Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 5

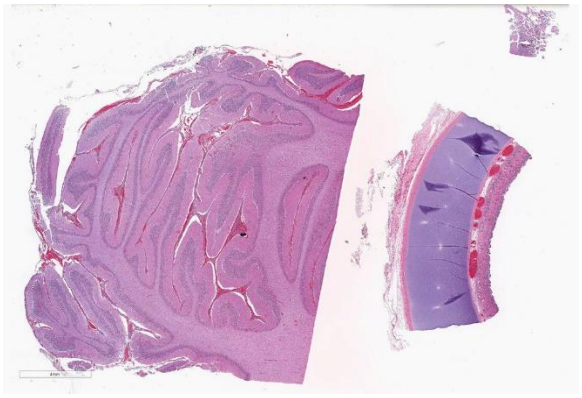
26 September 2018

CASE I: 1505184 (JPC 4066664-00).

Signalment: 14-month-old Holstein heifer.

History: Two 14-month-old Holstein heifers were presented to the large animal clinic as representatives from a heard outbreak, which affected five other heifers on the farm who had more severe signs and had subsequently died or were euthanized. This heifer (heifer A) died immediately prior to transit to the clinic, and had no known clinical signs. Her live herdmate (heifer B) had a 2-week-duration of clinical signs of unthriftiness, oral and cutaneous ulcers with ptyalism, and ocular signs including squinting, epiphora, and corneal ulcers. On physical exam, this heifer B was tachycardic and tachypneic with a rectal temperature of 103° Fahrenheit and generalized peripheral lymphadenomegaly. Heifers involved in the outbreak were also reported to have had cloudy eyes and appeared to be blind. Also housed in close physical proximity to the heifer herd were 5 sheep that had recently lambed. No treatments were performed at this time, and due to suspicion for malignant catarrhal fever, the live herdmate was euthanized without further clinical diagnostics or treatment.

Gross Pathology: Autopsy of the euthanized herdmate (heifer B) revealed severe crusting and ulcerative dermatitis and stomatitis including the following locations: perineal/perivulvar, periocular, mammary, perinasal/perioral and tongue. In addition, large raised to crateriform ulcers with adherent diphtheritic membranes were present throughout the rumen, as well as generalized lymphadenopathy, splenomegaly and corneal edema. Autopsy of the spontaneously dead heifer (heifer A) revealed meningeal edema and hyperemia with cerebellar coning, consistent with cerebellar

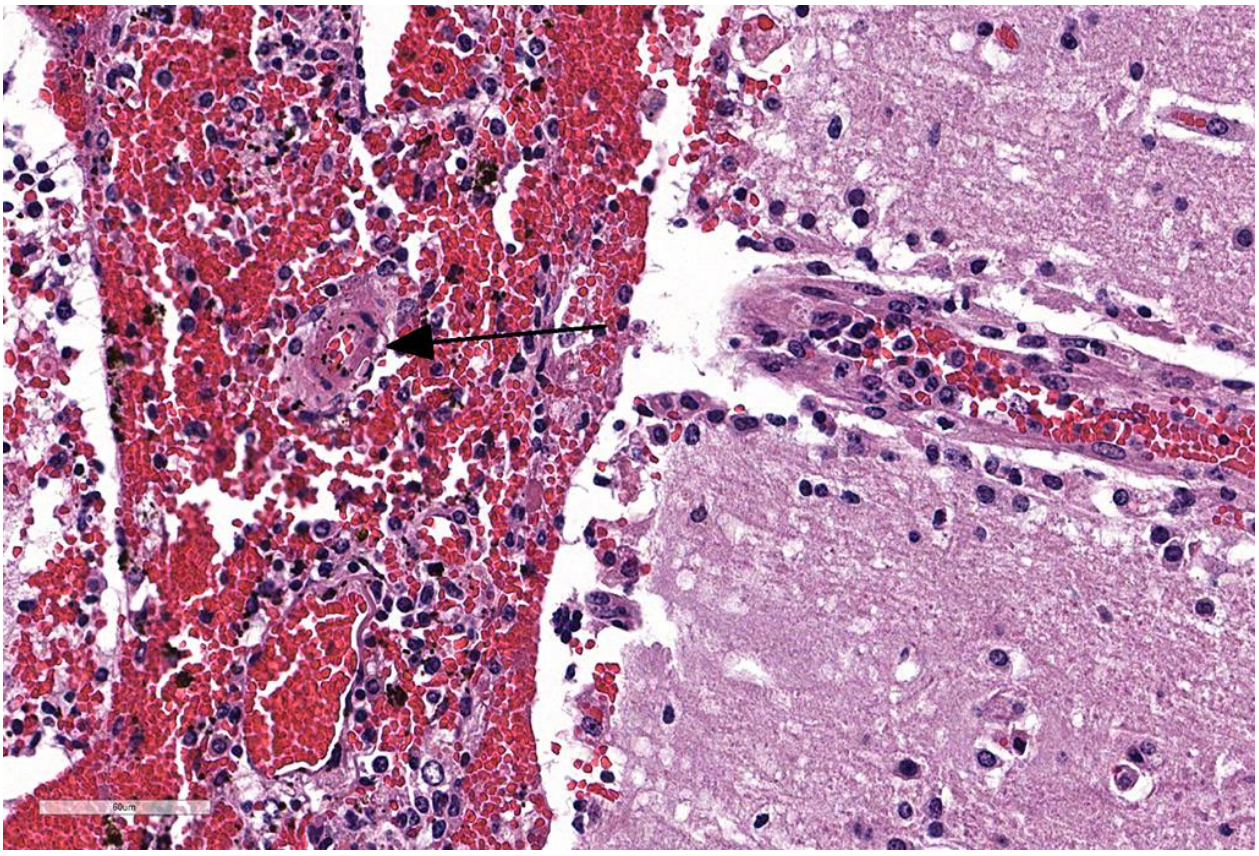


Cerebellum and trachea, ox. Sections of cerebellum and trachea are submitted for examination. Congestion and hemorrhage within the cerebellar leptomeninges as well as the tracheal submucosa are evident at low magnification. (HE, 5X)

herniation. The brain did not autofluoresce under exposure to ultraviolet light.

Laboratory results: A panel including serology and PCR tests was submitted on blood drawn from the live heifer (heifer B) immediately prior to euthanasia. A nested PCR test was positive for ovine herpesvirus-2 malignant catarrhal fever (MCF) virus, and negative for the alcelaphine herpesvirus-1 and 2 MCF viruses. Coronavirus PCR tests performed on intestinal contents were also negative. ELISA serology tests detected antibodies for bluetongue virus; however, PCR tests performed on serum for bluetongue virus were negative. No serum antibodies were detected by ELISA for foot and mouth disease virus, vesicular stomatitis virus (Indiana or New Jersey strains) or epizootic hemorrhagic disease of deer virus. Virus isolation performed on fresh lung, liver, kidney and spleen sections harvested immediately postmortem were negative for bovine viral diarrhea virus (BVDV) and infectious bovine rhinotracheitis (IBR) virus.

Microscopic Description: Cerebellum, Trachea (heifer A): Within the cerebellar leptomeninges and tracheal mucosa and submucosa, there is marked vasculitis with edema, multifocal to coalescing lympho-histiocytic infiltrates and hemorrhagic foci. The vasculitis is characterized by moderate numbers of lymphocytes, histiocytes, plasma cells and neutrophils that expand the tunica adventitia and transmigrate through the walls

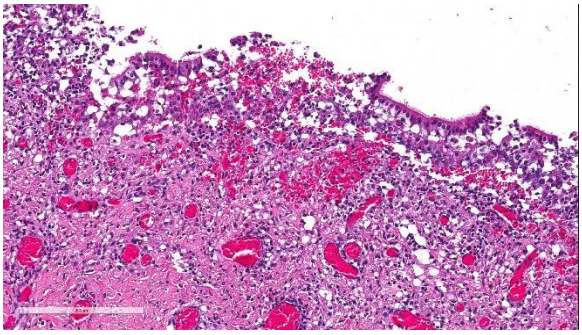


Cerebellum, ox. Higher magnification of the leptomeninges with abundant hemorrhage, and a lymphohistiocytic infiltration which extends down into Virchow-Robin's Space. There is protein and cellular debris in the wall of a small arteriole (vasculitis) and an infiltrate of lymphocytes and plasma cells in the leptomeninges as well as the adjacent Virchow-Robins' space. (HE, 215X)

of varying caliber arterioles and venules. Segments of vascular walls demonstrate pyknosis and karyorrhexis with fibrinoid necrosis and hemorrhagic foci that are also scattered within the cerebellar parenchyma.

Numerous discrete clear vacuoles surround Purkinje cells, which often demonstrate cytoplasmic hypereosinophilia (degeneration). The tracheal mucosal epithelium is multifocally attenuated, with loss of apical cilia, or necrotic and denuded and replaced by eosinophilic and karyorrhectic debris, necrotic neutrophils and extravasated erythrocytes. Similar inflammatory populations surround and infiltrate seromucinous glands.

The kidney and urinary bladder from heifer A had similar vascular changes as described above in addition to multifocal associated interstitial inflammation with tubular necrosis and regeneration in the kidneys, and ulceration of the transitional epithelium of the urinary bladder with submucosal hemorrhage. Similar vascular changes and inflammatory infiltrates were also present in the cutaneous, oral and rumen lesions of heifer B, and the choroid of both eyes. In skin and mucosal lesions, inflammatory infiltrates often extended from the submucosa and dermis into the overlying epidermis/epithelium, occasionally blurring the basement membrane. The epidermis and squamous mucosa of the tongue and rumen frequently contained deep erosions to ulcers covered by waves of serocellular crusts containing colonies of bacteria, grit and fragments of plant material. In other regions there was multifocal keratinocyte necrosis within all layers of the epidermis/epithelium characterized by cytoplasmic hyper-eosinophilia with loss of intercellular desmosomes and pyknosis. Marked paracortical hyperplasia was observed within enlarged peripheral lymph nodes of heifer B.



Trachea, ox. There is segmental necrosis of ciliated epithelium, and infiltration of the mucosa and underlying submucosa with numerous lymphocytes, and fewer histiocytes and neutrophils. There is also submucosal hemorrhage and edema. (HE, 216X)

Contributor's Morphologic Diagnoses: Cerebellum: Moderate-severe lympho-histiocytic meningitis and segmental necrotizing vasculitis with multifocal acute-subacute hemorrhage.

Trachea: Moderate multifocal acute erosive and ulcerative tracheitis with severe lymphohistiocytic necrotizing vasculitis, perivasculitis and adenitis.

Contributor's Comment: Malignant catarrhal fever (MCF) is a highly fatal lymphoproliferative viral disease reported worldwide affecting many wild and captive species of the order *Artiodactyla.*[5,9] The MCF virus group comprises ten gamma-herpesviruses, belonging to the recently assigned *Macavirus* genus[4,6,7], six of which are naturally pathogenic.[5,8] These include alcelaphine herpesvirus-1 (AIHV-1), alcelaphine herpesvirus-2 (AIHV-2), ovine herpesvirus-2 (OvHV-2), caprine herpesvirus-2, (CpHV-2), MCF of white-tailed deer (*Odocoileus virginianus)* (MCFV-WTD), and ibex-MCFV. Experimental infection with hippotragine herpesvirus-1 (HipHV-1) in domestic rabbits *(Oryctolagus cuniculus)* is documented, but no natural infections have been reported.[5,9] Non-pathologic members include: gemsbok-MCF, muskox-MCF, and aoudad-MCF, also named

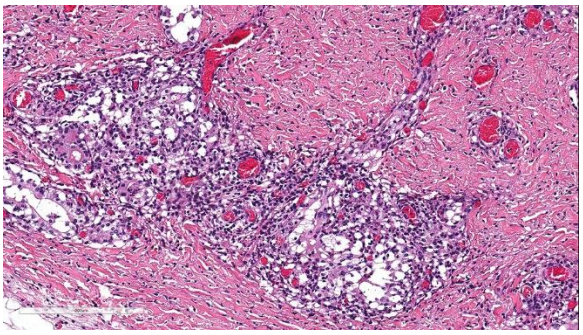
after their respective carrier species.[5,9] Identified natural host species including wildebeest *(Connochaetes taurinus),* domestic sheep *(Ovis aries),* wild and domestic goats *(Capra hircus)* and roan antelope *(Hippotragus equinus)* are a source of infection for susceptible, poorly adapted species such as American bison *(Bison bison),* many cervids, and domestic cattle *(Bos taurus).*

Of paramount economic importance to those managing bison, deer, and exotic game farms, as well as zoological collections, are alcelaphine herpesvirus-1 (AIHV-1) and ovine herpesvirus-2 (OvHV-2), the causative agents of wildebeest-associated MCF (WA-MCF), and sheep-associated MCF (SA-MCF) respectively. Susceptibility to infection by pathogenic MCF viruses and development of disease in non-reservoir hosts varies.[5] In general, Bali cattle/banteng *(Bos javanicus),* white-tailed deer *(Odocoileus virginianus),* Pere David's deer *(Elaphurus davidianus),* and American bison are highly susceptible to disease following infection; water buffalo *(Bubalus bubalis),* and many cervids are intermediately susceptible; domestic cattle and pigs *(Sus scrofa)* are mildly susceptible; and fallow deer *(Dama dama)* are resistant.[5,9] Additionally, laboratory animals such as rabbits (*Oryctolagus cuniculus)* and hamsters *(Mesocricetus auratus)* are susceptible to experimental infection.[5]

The clinical signs associated with WA-MCF and SA-MCF are indistinguishable.[5,9] In most cases, animals present with fever, depression, anorexia, and a constellation of clinical signs, termed the "head and eye" form, which includes: oral mucosal ulcers, ptyalism, oculonasal discharge, dyspnea, corneal edema, hypopyon and photophobia. Additionally, dysentery, profuse catarrhal and mucopurulent nasal discharge and generalized lymphadenopathy are characteristic of the intestinal form, and severe nasal mucosal inflammation and hemorrhagic gastroenteritis are characteristic of the peracute form. Hematuria, stranguria, and convulsions can also be seen.[9] In cattle infected with OvHV-2, there is usually an acute onset of clinical signs, but experimental studies show that some animals may present with disease months after infection.[9]

Bison are 100 times more likely to become infected, and 10,000 times more susceptible to developing MCF than cattle[5], but experimentally-induced MCF with OvHV-2 in bison yields milder clinical signs (oculonasal discharge, peripheral lymphadenopathy) and vascular lesions (arteritis, phlebitis) than in cattle.[9] CpHV-2 harbored asymptomatically in wild and domestic goats infects many species of deer and manifests usually as chronic weight loss, dermatitis and alopecia.[5] Goats also harbor OvHv-2, and may be the source of infection for white-tailed deer in MCFV-WTD, which is now known to affect other cervids such as the red brocket deer *(Mazama americana).*[5] CpHV-2 has also been linked to a recent report of MCF in a captive pudu *(Pudu puda).* It is important to note that viral shedding of OvHV-2 and AIHV-1 does not occur in clinically infected susceptible hosts[5,9], so an infected animal is not a threat to its herd mates.

Common histopathological findings include: mononuclear mucosal inflammation and necrosis in the gastrointestinal tract, respiratory tract and skin; lymphocytic arteritis/phlebitis and perivasculitis in several tissues; lymphoid hyperplasia (paracortical and interfollicular); and panophthalmitis. Other distinguishing histologic features of MCF in American bison are



Trachea, ox. There is diffuse necrosis of submucosal gland and infiltration by numerous neutrophils and perivascular accumulation of lymphocytes. (HE, 188X)

degeneration and apoptosis ofurothelium and hemorrhagic cystitis.[9] In cervids, most notably sika deer *(Cervus nippon),* granulomatous hyperplastic dermatitis and mural to perforating folliculitis are also seen. Widespread necrosis, intracytoplasmic viral inclusions and formation
of syncytia, typical of herpesviral infections, are not observed in MCF.

Differential diagnoses include: mucosa! disease/bovine viral diarrhea virus (BVDV), infectious
bovine rhinotracheitis (IBR), bluetongue virus, epizootic hemorrhagic disease, vesicular stomatitis, foot and mouth disease, rinderpest, and photosensitization. In this case, the clinical history, gross and histologic lesions, and positive PCR and serology tests confirmed Ovine herpesviral-2 associated MCF. Ancillary diagnostic tests in conjunction with histologic lesions allowed rule-out of the other differential etiologies listed above. Although the heifer had antibodies against Bluetongue virus, the negative PCR test was most consistent of prior exposure to the virus, but not active infection. Definitive diagnosis requires a combination of clinical signs in conjunction with positive serological test and/or confirmation with virus DNA detection in fresh or fixed tissue. This is due to the fact that infected, highly susceptible hosts may have negative titers, and that low levels of OvHV-2 have been detected in clinically healthy cattle.[5,9]

Key similarities and differences in transmission between alcelaphine and ovine gammaherpesvirus infections in reservoir hosts are listed below.[5,9]

AIHV-1:

- Harbored in wilderbeest as lifelong asymptomatic infection.
- Vertical and horizontal transmission is possible, though most infections are transmitted
  horizontally.
- Newborn calves are infected and continuously shed virus via ocular and nasal secretions. Viral shedding declines after about 3 months. Outbreaks in susceptible hosts are usually a seasonal phenomenon, corresponding to
  calving season in reservoir hosts.

OvHV-2:

- Harbored in sheep (and goats) as lifelong asymptomatic infection.
- Transmission via placenta or through milk/colostrum is rare, most infections transmitted horizontally.
- Lambs are born uninfected. Most are infected by 2 months of age from carrier adults. 6-9-month adolescents are responsible for most viral shedding predominantly via nasal secretions. Uninfected lambs remain susceptible to infection as adults.
- Sporadic outbreaks in susceptible hosts as well as seasonal outbreaks corresponding to calving season in carrier hosts.

Most of our understanding of MCF is derived from in vitro studies of AlHV-1, WA-MCF.[5,12]

Though OvHV-2 has yet to be cultured in vitro, the discovery that OvHV-2 viral particles are shed copiously in sheep nasal secretions has helped elucidate the pathogenesis of SA-MCF, and will be the focus of this discussion. We now know that the pathogenesis ofOvHV-2 infection in sheep (the natural host) involves a 3-stage replication cycle, characterized by entry and initial replication in the lung, maintenance with latent infection in lymphocytes, and exit via replication and shedding in nasal mucosal epithelium.[5] This differs from the 2-stage replication cycle in susceptible hosts with only entry, and maintenance (with latent and lytic viral replication), and no viral shedding.[5]

In general, sheep are better adapted to controlling the initial respiratory infection with OvHV-2, as evidenced by a significant increase in the level of expression of multiple immune-related genes when compared to susceptible species like bison and rabbits, who seem to have a less effective initial immune response in the lung.[5,9] The products of the following viral genes are used to determine infection patterns in OvHV-2: open reading frame (ORF) 25 (a major capsid protein associated with lytic viral replication), ORF73 (a latency-associated nuclear antigen), ORF50 (R-transactivator that induces latent to productive cell cycle switch), and ORF9 (a DNA polymerase)[5,9]

In sheep, lytic replication in the lower respiratory tract after initial infection is associated with detection of ORF25 transcripts. This is followed by a change in viral tropism from alveolar epithelial cells to lymphoid cells, and subsequent dissemination of latently infected T-lymphocytes. Although viral DNA may be detected in many tissues in sheep, ORF25 transcripts are rarely detected, indicating the prevalence of a latent stage in tissues in the natural host.[5] Although mechanisms of reactivation are unclear, shedding following reactivation occurs in the nasal mucosal epithelium.[5] Conversely, in MCF-susceptible species such as bison and rabbits infected with OvHV-2, dissemination occurs with a latent and lytic pattern of viral gene expression, with viral gene transcripts ORF25, 50 and 73 detected in multiple organs during MCF disease.[5] Systemic viral lytic replication is positively associated with severe illness in OvHV-2 infection as evidenced in bison and rabbits with SAMCF.[5] In contrast to OvHV-2, studies with AIHV-1 infection in rabbits (a susceptible species) indicate that the virus is not productive during MCF disease, with low expression of viral genes (< 10% of viral genome) and very low detection of viral particles in tissues.[10] Palmeira et al. (2013) confirmed the expression of ORF73 (the latency-associated nuclear antigen) during active MCF disease, without detectable expression of other ORF genes necessary for viral production, giving supportive evidence that active WA-MCF disease occurs with a latent viral infection. These findings were further confirmed when experimental nebulization of rabbits with an ORF73-deleted recombinant virus, showed effective initial host infection *without* the development of MCF lesions. Since it was also demonstrated at this time that AIHV-1 ORF73-null viral infection elicits an antibody response, this null-virus is currently being explored as a potential candidatefor vaccine development.[10]

In susceptible host species, lesions are associated with multisystemic lympho-histiocytic vasculitis and vascular necrosis, producing multiorgan inflammation, hemorrhage and ulcers. It is controversial whether the lympho-proliferation that results in generalized lymphadenopathy and

immunosuppression is a direct result of viral infection of T lymphocytes, or a triggered dysregulation of uninfected I- lymphocytes.[5] In MCF disease, cytotoxic T lymphocytes are the incriminated cell type in vascular lesions.[5,11] In OvHV-2, CD8+ perforin+ cytotoxic T cells
as well as CD4+ perforin- regulatory T-cells and B-cells, are involved in vascular lesions.[5,8]
IL-10, *TNFa*, IFNy, and IL-4 are demonstrated, with no expression of IL-1~ and IL-2 in OvHV-2 cell lines.[5] In AlHV-1, CD3+ CD8+ CD4-cytotoxic T-cells with increased expression of activated surface markers CD25 and CD44, high levels of IFNy, and perforin, and reduced levels
IL-2 are characteristic.[4,5] It appears that suppression of IL-2 production and signaling, and thus, suppression of immune regulation is common to the pathogenesis of disease in both ALHV-1 and OvHV-2 MCF infections.[5]

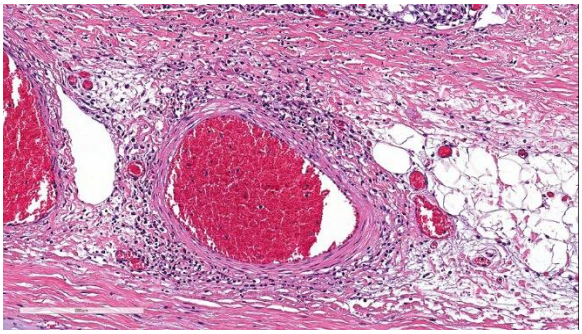
Interestingly, in a recent study of OvHV-2 in bison, cytoplasmic ORF25 protein was demonstrated in perivascular fibroblasts, with no detection in endothelium, vascular leiomyocytes or transmigrating leukocytes, presenting yet another potential avenue for the pathogenesis of vascular lesions in MCF.[7]

The lesions in these two heifers included a wide spectrum of clinical signs, as well as gross and histologic lesions seen with MCF disease, including cutaneous, gastrointestinal (tongue and rumen), urinary, respiratory, ocular and neurologic manifestations. This herd outbreak serves as a reminder of the various forms of MCF, the importance of biosecurity regarding separation of ruminant species in a production operation, and
emphasizes the multiple modalities required to make a definitive etiologic diagnosis.
JPC Diagnosis: 1. Cerebellum: Vasculitis, necrotizing and lymphocytic, multifocal, moderate, with hemorrhage, neuronal necrosis, and diffuse lymphohistiocytic meningitis.
2. Trachea: Vasculitis, necrotizing and lymphocytic, diffuse, moderate, with hemorrhage and mucosal and submucosal gland necrosis.

Conference Comment: The contributor has done an excellent job summarizing the condition knowns as malignant catarrhal fever, from species affected, basic pathogenesis, and molecular aspects of this disease.

American bison are considered one of the most sensitive species to the various viruses which may cause MCF, particularly that of OHV-2. Dr. Donal O'Toole has written and published extensively on this topic[7,8,9], and his comments certainly bear summarization for this species, one of the U.S. most majestic symbols.

Bison with MCF due to OvHV-2 are most often found dead or dying, due to the large size of bison pastures and the inherent difficulties in patrolling these large areas of land. Mortality among bison heard approaches 100%, especially in those in feedlots. Some feedlots experience up to 75% of all deaths as a result of MCF. Aerosol transmission is an important route of exposure between bison in sheep, especially in sale barns.[9]



Trachea, ox. Submucosal arterioles often are surrounded by a lymphocytic infiltrate.

Acute clinical disease in bison predominates over subclinical and chronic forms. In natural outbreaks, preclinical pneumonia is inconspicuous or may be masked by aspiration pneumonia of the clinical disease. Characteristic gross findings in terminal animals include oculonasal discharge, hemorrhagic cystitis, necrohemorrhagic typhlocolitis, ulcerative rhinitis, stomatitis, pharyngitis, laryngitis, and esophagitis.[9] Abortions may also occur. Due to its prevalence in affected animals and rarity in other conditions, hemorrhagic cystitis may be used by owners in making a provisional diagnosis of this condition. Generalized lymph node hyperplasia is usually present, but rarely as pronounced as in cattle. Ulceration in the forestomachs and alimentary tract is common. Traumatic lesions due to goring may be seen and may be incorrectly assumed to be the sole cause of illness.

Histologic lesions are similar to that discussed with cattle; in addition, degeneration of urothelium is a consistent and helpful feature in fresh carcasses. Fibrinoid necrosis is less common bison than in cattle. Myocardial necrosis may be seen in more longstanding cases as a result of exertional myopathy.[9]

Sampling for OHV-2 is occasionally problematic as the distribution of OHV-2 antigen may not necessarily reflect the pansystemic nature of disease. Sampling a minimum of 2 tissues, both lymphoid and nonlymphoid is recommended, and OHV-2 DNA may also be found in peripheral blood of clinically healthy bison. Interestingly in spite of the widespread and often severe lesions, no viral particles are detectable ultrastructurally, and the expression of viral antigens in tissue is limited. It is generally assumed that the small fraction of infected lymphocytes induces proliferation and deregulation of uninfected cells, which may induce lesions due to non-specific cytotoxic effects.[9]

Contributing Institution:

University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology
http://www.vet.upenn.edu/research/academic-departments/pathobiology/pathology-toxicology

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CASE II: S1408459 (JPC 4066395-00).

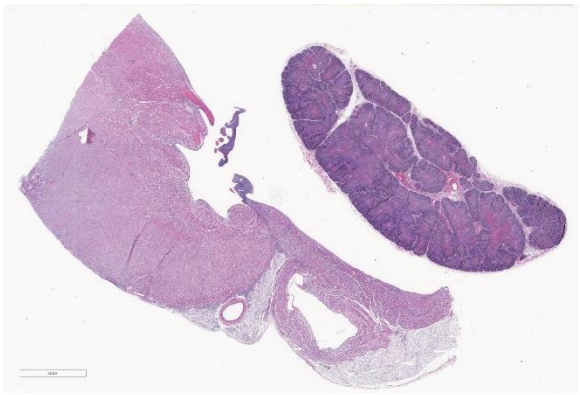
Signalment: An aborted Angus bull calf, *Bos taurus*, bovine

History: An aborted Angus bull calf of approximately 6 month gestation; second first time heifer to have aborted fetus on the previously non-grazed ranch.

Gross Pathology: A 10.56 kg Angus bull calf fetus with crown-to-rump length of 54 cm and in good post-mortem state. Lung lobes were diffusely red and samples sank in 10% formalin [non-inflated lung]. The liver weighed 0.84 kg, was deep red-brown, nodular, markedly enlarged with rounded margins, and had a cobblestone appearance with multiple, pinpoint white foci scattered throughout. Spleen and thymus were markedly enlarged and diffusely lymph nodes were enlarged and prominent.

No gross lesions / abnormalities were noted in other viscera, brain, umbilicus, cranial vault and in bones and joints of all limbs. Placenta was not submitted.

Laboratory results: Tissues tested negative for multiple agents on a full bovine abortion panel which included *Leptospira* sp., *Salmonella* sp., *Campylobacter* sp., *Brucella* sp., bovine herpes virus 1, and bovine viral diarrhea virus [BVDv]. Liver minerals including selenium were within acceptable concentrations.
Submitted dam serum had marginally low magnesium levels, and was negative for antibodies to *Leptospira* spp., BVDv-1,



Heart and thymus, aborted calf: There is multifocal thymic hemorrhage (incidental), and a cellular infiltrate can be seen within the myocardium and epicardium and infiltrating epicardial fat. (HE, 5X)

BVDv-2, BHV-1, *Neospora caninum* and *Brucella abortus*.

Microscopic Description: Slides contain a section each of thymus and heart.

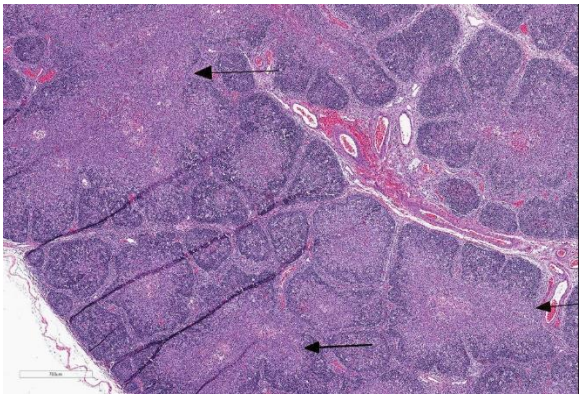
Thymus: There is marked depletion of cortical thymocytes affecting most if not all follicles, scattered foci of lymphocytolysis, marked histiocytic infiltration of the medulla and septa with occasional giant cell formation, and focal interlobular hemorrhages.
Heart: There are mild to moderate histiocytic infiltrates and fewer numbers of lymphocytes and plasma cells around small and medium caliber vessels throughout the epicardium, myocardium and epicardial fat.

Other findings: Mild to marked perivascular [vasculitis] and interstitial predominantly histiocytic infiltrates [fewer lymphocytes and plasma cells] in brain, lung, liver, kidney, spleen, lymph node, intestine, diaphragm, adrenal gland, and urinary bladder. There was marked lymphoid hyperplasia in spleen and lymph nodes in addition to infiltration by macrophages. Tissues were negative for *Mycobacterium* sp. on acid-fast stains.

Contributor's Morphologic Diagnoses: Thymus: Thymitis, histiocytic to granulomatous, marked, diffuse with loss of cortical thymocytes and lymphocytolysis, consistent with epizootic bovine abortion.

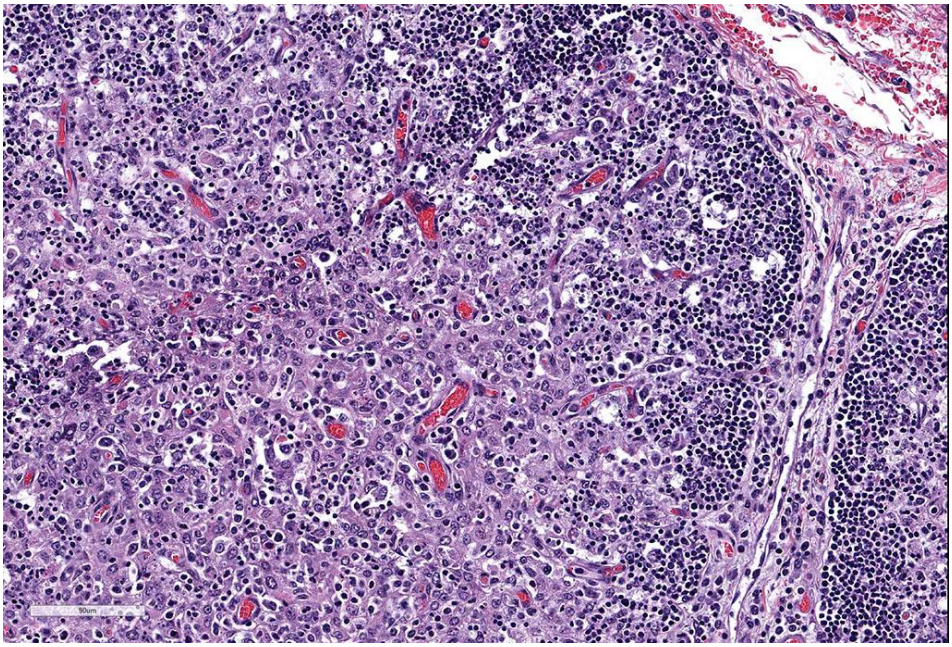
Heart: Vasculitis, histiocytic and lympho-plasmacytic, multifocal, moderate.

Contributor's Comment: Lesions are similar in submitted slides though there may be some variation in severity and extent. The clinical history, gross findings and microscopic lesions particularly those noted in the thymus, spleen and lymph nodes are considered characteristic of epizootic bovine abortion [EBA].



Thymus, aborted calf: The thymic medulla is markedly expanded by an infiltrate of large numbers of macrophages (arrows). (HE, 32X)

EBA, also known as foothill abortion, is a tick-born infection of cattle that results in late term abortions of fetuses, stillbirths, or the birth of weak calves. The name of the disease is misleading given that the disease is endemic, and distribution is confined to the range of the vector, the argasid tick *Ornithodoros coriaceus*, which includes foothills of California, and adjacent states of Nevada, Oregon and Northern Mexico.[1,6,13] The tick is thought to transmit the agent from deer to cattle, and naïve cattle i.e. first time heifers or pregnant cows mainly beef cattle grazing for the first time in a tick-infested area, are primarily at risk.[7,10] Infection from dam to fetus commonly occurs at 2-6 months gestation resulting in chronic disease in the fetus.[7] The etiologic agent of EBA (aoEBA) has been identified as a novel organism in the



Heart and thymus, aborted calf: Higher magnification of the numerous macrophages infiltrating the thymic medulla. (HE, 320X)

class Deltaproteobacteria in the order *Myxococcales*. [10]

Clinical findings include petechial hemorrhages of conjunctiva, tongue, oral mucous membranes and tracheal mucosa, palpable lymph nodes, and often ascites. Grossly there is splenomegaly, lymphadenomegaly, enlarged nodular liver, and enlarged thymus with hemorrhages.[1,7,9] Microscopic lesions consist of marked lymphoid hyperplasia of lymph nodes and spleen accompanied by mononuclear, predominantly histiocytic, infiltration of medulla and sinuses. Histiocytes diffusely infiltrate medulla and septae of the thymus, and are present around vessels in multiple organs sometimes with giant cell formation.[1,7,8,11] Bacterial rods, 1.5 to 3 µm long can often be seen in cytoplasm of histiocytes using Steiner's silver stains, and there is positive cytoplasmic EBA immunohistochemical staining confined to foci of inflammation in multiple tissues.[1]

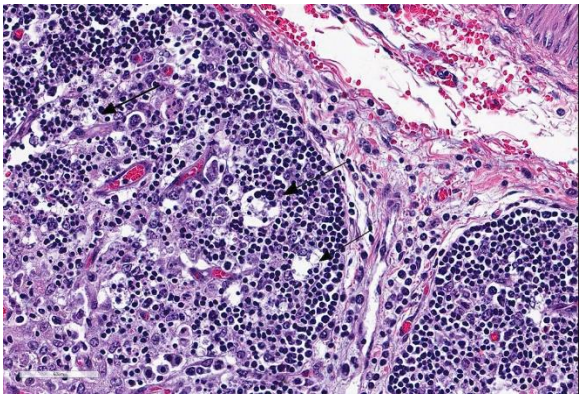
Diagnosis is based on compatible clinical history, gross and microscopic lesions, elevated fetal immunoglobulins particularly IgG, and exclusion of other agents commonly associated with bovine abortion.[2,3]. Supplemental diagnostic tests to identify agent in tissues include histochemical [Steiner's silver] and immunohistochemical stains,[1] and TaqMan polymerase chain reaction.[4,10]

Successful transmission of the disease has been achieved by inoculation of thymus from EBA-positive fetuses in naïve pregnant cows[12], and viability of the agent had been proved following serial passages and propagation of deltaproteobacterium in immunodeficient mice.[2] While the tick *O. coriaceus* is identified as the known vector for the deltaproteobacterium, co-infection of the tick with *Borrelia coriaceae* has been reported, and antibody cross-reaction with *Borrelia* spp. in fetal tissues is possible.[14]

JPC Diagnosis: 1. Thymus: Thymitis, granulomatous, diffuse, marked, with marked lymphoid depletion.

2. Heart: Pancarditis, granulomatous, diffuse, mild.

Conference Comment: Since the submission of this case in 2015, additional characterization of this agent and a name change has occurred. The organism is



Thymus, aborted calf: The cortex is markedly depleted, with lymphocyte karyorrhexis and tingible body macrophages (HE, 400X)

currently identified as *Pajaroellobacter abortibovis*, after the Pajaroello tick, (*Ornithodorus coriaceous*) which transmits the disease (and which was identified as the vector in 1976). The name "Pajaroello" (pa-har-way"-o) comes from the Spanish "paja" meaning straw and "huello" meaning the underside of a hoof, is also called to the grayback or leatherback tick, and is found only in California and Mexico extending from Humboldt County on the north to the Mexican isthmus of Tehuantepec.[7]

Phylogenetic characterization of this yet-to-be-cultured bacterium is currently based on analysis of the 16S ribosomal RNA gene, which places the bacterium in the order Myxococcales, suborder Sorangiineae, family Polyangiaceae and the bacterium is most closely related to *Sorangium cellulosum*. [5] Modified Gram staining, combined with transmission electron microscopy, provides strong evidence that the bacteria is gram-negative.[5] PCR analysis has demonstrated *P. abortibovis* to be a myxobacterium a member of a family of gram-negative rods typically inhabiting soil. These bacteria are referred to as "gliding" bacteria as many produce a polysaccharide slime layer to aid in their motility.[5] Its most closely related relative, *S. cellulosum* carries the largest genome of any known bacterium. In addition, its 16S ribosomal sequence establishes a distant connection to another deltaproteobacterium, *Lawsonia intracellularis*.[5]

Interestingly, the bite of the pajaroella tick (*Ornithodoros coriaceous*) has for many years been the subject of much misinformation and great myth. "A bite more severe to than that of the rattlesnake" is largely the product of the overactive imagination of the lay press, with hyperbolic early reports describing the bite as "intolerably sharp and painful… with intermittent irritation, irritability, and numbness" persisting for weeks to months. Local folklore in one area reported that "three bites would result in certain death."[7] The truth of the matter is that the macule or papule resulting from the bite of this tick is similar grossly and histologically to those of other ticks of a similar size.[7]

Contributing Institution:

California Animal Health and Food Safety Laboratory, University of California Davis, 105 W.
Central Avenue, San Bernardino, CA 92374
http://www.vetmed.ucdavis.edu/cahfs/

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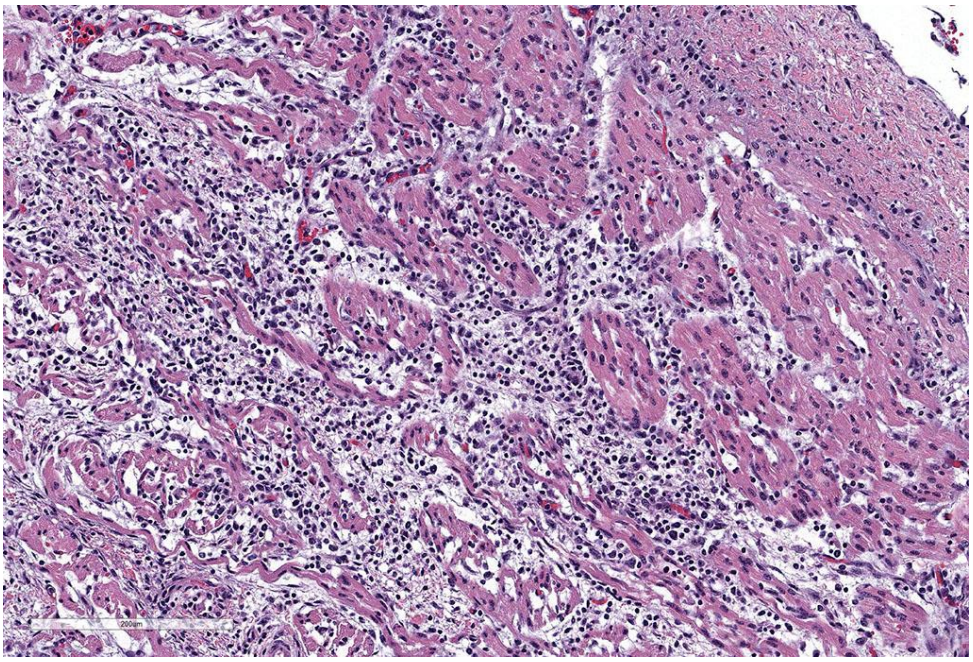

Heart, aborted calf: There is patchy infiltration of the myocardium and separation of myofibers by moderate numbers of histiocytes and lymphocytes. (HE, 183X).

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CASE III: 13749 (JPC 4033383-00).

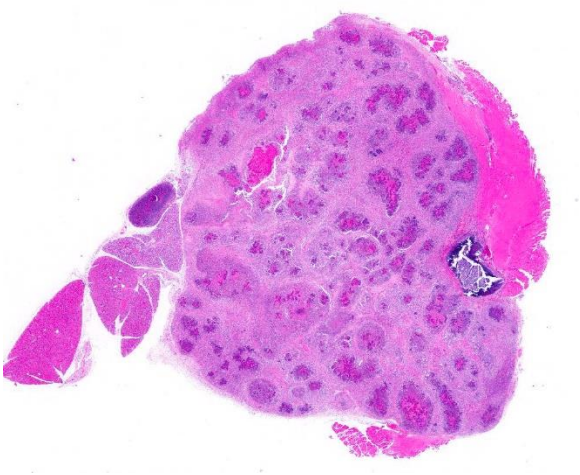
Signalment: 7-month-old B6;129 wild type mice (hybrid C57BL/6J; 129X1/SvJ), male.

History: The mouse belonged to a WT +/+ control group in a study using genetically engineered mice on a B6;129 background. It was euthanatized at the age of 7 months for ethical reasons due to a rapidly growing submandibular mass. No other animal of the study was affected.

Gross Pathology: A multinodular tan mass replaced and destroyed most of the left submandibular salivary gland. After section the mass, measuring 2cmx1cm, appeared encapsulated and filled with white to tan caseous material.

Laboratory results: None provided.

Microscopic Description: The sub-mandibular salivary gland is almost entirely replaced by a well-circumscribed multifocal coalescing inflammatory lesion. Most foci are identical and centered on a core of eosinophilic karyolitic or karyorrhectic necrotic cell debris, pyknotic and karyorrhectic neutrophils (suppuration) and cocci colonies circumscribed by a radiating band of hyaline eosinophilic material (Splendore-Hoeppli phenomenon). A peripheral rim of activated macrophages, epithelioid macrophages, multinucleated giant cells, lymphocytes, fibroplasia and



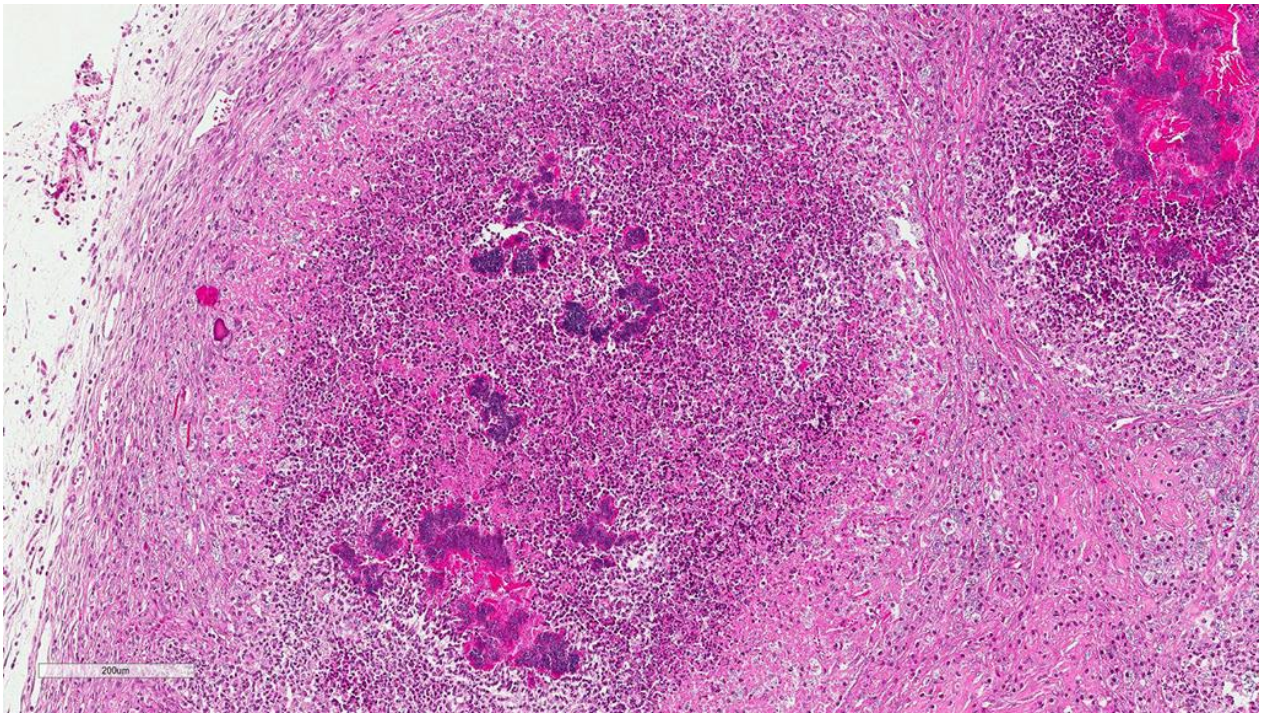
Subcutaneous tissue, mouse: The subcutis ventral to the ear is expanded by numerous coalescing pyogranulomas which infiltrate the adjacent salivary gland and lymph node. (HE, 5X)

fibrosis form the external limit of those pyogranulomas. Macrophages and giant multinucleated cells often display a markedly enlarged cytoplasm filled with myriads of cocci. Those bacteria-filled macrophages are also observed within the fibrotic bands. Necrotic centers display multifocal dystrophic mineralization.

Eosinophilic macrophages and acicular or rectangular eosinophilic birefringent crystals are observed within the pyogranulomas. Crystals are found free in the extracellular space and less commonly in the cytoplasm of activated macrophages and giant cells.

A Gram coloration was performed and revealed the presence of abundant gram-positive cocci, 1 to 2 um in diameter, consistent with staphylococci.

Contributor's Morphologic Diagnoses: 1. Multifocal coalescing chronic pyo-granulomatous sialoadenitis with cocci circumscribed by Splendore-Hoeppli phenomenon (botryomycosis).



Subcutaneous tissue, mouse: Pyogranulomas are centered on colonies of cocci which are surrounded by eosinophilic Splendore-Hoeppli material. (HE, 108X)

2. Presence of acicular and rectangular eosinophilic crystals into macrophages, giant cells, and extracellular space.

Contributor's Comment: Botryomycosis is a chronic suppurative infection characterized by a granulomatous inflammatory response to bacterial pathogens; it may present with cutaneous or, less commonly, visceral involvement. The term botryomycosis is derived from the Greek word *botrys* (meaning "bunch of grapes") and *mycosis* (a misnomer, due to the presumed fungal etiology in early descriptions). In Human, the major etiologic agent of botryomycosis is *Staphylococcus aureus* (40%) followed by *Pseudomonas aeruginosa* (20%), *coagulase-negative staphylococci*, *Streptococcus spp*, *Escherichia coli*, and *Proteus spp*[1]. The Spendore-Hoeppli phenomenon consists of antigen-antibody precipitates, immuno-globulin G, C3 complement, tissue debris and fibrin[1].

Staphylococci are commensal bacteria that inhabit the skin and mucous membrane and that can become pathogenic under certain circumstances. In the mouse, disease has been associated with *Staphylococcus aureus* and *S. xylosus*, causing necrotizing dermatitis, chronic suppurative infection of cutaneous adnexae, conjunctiva, periorbital tissues, preputial glands and regional lymph nodes[2]. The Splendore-Hoeppli phenomenon is classically observed in staphyloccocal lymphadenitis[2] and in chronic skin infection affecting immune compromised mice such as agammaglobulinemic or nude mice[3]. Botryoid abscess of a salivary gland is to our knowledge uncommon, especially in immunocompetent mice, and has not yet been documented.

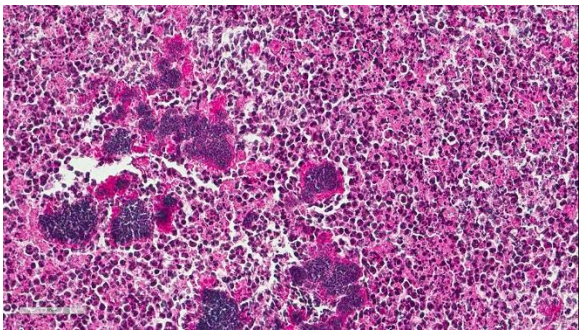
The incidence of eosinophilic crystals is high in aging B6;129 mice, a hybrid background often encountered in studies using genetically engineered mice. It is observed in lungs (36% of males and 65% of females) and nasal

turbinates (4% of males and 12% of females)[4]. The so-called eosinophilic crystalline pneumonia is a major cause of death in 129/SvJ mice and immuno-histochemical studies show that crystals are composed predominantly of Ym1 protein, a chitinase-like protein known to be secreted by activated macrophages[5]. In our case, the context of chronic granulomatous inflammation with numerous activated macrophages is very likely the direct cause of Ym1 protein accumulation and crystallization.

JPC Diagnosis: Subcutis: Cellulitis, pyogranulomatous, focally extensive, severe with numerous colonies of cocci, Splendore-Hoeppli material, with mild to moderate granulomatous lymphadenitis and sialoadenitis.

Conference Comment: Botryomycosis is a chronic pyogranulomatous disease which is as well known in mice as it is poorly named. This inflammatory lesion is seen in a wide range of mammals including man, mice, dogs, cattle[6], swine, apes, and elephants, and likely many other in species. The lesion, which consists of colonies of bacteria surrounded by aggregates of antigen-antibody complexes (known as Splendore-Hoeppli material), goes also by the name of "bacterial pseudomycetoma", "bacterial pseudomycosis", "bacterial granuloma", and "granulomatosis". It has no connection to fungal infections, other than a passing resemblance to mycetomas, or deep fungal infections which are characterized by "grains".

Historically, the first cases of botryomycosis were observed in the late 1800s in post-surgical cattle and horses, with the first actual description of botryomycosis in a horse in 1870 by Bollinger. The first human case of botryomycosis was not reported until 1913.



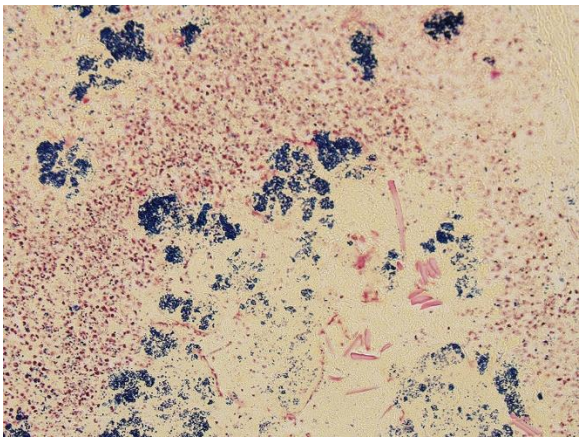
Subcutaneous tissue, mouse: Higher magnification of the center of the pyogranulomas. (HE, 108X)

The causality of *Staphylococcus aureus* and description of Splendore-Hoeppli phenomenon was first made in 1919.[4]

Botryomycosis is an uncommon disease in humans with approximately 200 cases reported to date. Case distribution is worldwide, and the disease predominates 2:1 men with no age predilection. 75% of reported cases are cutaneous, with the rest visceral. Accidental or postoperative skin trauma and altered immune function are considered major contributing factors.[4]

The pathogenesis of botryomycosis in humans and animals has not been explicitly defined, but several factors appear to be necessary. Most causative microorganisms are of low virulence. The contributor lists the commonly implicated organisms (above). Implicated anaerobes include *Propionibacterium acnes* and *Bacteroides* species. Rare causes include *Actinobacillus lignieresi*, *Serratia marcescens*, *Pneumocystis jirovecii*, and *Moraxella* sp[4].

A wide range of immune dysfunctions have been associated with the development of botryomycosis in humans and animals.[4] It has been found more often in animals with deficient cellular immune responses including lymphopenia (nude mice) and various hypoglobulinemic syndromes (agammaglobulinemic mice).



Subcutaneous tissue, mouse. A tissue Gram stain demonstrates the gram-positivity of the cocci, as well as highlighting shards of crystalline protein within the pyogranulomas. (Brown-Brenn, 200X)

Cutaneous botryomycosis require skin trauma in most cases, as low-virulence bacteria are generally unable to cross an intact barrier. The inoculum of bacteria must be high enough to evade the body's immediate immune response, yet low enough not to cause massive necrosis or systemic disease. In all but severely immuno-suppressed patients, the Splendore-Hoeppli phenomenon (SHP) is present. SHP assists in clustering the bacteria to create a stage of resistance. The major component of SHP is antigen-antibody complexes, but other substances are often present, to include immunoglobulin G, C3 fragments of complement, fibrin, and cellular debris.[4]

Both cutaneous and visceral forms of botryomycosis are well documented in man in animals. In humans, cutaneous forms most often affect expose parts of the body including the hands, feet, head, and neck. Visceral forms may be seen in almost 10 organ and in humans often accompanies prolonged hospital stay's, especially in immunosuppressed patients. The most frequent visceral organs involved or the lungs, and are often associated with patient suffering from cystic fibrosis. HIV/AIDS is also a common predisposing condition.[4]

Contributing Institution:
Department of Pathology, Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering - ONIRIS, 44 307 Nantes Cedex 03, France.
www.oniris-nantes.fr

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CASE IV: Case 2 (JPC 4068765-00).

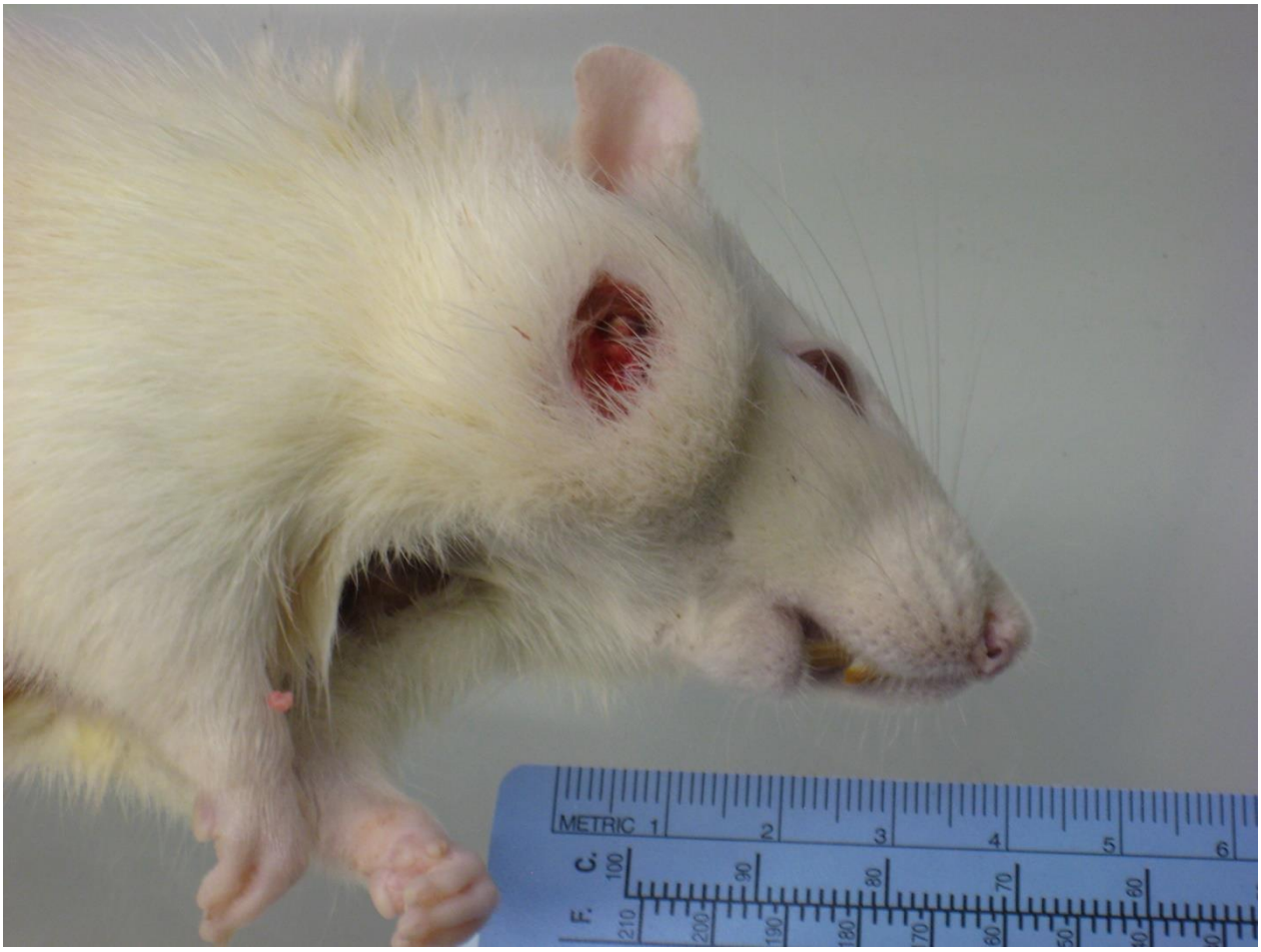
Signalment: 6 month old, female, Harlan Sprague-Dawley rat, (*Rattus norvegicus*)

History: Moribund sacrifice due to ulcerated facial mass; several animals affected.

Gross Pathology: A multinodular tan mass replaced and destroyed most of the left submandibular salivary gland. After section the mass, measuring 2cmx1cm, appeared encapsulated and filled with white to tan caseous material.

Laboratory results: None provided.

Microscopic Description: Facial mass: An approximately 2 x 2cm, well-circumscribed, unencapsulated, multilobular, and cystic neoplasm is expanding and compressing the subcutis. The neoplasm is composed of polygonal cells arranged in islands, cords, and trabeculae supported by moderate fibrovascular stroma. Neoplastic islands and trabeculae are often bordered by a peripheral layer of flattened to cuboidal epithelial cells with scant, eosinophilic cytoplasm, and condensed round to oval nuclei (basaloid reserve cells) and also contain a central area comprised of polygonal cells with abundant, microvacuolated cytoplasm and round nuclei with finely stippled chromatin and 1-3 variably distinct nucleoli (sebaceous

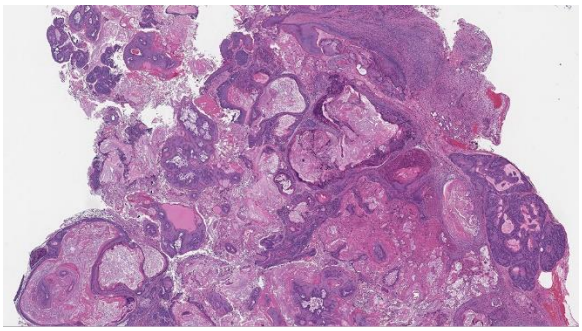


Facial mass, rat. An approximately 2 x 2cm, ulcerated nodule is at the base of the right ear. (Photo courtesy of: NIEHS/NTP, http://ntp.niehs.nih.gov/nnl/)

differentiation). Anisocytosis and aniso-karyosis are minimal and the mitotic rate for both cell populations is less than 1 mitotic figure per 40X field. Also within the neoplasm, are multifocal areas of squamous epithelial cells undergoing gradual keratinization, lining cystic spaces filled with lamellar whorls of keratin, moderate numbers of viable and degenerative neutrophils, smooth proteinaceous material (sebum), and variable amounts of necrotic debris (cystic degeneration and ductal differentiation). The neoplasm is focally disrupted and replaced by large numbers of epithelioid macrophages with scattered neutrophils and multinucleated giant cells (pyogranulomatous inflammation), reactive fibroblasts with perpendicularly arranged small caliber vessels lined by hypertrophied endothelium (granulation tissue) admixed with collagen, lamellations of keratin, and necrotic and proteinaceous debris. Some of these areas appear to be associated with focally extensive ulceration. There are also multifocal areas of mild hemorrhage scattered throughout the neoplasm.

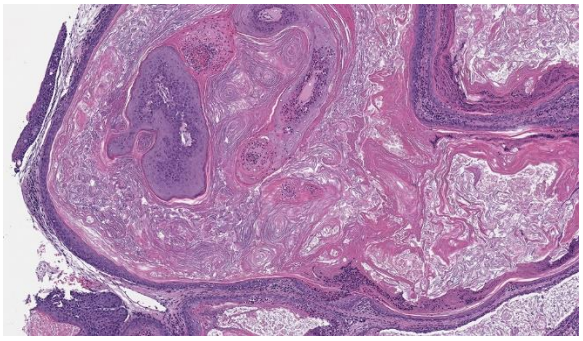
Contributor's Morphologic Diagnoses: Zymbal's gland: Adenoma.

Contributor's Comment: The Zymbal's gland or auditory sebaceous gland is a specialized sebaceous gland unique to rodents and insectivores.[3,6,7] This compound branched acinar gland sits anterioventral to the external ear meatus and is composed of 3-4 triangular lobes. These lobes are characterized by clusters of saccular acini surrounding a prominent interlobular duct that drains into a short excretory duct. The periphery of each acinus is lined by an incomplete layer of flattened to cuboidal cells containing hyperchromatic nuclei and scant cytoplasm (basaloid reserve cells) while the center is comprised of large polygonal cells with pale, foamy cytoplasm due to the progressive accumulation of lipid vacuoles.[6] Two smaller sebaceous glands underlying the ear canal epithelium are sometimes considered to be a component of the Zymbal's gland; these glands are holocrine glands, as their secretory products are formed from degenerative mature acinar cells that disintegrate and discharge into the ducts.[3,4,6]



Facial mass, rat. A well-circumscribed, multilobular, unencapsulated, and cystic neoplasm is expanding and compressing the subcutaneous tissue. Note remnants of more normal sebaceous (Zymbal's) gland along the periphery. (HE, 1X) (Photo courtesy of: NIEHS/NTP, http://ntp.niehs.nih.gov/nnl/)

Spontaneous neoplasms of the Zymbal's gland are uncommon in Fisher 344 (F344) and Harlan-Sprague Dawley rats and rarely occur in the mouse.[1,5,6] The classic presentation for a Zymbal's gland tumor is an ulcerated mass on the side of the face or the base of the ear. Early neoplasms are typically firm, well-circumscribed, freely moveable, subcutaneous masses whereas larger neoplasms are frequently ulcerated, with or without extension into the ear canal, and may exude caseous or purulent material on cut section. Most Zymbal's gland neoplasms exhibit histological features of malignancy with local invasion, cellular atypia, necrosis and high mitotic activity, however, metastasis is not reported to occur. [3,4,6] Benign neoplasms, adenomas or papillomas, are generally smaller, well-circumscribed masses with minimal cellular atypia and



Facial mass, rat. Higher magnification of one f the lobules with a central cystic area lined by proliferating squamous epithelium which largely recapitulates normal epidermal maturation in verrucous projections into the cystic space. (HE. 10X) (Photo courtesy of: NIEHS/NTP, http://ntp.niehs.nih.gov/nnl/)

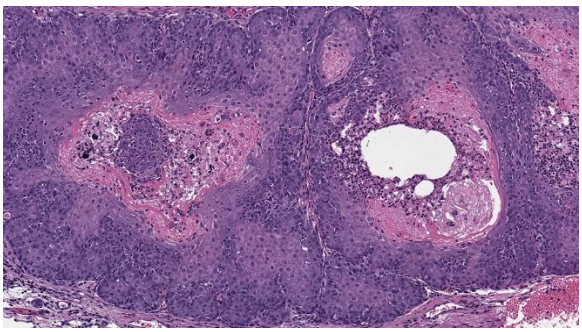
invasion. Zymbal's gland carcinomas are less well-circumscribed with invasion of the surrounding tissues and frequent cystic cavities filled with mixtures of proteinaceous fluid (sebum), keratin and necrotic debris. The neoplastic sebaceous and squamous cells that comprise the malignant neoplasms are very pleomorphic and anaplastic and some tumors are composed almost entirely of squamous epithelium.[4,6]

While spontaneous Zymbal's gland neoplasms are infrequently observed, these types of tumors are easily induced by a variety of carcinogens, particularly aromatic amines like benzene.[3,4,7] The Zymbal's gland lacks transacylase and sulfotransferase activity, but is able to hydroxylate compounds via cytochrome p450-dependent enzymatic pathways. Detectable cytochrome p450 activity within these tumors suggests that the formation of active metabolites within the affected gland may contribute to tumorigenesis. In a survey conducted by the National Cancer Institute, 8% of rats treated with carcinogenic compounds developed Zymbal's gland neoplasms (where N = 526 different chemical studies), yet tumor incidence increased to 14% among rats treated with those chemicals known to be mutagenic for *Salmonella typhimurium* (N = 214); this indicates that those chemicals typically causing neoplastic transformation in specialized sebaceous glands, like the Zymbal's gland, are typically mutagenic compounds.[2,6] The rat in this case was part of an 18-month carcinogenicity study for a known carcinogen with no reported mutagenic activity.

JPC Diagnosis: Zymbal's gland: Adenoma.

Conference Comment: Due to their unique location and fairly characteristic histologic presentation, Zymbal's gland neoplasms are one of the most well-known neoplasms in the rat. The contributor has provided a concise but thorough review of Zymbal's gland neoplasia in the rat. However, it should be noted that the Zymbal's gland undergoes other degenerative, inflammatory, vascular and other non-neoplastic changes, most similar to those observed in the mammary gland.

Non-neoplastic changes of degeneration, single cell necrosis (apoptosis), necrosis, and regeneration changes may all be seen in the ductal and sebaceous epithelium[4] following administration of toxins, and cystic



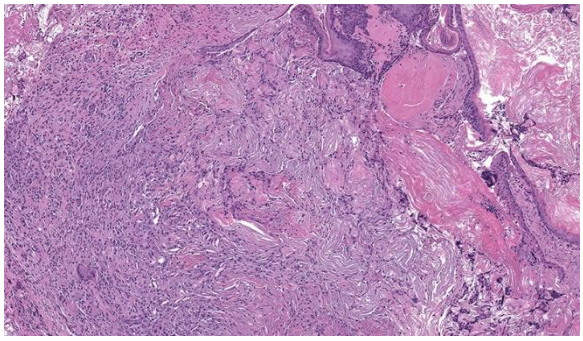
Facial mass, rat. Squamous epithelial cells exhibit gradual keratinization and line cystic spaces filled with lamellar whorls of keratin, moderate numbers of viable and degenerative neutrophils, smooth proteinaceous material (sebum), and variable amounts of necrotic debris (cystic degeneration and ductal differentiation). HE. 10X. (Photo courtesy of: NIEHS/NTP, http://ntp.niehs.nih.gov/nnl/)

degeneration of the ducts may be seen as an aging change. Zymbal's glands are uncommonly inflamed, but may be home to the typical range of inflammatory cells, largely depending upon the chronicity of the insult. It may be a site for metastasis in lymphoma or other neoplasms of hematopoietic origin.[4] Both hyperplastic and atrophic changes are occasionally observed in acinar epithelium. The retention of normal tissue architecture aids in differentiating adenoma from hyperplastic lesions, and adenomas will also have increased numbers of basal cells.[4]

Neoplasms of Zymbals's glands include adenomas (as seen in this case), carcinomas, and squamous papillomas, which may closely resemble tumors of the glandular epithelium, and often pose a diagnostic dilemma.

Carcinomas may be differentiated from adenomas due to the presence of large, irregular acini lacking ducts, and the presence of invasive growth with nests or cords of squamous epithelium penetrating the basal lamina and invading the underlying dermis and skeletal muscle.[4] Close examination will reveal abnormalities in cellular differentiation of cells above the basal layer; there is variation in size and staining of nuclei, atypical mitotic figures, and loss of intracellular bridges.[4]

A more difficult differential diagnosis is squamous papilloma of the Zymbal's gland (WSC 2015-2016, Conference 22, Case 3.) These neoplasms arise from the main duct epithelium and have a complex arborizing structure with maturing squamous epithelium overlying fibrous cores. One of the keys for the diagnosis of squamous papilloma of the Zymbal's gland is the absence of sebaceous differentiation and the entirely keratinaceous



Facial mass, rat. Marked granulomatous inflammation in areas of ulceration and extrusion of keratin debris into the fibrovascular core of the neoplasm (HE. 10X.) (Photo courtesy of: NIEHS/NTP, http://ntp.niehs.nih.gov/nnl/)

nature of the contents in the cystic area of the neoplasm. [4]

Contributing Institution:
NIEHS/NTP
http://ntp.niehs.nih.gov/nnl/

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