyphae.

Conference Comment: Distinguishing the ruminant forestomachs histologically can be daunting, particularly with advanced disease. Here are a few key features that may simplify the process.

The rumen contains small tongue-shaped papillae which become more substantial as the animal is weaned and begins eating more roughage. The mucosal epithelium has three functions: protection, metabolism, and absorption and is keratinized to protect against rough, fibrous ingesta. Within the deeper strata, short chain volatile fatty acids are metabolized (butyric, acetic, and

propionic acids).

Microscopically, what sets the rumen apart is the shape of the papillae and the lack of lamina muscularis extension into the papillae.¹

Next, the reticulum is composed of long, thin primary lamellae with knob-like projections extending off termed secondary lamellae and permanent interconnecting folds (reticular crests) giving the gross appearance of

a honeycomb. The reticulum is also lined by keratinized stratified squamous epithelium and the key microscopic feature are small slits of lamina muscularis within the tips of the primary lamellae.¹

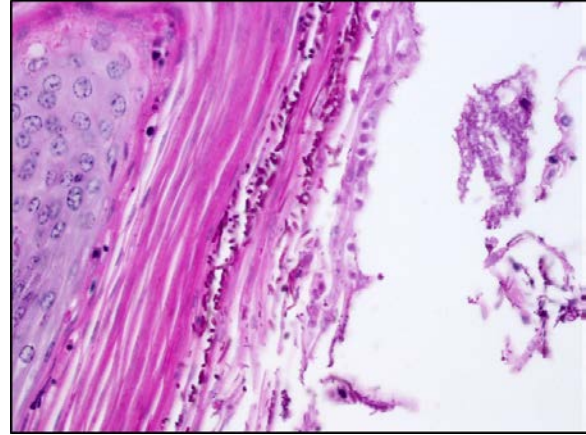
Finally, the omasum has even more folded lamellae which extend like pages of a book grossly and are lined by keratinized stratified squamous epithelium, all be it not as keratinized as the ruminal papillae. In the omasum, form follows function, and there is a much thicker lamina muscularis beneath the lamina propria that extends into the entire length of the lamellae providing the ability to squeeze ingesta into the abomasum.¹

Both *Candida* spp. and zygomycetes are normal flora in the ruminant gastrointestinal tract.

Zygomycosis comprises numerous etiologic agents which can manifest clinically as

cutaneous, subcutaneous, systemic (pulmonary or gastrointestinal), or rhinocerebral infections. The microscopic hallmark of all zygomycetes is the invasive mycelial form in tissues which is infrequently septate and exhibits non-dichotomous branching with non-parallel walls and rare bulbous dilatations. The hyphae are often surrounded by eosinophilic granular material (Splendore-Hoeppli phenomenon) and angioinvasion is common particularly with Mucorales. The main microscopic differential for zygomycosis is pythiosis and must be distinguished using immunohistochemistry, culture, or PCR. In addition to mycotic rumenitis, zygomycetes are a common cause of abortion in ruminants and a differential for granulomatous lymphadenitis.^{3,7,11}

In contrast with zygomycosis, *Candida* spp. are less invasive and often associated with prolonged antibiotic use, immunosuppression, concurrent disease, or stress. Anorexia in chronically ill animals results in increased keratin along the surface of mucus membranes which may provide a fertile environment for *Candida* to hang on and grow. An important determinant of virulence amongst *Candida* spp. is the presence of adhesins which aide in binding to host tissues. Specifically, yeast bind mannose receptors and hyphae bind complement receptor 3 (CR3) and the Fc-gamma receptor. Adherence and resistance to antifungal drugs is facilitated by biofilm formation. Additionally, *Candida* species produce enzymes to degrade proteins in the extracellular matrix which aides in dissemination (aspartyl proteinases), resist oxidative killing by phagocytes (catalase), and block neutrophil degranulation (adenosine). Microscopically, *Candida* spp. are seen in three, often comingling, forms: pseudohyphae, hyphae, and yeast. The pseudohyphal form is composed of chains of



Rumen, calf: Numerous ovoid yeasts are present within the keratin layers of the mucosa. Keratin is commonly seen on the rumenal papillae of nursing calves. (Periodic acid-Schiff, 400X)

blastoconidia and distinguished from the hyphal form by narrowed points of attachment of adjacent yeast. The yeast form can often be seen reproducing by narrow-based budding.^{3,10,11}

Conference attendees viewed PAS, GMS, and PTAH immunohistochemical stains to identify organisms and fibrin thrombi present in the section. Polymerized fibrin was identified first at the periphery of affected vessels, plugging endothelial holes, and eventually filling the lumen. While the pathogenicity of the fungal hyphae, present transmurally and often within the walls of necrotic vessels were in doubt, the damage resulting from presumptive yeast forms of *Candida* present largely in the hyperkeratotic layer and mucosa was a source of spirited discussion.

In addition, the possibility of this calf as a “ruminal drinker” (a calf with abnormal closure of the rumenoreticular groove during nursing, which directs milk directly into the stomach) was discussed, the history of tube-feeding was also cited as a possibility for the introduction of milk into the developing rumen, where it might be used as a culture

medium for a range of commensal bacterial and fungal agent.

Contributing Institution:

<http://ivabs.massey.ac.nz>

<http://vet-school.massey.ac.nz>

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CASE IV: Case 2 (JPC 4101147).

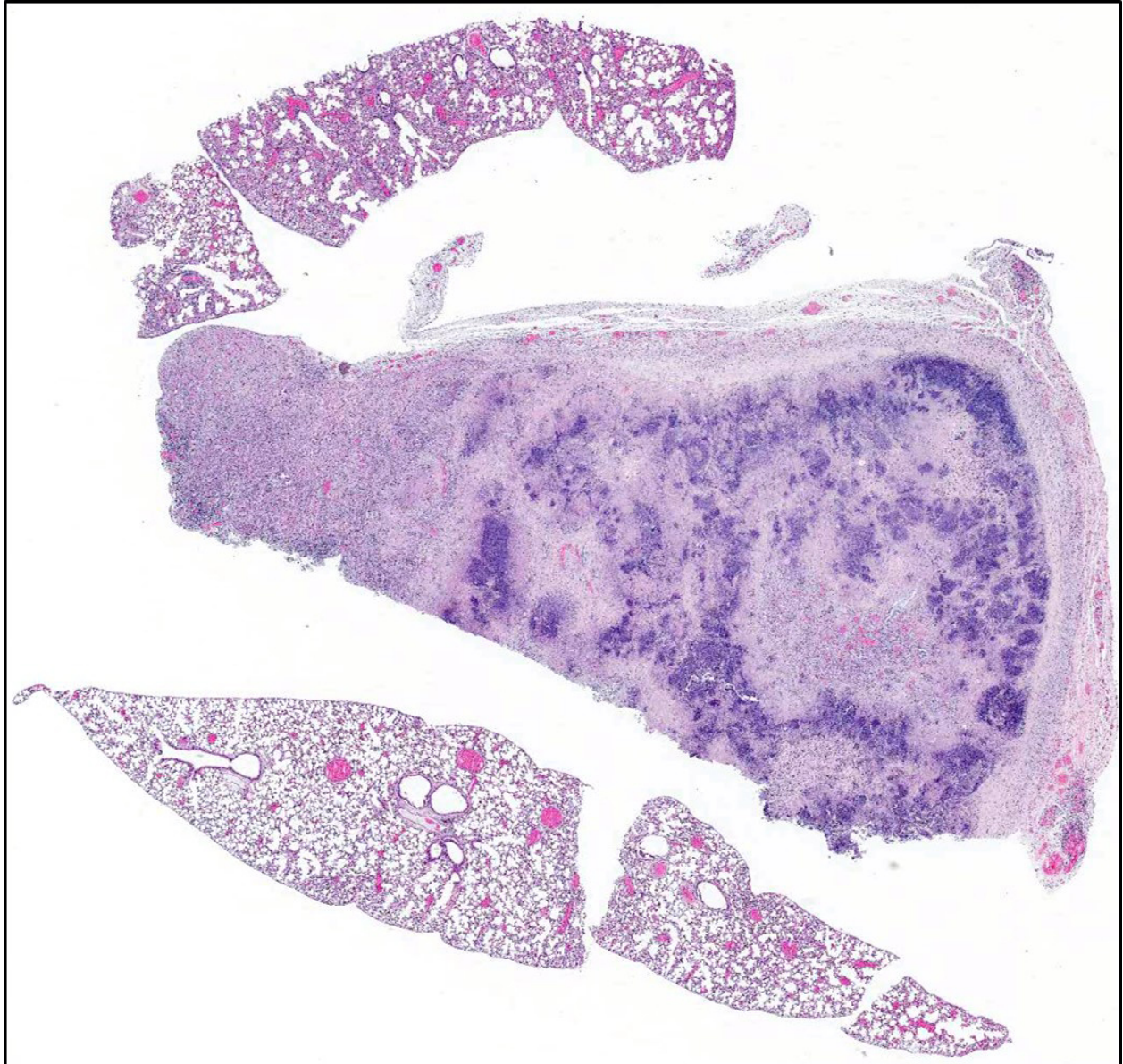
Signalment: 3.5-month-old, female, CyBB knockout (*Mus musculus*), mouse.

History: The animal had been used as a breeder and was found dead. Dystocia was the top differential diagnosis.

Gross Pathology: There is a mass replacing the caudal right lung lobe that contains inspissated white-tan material (abscess), with few scattered tan pinpoint-1mm foci throughout all lobes. There is bloody discharge from the vulva. The uterus is gravid with 2-3 pups in each horn. The spleen is enlarged 5- fold.

Laboratory results: Bacterial culture yielded positive growth for *Klebsiella pneumoniae ssp pneumonia* from heart blood, as well as swabs from the lung abscess.

Microscopic Description: Lung: There is marked expansion and replacement of architecture by a central region of lytic cell debris, as well as lytic and degenerate neutrophils surrounded by an outer layer of collagen and fibroblasts (abscess). Within some sections, where inflammation is less extensive, there are alveoli lined by cuboidal



Lung, mouse: Five section of lung are submitted. One section contains a large, subpleural area of necrosis with a prominent basophilic infiltrate of degenerate neutrophils and cellular debris. (HE, 6X)

epithelium (type 2 pneumocyte hyperplasia), and are filled with degenerate neutrophils, epithelioid macrophages and multinucleated giant cells with variably-sized eosinophilic rhomboid crystals. There is compression of adjacent relatively normal parenchyma, where alveoli are filled with similar inflammatory cells containing crystals, which are also present in large numbers in peripheral alveolar spaces in non-abscessed

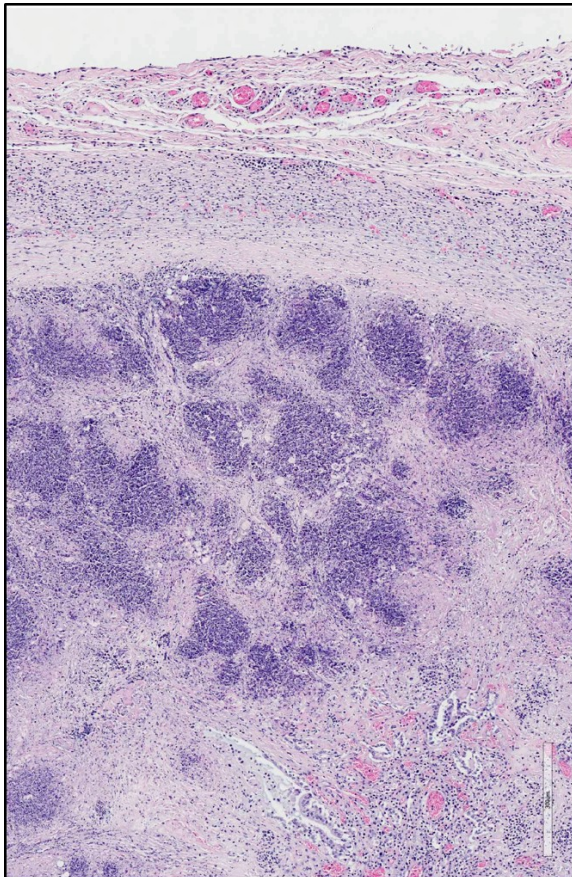
lung sections. Similar crystals are also present free within the lumens of airways. The associated pleura is expanded by immature fibrous connective tissue, macrophages, lymphocytes and plasma cells, and congested small blood vessels.

Contributor's Morphologic Diagnosis:

Lung: Multifocal to coalescing suppurative pneumonia with abscesses; Multifocal to

coalescing granulomatous pneumonia with intracytoplasmic eosinophilic crystals (acidophil macrophage pneumonia)

Contributor's Comment: The submitted slide illustrates two relatively classic lesions seen in mice: bacterial pneumonia with abscessation and acidophil macrophage pneumonia. *Klebsiella pneumoniae* is an enteric commensal bacterium and is considered an opportunistic pathogen. It has been associated with suppurative lesions of the female reproductive tract in aged B6C3F mice, as well as perianal dermatitis, preputial abscesses, otitis and bacteremic disease (leading to pneumonia and abscesses in other organs) in mice of all ages and strains.¹



Lung, mouse: Higher magnification of the areas of necrosis, which are partially encapsulated by a fibrous capsule. (HE 88X)

Although not shown, this animal also had necrosuppurative metritis and similar inflammation affecting numerous organs, indicating bacteremia.

Acidophil macrophage pneumonia (AMP), also known as eosinophilic crystalline pneumonia, typically occurs in older animals with B6, 129 and Swiss mice being more susceptible.¹ These crystals have been shown to be composed of iron, α -1 antitrypsin, immunoglobulin and granulocytic breakdown products, as well as Ym1 chitinase.^{1,5} AMP is typically an incidental finding, but can cause mortality in severe cases.^{1,3}

Both bacterial pneumonia and acidophil macrophage pneumonia, along with splenomegaly, are documented to occur in the present genetically engineered mouse (homozygous null CyBB, or cytochrome b-245, beta polypeptide).² This transgenic mouse is used to model chronic granulomatous disease in humans and is considered to be highly susceptible to bacterial infections.^{2,4} These mice are also highly susceptible to fungal infections; however, none were observed on H&E stained slides.

JPC Diagnosis: 1. Lung: Pneumonia, necrosuppurative, multifocal to coalescing, severe.

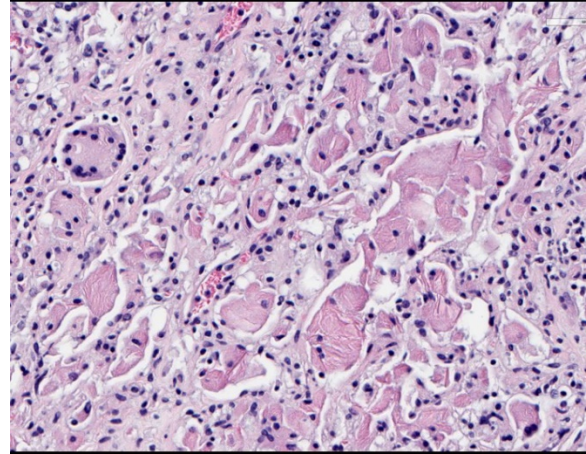
2. Lung, Pneumonia, granulomatous, diffuse, moderate, with numerous intra-histiocytic intracytoplasmic, eosinophilic crystals.

Conference Comment: *Klebsiella* spp. are common commensal bacteria of the gastrointestinal tract of mice in the family Enterobacteriaceae. These bacteria, although usually quiescent, have the ability to become opportunistic pathogens. In mice (particularly immunodeficient mice), several

disease processes have been linked to *Klebsiella* spp. including: suppurative endometritis, perianal dermatitis, preputial abscesses, otitis, tooth infections, urogenital infections, pneumonia, and bacteremic disease (cervical lymphadenopathy, hepatic and renal abscesses, empyema, myocarditis, and thrombosis). Lesions are not characteristic and diagnosis is dependent upon virus isolation with either *Klebsiella pneumoniae* or *K. oxytoca* being the most common isolates.¹

Acidophilic macrophage pneumonia (AMP) is characterized by accumulation of eosinophilic crystals within macrophages and free within alveolar spaces and is often an incidental finding in certain strains of mice (listed by the contributor above). In severely affected mice, the lungs are grossly red to tan and do not collapse. Ultrastructurally, the crystals are needle-shaped or rhomboidal with a clear lattice structure which repeats every 3-5 nm and are quite complex, composed of Ym1 and Ym2 chitinase, containing iron, alpha-1 antitrypsin, immunoglobulin, and breakdown products of granulocytes. This ultrastructural appearance is similar to Charcot-Leyden crystals associated with eosinophil-rich diseases in humans and non-human primates. Older mice frequently have a small number of alveolar macrophages containing crystals which become pathologic when a disease process impairs clearance of alveolar exudate and the crystal-laden macrophages accumulate and fill vital air spaces leading to dyspnea. Other locations in predisposed mice also contain Ym1 and Ym2 chitinase including: epithelium of the olfactory, nasal respiratory, middle ear, trachea, lung, stomach, gall bladder, bile duct, and pancreatic duct.¹

Conference participants were hesitant to describe this lesion as an abscess because it is lacking a definitive capsule and there is



Lung, mouse: Adjacent alveoli (as well as those in other submitted sections), contain numerous epithelioid macrophages and Langhans' type multinucleated macrophages contain numerous acicular eosinophilic crystals. (HE 400X)

normal tissue in between areas of lytic necrosis, preferring this to represent multifocal to coalescing areas of lytic necrosis.

Contributing Institution:

<http://www.yerkes.emory.edu/research/divisions/pathology/index.html>

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