Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #14 — 11 December 2024

CASE I:

Signalment:
Adult male limousine bull, (*Bos taurus*)

History:
Unknown.

Gross Pathology:
Post-mortem examination revealed:

- Diffuse alopecia and lichenification and multifocal erythematous nodules (Fig. 1)
- Multifocal granulomatous scleritis and conjunctivitis (Fig. 2)
- Multifocal granulomatous orchitis (Fig. 3)

No other macroscopic lesions were present.

Microscopic Description:
Epiglottis: Multifocally, in the lamina propria and within the mucous gland interstitial tissue, a moderate number of round, 400µm in diameter, protozoal cysts are visible. Cysts are composed of a multilayer wall; the following wall parts are recognizable: an external fibrillary host 'collagen layer; a thick, homogenous eosinophilic outer layer; and a thin inner layer composed host cell cytoplasm, containing multiple flattened nuclei with prominent nucleoli. The parasitophorous vacuole within the host's cell cytoplasm is 250µm in size, and contains a large number of densely packed, 3-5 µm crescentic bradyzoites (Figure 4). The inflammation around the cysts is almost absent or minimal, and mainly composed of macrophages.

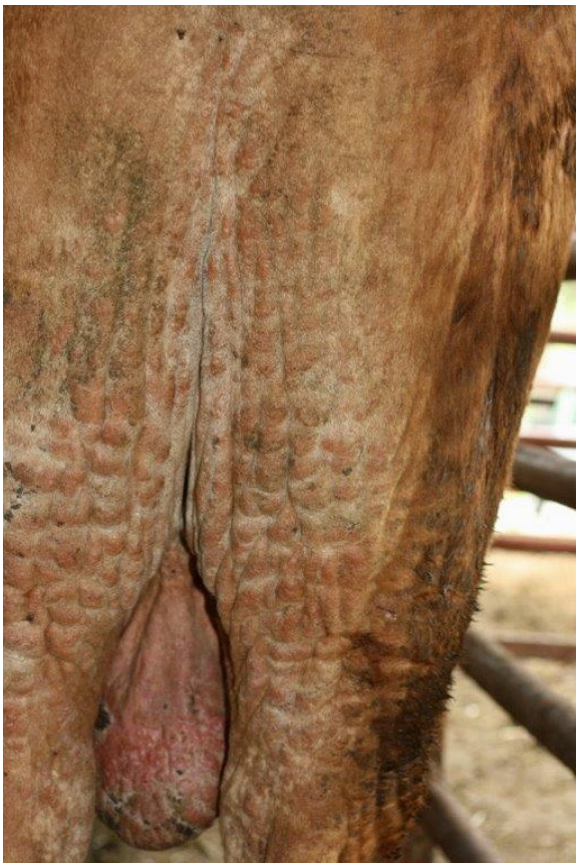

Figure 1-1. Haired skin, ox. The skin over the caudal hind legs is alopecic and markedly thickened. (*Photo courtesy of:* Laboratoire d'Histopathologie animale, Vetagro Sup, Campus vétérinaire, www.vetagro-sup.fr)

Contributor's Morphologic Diagnosis:
Epiglottis: epiglottitis, granulomatous, multifocal, minimal, with moderate number of protozoal cysts; etiology consistent with *Besnoitia besnoitii,* bovine.

Figure 1-2. Eye, ox. There are numerous protozoal cysts within the sclera (arrows). (*Photo courtesy of:* Laboratoire d'Histopathologie animale, Vetagro Sup, Campus vétérinaire, www.vetagro-sup.fr)

Contributor's Comment:
Besnoitia spp are obligatory intracellular apicomplexan protozoal parasites belonging to the *Sarcocystidae* family. Several species have been identified, affecting both wild and domestic animals. (Table 1)

Besnoitia besnoiti is the causative agent of bovine besnoitiosis. The parasite is present worldwide. Bovine besnoitiosis has an outsized economic impact because of mortality, which can reach up to 10% of clinically infected animals in Africa. Similarly, poor body condition of surviving animals may result in culling or decreased market value.[6] The disease presents seasonal variations, but this does depend on the geographical region of affected animals.[6,8] Sex predisposition is also variable;[8] young adults are more affected by the severe clinical forms than animals of less than 18 months or older than 4 years.[6]

The life cycle of *Besnoitia* is known only for 4 species, for which felidae were recognized as the definitive host.[5,8] For the other instances which include *Besnoitia besnoitii*, the definitive host, mode of transmission, and pathogenesis are not completely understood.[8] In the species for which life cycle is known, the parasite is located in the intestine and the sporulated oocysts are released with feces. The exact mode of transmission is unknown, though blood-sucking insects and direct transmission between intermediate hosts are considered possible routes.[6,8] In the first phase of intermediate host infection, tachyzoites replicate in endothelial cells, monocytes, and neutrophils. They then move into peripheral tissues where they invade fibroblasts, myofibroblasts, endothelial cells, and/or smooth muscle cells and form bradyzoite-containing cysts. This intracellular replication induces profound changes in the hosting cell with development of parasitic cysts. The definitive host is infected by ingesting cysts containing tissues.[8]

Bovine besnoitiosis has acute, subacute and chronic phases. In the acute phase, tachyzoites replicate within endothelial cells, causing vasculitis and thrombosis. Clinically, fever, anorexia, rhinitis, conjunctivitis and photophobia, subcutaneous edema and peripheral lymph node hypertrophy can be observed. In the subacute phase, tachyzoites move to peripheral tissues and invade mesenchymal cells. Small cysts can be seen in the sclera, and chronic dermatitis starts to develop. The chronic form is linked to cysts maturation and eventually associated inflammation.

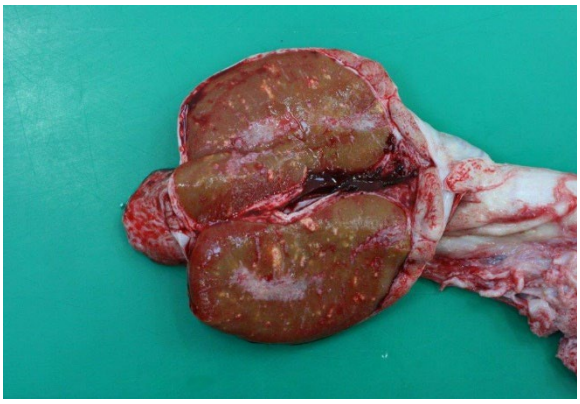

Figure 1-3. Testis, ox. There are numerous foci of granulomatous inflammation surrounding protozoal cysts within the testis. (*Photo courtesy of:* Laboratoire d'Histopathologie animale, Vetagro Sup, Campus vétérinaire, www.vetagro-sup.fr)

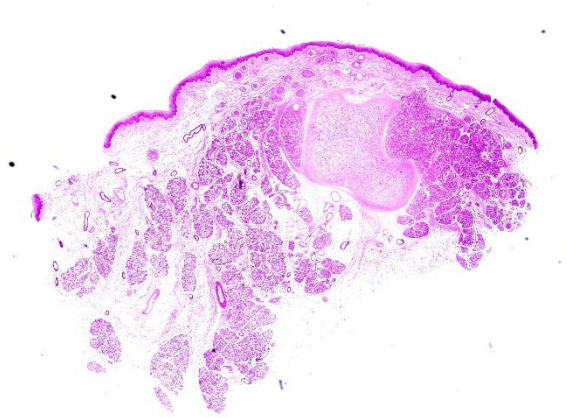

Figure 1-4. Epiglottis, ox. One section of epiglottis is submitted for examination. (HE, 5X)

Chronic dermatitis with alopecia and lichenification are often present and they can be aggravated by secondary cutaneous bacterial and fungal infections. Sterility, due to previous inflammatory reaction and vasculitis in the testis, can also be observed.[2,3,6]

Acute besnoitiosis is difficult to diagnose, while in chronic cases the histologic evidence of intra-cellular cysts is pathognomonic.[4] Today, no treatments exists.[6]

Grossly and histologically, the most affected organs are skin, upper respiratory mucosae, sclera and conjunctiva, and muscular and testicular connective.[2,6] Cysts can measure up to 0.5mm, being visible as small nodules at naked eye.[1,8]

Histologically, in the acute phase, moderate perivascular infiltrates composed of lymphocytes, plasma cells, macrophages and eosinophils are present in the dermis. Dermal edema is a consistent finding though hemorrhages are variable. Few tachyzoites can be visible in parasitophorous vacuoles within endothelial cells: they have a crescentic shape with an eosinophilic cytoplasm. Nevertheless, they can be better identified by immunohistochemistry.[4]

In the subacute and chronic phases, edema and vascular lesions regress and cysts form.

Cyst development is characterized by an increase in intracellular zoite number, host cell modifications (including increase in size, multinucleation, anisokaryosis, and prominent nucleoli) and development of a cyst wall. At the end of their maturation (34-50 days post seroconversion), cysts can be up to 400µm in size, and their size is correlated to their age.[1,4] The cyst wall is formed by 4 layers comprising an outer condensed collagen layer, an eosinophilic 30µm thick layer, a thin homogenous basophilic layer (not always visible, especially in older cysts), and a rim composed of scant cytoplasm containing multiple enlarged nuclei. The multilayer wall lines a parasitophororus vacuole containing many basophilic bradyzoites.[4] The inflammatory reaction is almost absent however. When present, especially secondary to cyst 'rupture, it is composed of macrophages, lymphocytes and plasma cells with few eosinophils. In the skin, cysts are mainly located in the papillary dermis.[1,4,8]

Table 1.

Intermediate Host	*Besnoitia spp.*	Definitive Host
Cattle	*B. besnoiti*	Unknown
Equids	*B. bennetti*	Unknown
Goats, sheep	*B. caprae*	Unknown
Reindeer, caribou	*B. tarandi*	Unknown
Rabbits	*B. oryctofelis*	Felidae
N. American opossums	*B. darlingi*	Bobcat[7]
Southern plain woodrat	*B. neotomofelis*	Felidae
Rodents	*B. wallacei*	Felidae

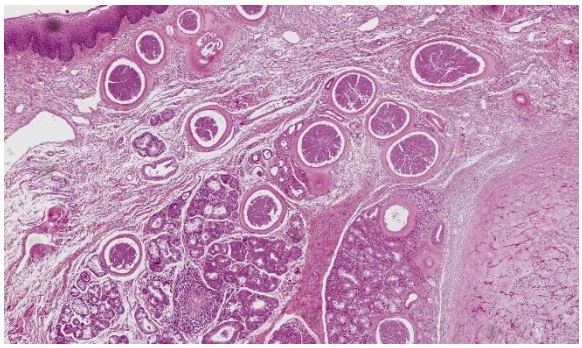

Figure 1-5. Epiglottis, ox. There are numerous apicomplexan cysts within the submucosa of the epiglottis. (HE, 146X)

Contributing Institution:
www.vetagro-sup.fr

JPC Diagnosis:
Epiglottis: Epiglottitis, granulomatous, multifocal, mild, with apicomplexan cysts.

JPC Comment:
This week's moderator was Dr. Corrie Brown from the University of Georgia who focused on infectious and transboundary diseases with conference participants. Although the Wednesday Slide Conference archives are (thankfully perhaps) not replete with Foreign Animal Disease examples, Dr. Brown nonetheless selected four cases to delve into comparative pathogenesis.

In this first case, the contributor provides several interesting gross images which are characteristic of *Besnoitia* infection. Conference participants were not entirely sure of tissue identification for the submitted histologic image, though the presence of tissue cysts, their predilection for the epiglottis, and a large wedge of cartilaginous tissue were helpful landmarks. Bradyzoites within tissue cysts were readily apparent on H&E alone, though PAS was similarly useful. In some smaller cysts, cell nuclei were still visible and peripheralized by parasitophorous vacuoles containing bradyzoites. Although not stated by the contributor, the presence of cysts within both connective tissue and glands suggests that one or more of fibroblasts, myofibroblasts, or endothelial cells were the site of tachyzoite replication in this case.

Similar to other emerging diseases, *Besnoitia* has been increasingly identified within European cattle, to include further northern locations such as Ireland.[9] As biting insects may play a role in the transmission of these protozoans, both prolonged periods of warmer temperatures and extreme temperatures due to global climate change likely aid in sustained development and dispersion of *Stomoxys* and *Tabanus*.

Dr. Brown emphasized the viewpoint of small-scale animal agriculture (i.e. 'smallholders'[10]) and the stakes at play in transboundary diseases. As each individual animal may represent significant economic value from production of meat, milk, and/or fiber, the impact of *Besnoitia* can be felt several times over. Though death is the most severe loss for smallholders of affected animals, loss of productivity (meat, milk) as well as poor quality hide in surviving animals is also a loss of income. Likewise, permanent infertility of bulls may rob smallholders of a

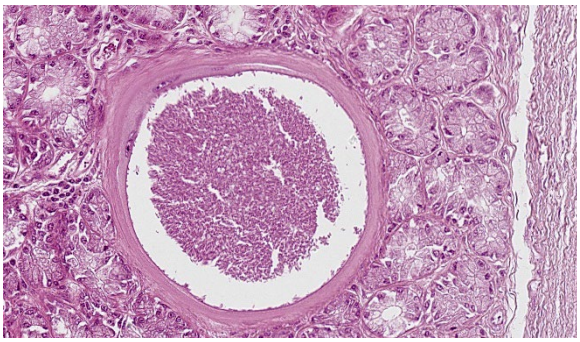

Figure 1-6. Epiglottis, ox. High magnification of a 250um apicomplexan cyst. The cyst is typical of *Besnotia* with a 5-10 um thick rim of host cell cytoplasm with multiple enlarged but flattened nuclei which in turn surround a parasitophorous vacuole containing numerous, densely packed crescentic 3-5 um bradyzoites. (HE, 560X)

prosperous future. Therefore, understanding and control of these diseases plays an important role in global food security and overall stability.

References:

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7. Verma SK, Cerqueira-Cézar CK, Murata FHA, Lovallo MJ, Rosenthal BM, Dubey JP. Bobcats (Lynx rufus) are natural definitive host of Besnoitia darlingi. *Vet Parasitol*. 2017 Dec 15;248:84–89.
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10. Lifestock International. About Us. https://lifestock.org/

CASE II:

Signalment:
Twin Oberhasli goat fetuses (kid A, kid B), *Capra aegagrus*.

History:
Received for necropsy examination in February were the bodies of twin Oberhasli goat fetuses (kid A, kid B) with placenta, reportedly stillborn/abortion. The history as stated by the submitter stated site of occurrence in San Pedro Valley River area of Arizona in the United states. Site on river, with a massive mosquito problem every year, also possible ticks; do treat to prevent but not usually effective. Unknown breeding date but doe was approximately 1-2 weeks from kidding based on ligaments starting to soften. Herd of 10, goats in good nutritional status. Put into fresh pasture 3 days prior to abortion with belly-high weeds, suspect mustard weed yellow flowers; pasture during day, hay at night, mineral supplement once weekly, dewormed twice annually, CDT immunization annually in March. Tested for Q fever, Caseous Lymphadenitis, Caprine Arthritis Encephalitis virus annually with specialty laboratory. These are the first kids born pre-term on farm in 20 years. Q fever reported in animals at another farm in area previously with one noted human testing positive from incident. Farm is a closed site, goats are inbred with only 1 buck,

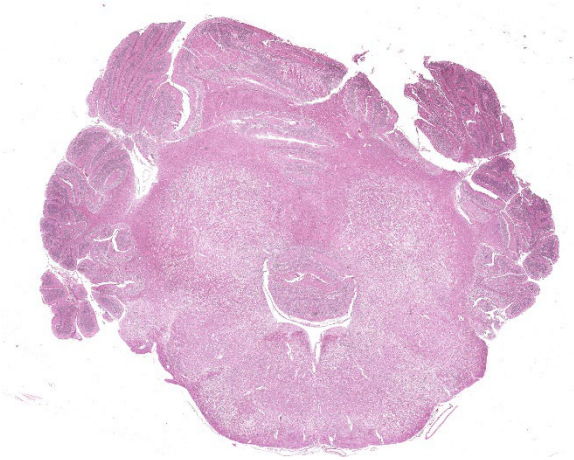

Figure 2-1. Metencephalon, kid. One section of the cerebellum and brainstem are submitted for examination. The cerebellum is mildly hypoplastic with decreased thickness of folia which collapse on themselves. There is diffuse vacuolation of the brainstem parenchyma with marked dilation of axonal sheaths. (HE, 6X)

two generations inbred; buck is sire of dam and of preterm kids. Buck has malformed head. Fetal bodies may be bruised due to doe pawing aggressively at them.

Gross Pathology:
Examined are twin goat fetuses (kid A, kid B) and placenta, all of which are in good post-mortem condition. Kid A is a 4 lb. female with a 47 cm crown to rump length that is well-haired across the body surfaces. Kid A has mild vascular congestion of the meninges but is otherwise unremarkable on external evaluation. Kid B is a 4.5 lb. male with a 37.5 cm crown to rump length that is well-haired across the body. Kid B has prominent brachygnathism (craniofacial deformity). There is widespread, moderate edema of the body wall as well as a large amount of partially clotted blood free within the peritoneal cavity (maternal or partum induced trauma vs. tissue edema & hemoperitoneum). The placenta is 0.75 lb. and appears complete, with no grossly evident lesions; environmental debris is present on surfaces.

Laboratory Results:
Bunyavirus & Cache Valley Virus gel-based PCR, Liver/Lung/Placenta (Texas Veterinary Medical Diagnostic Laboratory): Cache Valley Virus *detected*.

Coxiella burnetii Q Fever rtPCR, Pooled Liver/Lung/Placenta (Texas Veterinary Medical Diagnostic Laboratory): Not detected.

Abortion Panel Livestock Bacterial Culture, Pooled Liver/Lung/Placenta (Texas Veterinary Medical Diagnostic Laboratory): Mixed bacterial growth, negative specialized culture for *Brucella* sp. and negative specialized culture for *Campylobacter* sp.

Trace Mineral Panel, Liver (Texas Veterinary Medical Diagnostic Laboratory): cobalt 0.16 ug/g (no reference range), copper 267.42 ug/g normal (normal: 100-600), iron 324.53 ug/g normal (normal: 200-520), manganese 14.54 ug/g normal (normal: 8.0-24.0), molybdenum 0.85 ug/g below normal (normal: >1.24 ug/g), selenium 2.32 ug/g normal (normal: 1.00-4.80), zinc 252.55 ug/g normal (normal: 100-480).

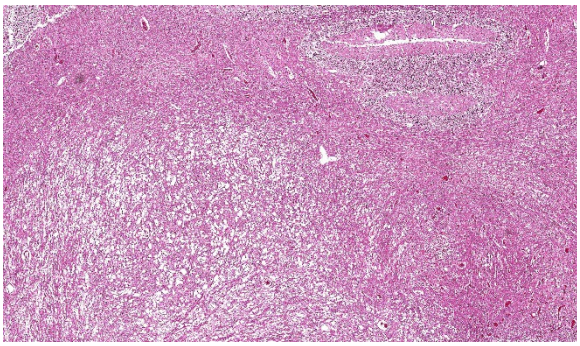

Figure 2-2. Metencephalon, kid. Higher magnification of the dilated axon sheaths within the brainstem. (HE, 44X)

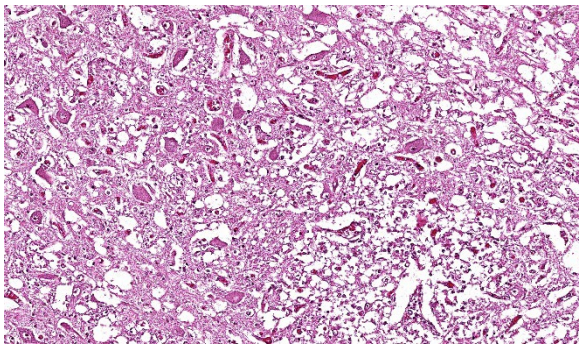

Figure 2-3. Brainstem, kid. Brainstem nuclei are hypocellular with marked loss of neurons and dilation of axon sheaths at right. (HE, 248X)

Microscopic Description:
Brain: The brainstem, and to a lesser extent the cerebellum, has variably mild to marked rarefaction of the neuropil, expanded clear Virchow Robin spaces, white matter dilated axonal tracts rarely containing swollen axons, rare random small areas with loss of cellularity with small basophilic aggregates, and variably diminished to normal nuclear density within the granular layer of the cerebellum (kid B). The grey matter of the cerebrum appears hypercellular with areas of diminished or loss layering (kid A, kid B). There is mild to moderate vascular congestion throughout the brain (kid A, kid B).

Placental meconium staining noted (not represented on JPC WSC slide submission). No significant lesions noted on histologic evaluation of major organs from kid A, kid B.

Contributor's Morphologic Diagnosis:
Brain: Rarefaction, hypoplasia, degeneration, locally extensive, grey and white matter, brainstem and cerebellum, marked (kid B)

Contributor's Comment:
Gross, microscopic, and molecular diagnostics collectively confirmed Cache Valley virus in this case. Cache Valley virus (CVV) belongs to the viral family *Bunyaviridae*, genus *Orthobunyaviridae*, serogroup Bunyamwera.[3] Cache Valley virus, Akabane virus, and Schmallenberg virus are each members of the genus *Orthobunyaviridae*. Orthobunyaviruses are usually maintained through arthropod-vertebrate-arthropod cycles.[3] These viruses have a notable tropism for fetal tissues.[3] In utero infection by these viruses causes prenatal losses and congenital deformities, including arthrogryposis and lesions in the brain ranging from hydrocephalus to hydranencephaly in certain mammalian hosts.[3,4,6]

Cache Valley virus is most frequently reported in sheep, but has been reported in other mammalian species.[3,4] Cache Valley virus has been reported as a cause of embryonic losses, fetal malformations, abortions, and stillbirths in sheep and goats.[1,3,4,6] Investigations on in utero infection of ovine fetuses show highest mortality with infection between 27-35 days gestation, highest incidence of congenital anomalies at 36-45 days gestation.[6] Typical of orthobunyaviruses, Cache Valley virus is transmitted to mammals by infected carrier arthropod vectors, with the viral identification confirmed in certain mosquitoes as well as biting midges broadly across North America.[3,4,6]

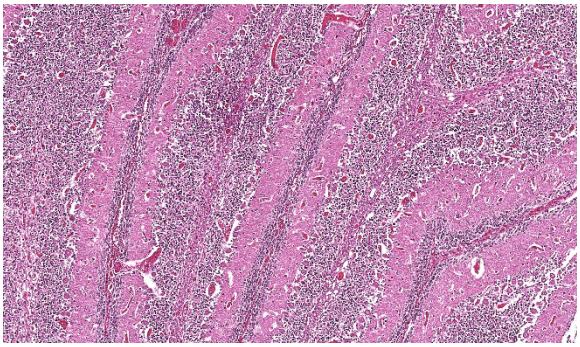

Figure 2-4. Cerebellum, kid. Cerebellar folia are markedly thinned, largely as a result of diminished nuclei within the granular layer. (HE, 146X)

Humans may potentially be infected if bitten by infected arthropod vectors, but direct mammal to mammal transmission has not been identified. Overall reported cases of Cache Valley virus causing clinical disease in humans is rare.[7]

Contributing Institution:
Arizona Veterinary Diagnostic Laboratory

https://azvdl.arizona.edu/

JPC Diagnosis:
Metencephalon: Neuronal necrosis and loss, multifocal, severe, with cerebellar hypoplasia

JPC Comment:
We thank the contributor for sharing this case with us. In particular, the long clinical history made for a good discussion of differentials and assessment of overall slide features. The group discussed ruminant abortion at length. Although Cache Valley virus was not among the group's morphologic diagnoses based on review of the slide alone, other similar agents such as border disease (pestivirus) and caprine arthritis encephalitis (lentivirus) were popular picks.

We differed from the contributor in our morphologic diagnosis concerning the matter of myelination. The asymmetry in measured crown-rump length is interesting and suggests that one twin likely died several weeks before its sibling and the accompanying abortion. Dr. Brown weighed the potential lack of myelination within the developing brain of this fetus (i.e. myelination occurs in greater proportion later in development) as another possible interpretation of the rarefaction that is evident from subgross. Additionally, the lack of spheroids (axonal degeneration correlate) is suggestive that few mature axons had yet developed in this animal. Nonetheless, we were surprised at the lack of overt autolysis in the brain given the timeline we inferred.

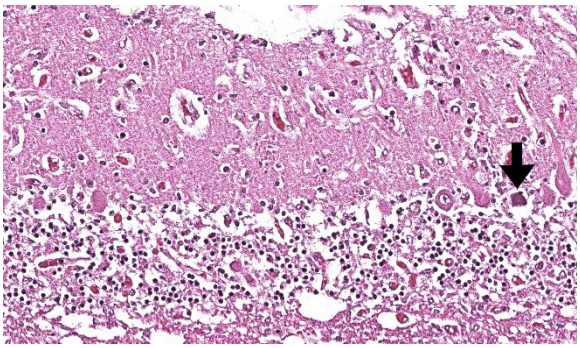

Figure 2-5. Cerebellum, kid. There are large gaps in the Purkinje cell layer and focal Purkinje cell necrosis (arrow). (HE, 477X)

We agreed with the contributor's assessment of cerebellar hypoplasia in this case. From subgross, folia were small with thin layers and increased space between folia were suggestive of this decrease in size. The accompanying necrosis, degeneration, and loss of Purkinje neurons reinforced this interpretation. Subsequently, grey matter neurons were also affected.

The pathogenesis and early lesions of CVV have been previously described.[5] Key gross findings included spinal deformity, arthrogryposis and oligohydramnios. Key microscopic findings in fetuses include necrosis in the central nervous system and skeletal muscle of 7 to 14 days postinfection, and hydrocephalus, micromyelia, and muscular loss after 21 to 28 days postinfection.[5]

The epidemiology of CVV remains incompletely understood, to include major transmission vectors and amplifying hosts across regions of North America.[2] The role of climate change in distribution of CVV across wider geography is speculative, though CVV isolation from mosquito vectors is more common later in the season (late summer and early fall).[2] Longer periods of warmer weather may also extend the breeding period for reservoir hosts and increase the number of naïve hosts to propagate the virus further.

References:

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CASE III:

Signalment:
6-week-old, crossbred pig, *Sus scrofa domesticus*

Figure 3-1. Lung, pig. Gross appearance of the lungs from a pig experimentally inoculared with HPAI. The section was taken from the tip of the right cardiac lobe (arrows). (*Photo courtesy of*: USDA/ARS-National Animal Disease Center, 1920 Dayton Avenue, Ames Iowa 50010)

History:
Pig from an experimental inoculation study.

Microscopic Description:
Lung. Section of lung in which there is minimal to mild dilation of bronchi and bronchioles. These airways are empty or partially filled by fibrin, neutrophils, cell debris and macrophages. The airway epithelium is segmentally eroded in multifocal areas and small amounts of cell debris cover these regions. Other bronchi and bronchioles are lined by hypertrophied epithelial cells with large, nonbasilar nuclei and occasional mitotic figures (epithelial regeneration). In these conducting airways, there are intraepithelial lymphocytes and neutrophils. The lamina propria of some bronchi have minimal to mild infiltrates of lymphocytes and plasma cells. Some bronchi, bronchioles, small arteries, and veins have mild to moderate adventitial infiltrates of

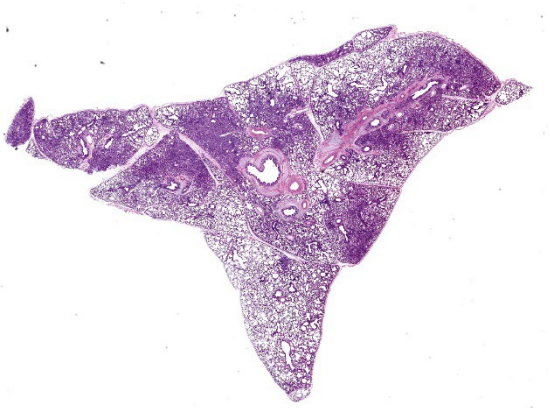

Figure 3-2. Lung, pig. There are multifocal to coalescing areas of peribronchial hypercellularity and atelectasis, as well as diffuse thickening of alveolar septa throughout the section. (HE, 7X)

lymphocytes and plasma cells that occasionally form nodular aggregates. There is multifocal minimal to moderate thickening of alveolar septae commonly around airways due to mild multifocal hyperplasia of type II cells and infiltrates of lymphocytes and macrophages. The alveolar lumens of multiple lobules contain seroproteinaceous fluid, cell debris, fibrin, occasional macrophages, and neutrophils. The interlobular septae are expanded by clear space (edema) and a mixed inflammatory infiltrate composed predominately of lymphocytes, macrophages, and plasma cells.

Contributor's Morphologic Diagnosis:
Lung: Mild to moderate multifocal acute necrotizing bronchitis and bronchiolitis with moderate to marked peribronchiolar and peribronchiolar interstitial pneumonia

Contributor's Comment:
The lung in this case featured macroscopic lesions consistent with influenza A virus infection and was from a pig experimentally inoculated with a highly pathogenic strain of avian influenza (HPAI; A/bald eagle/Florida/W22-134-OP/2022; Genotype B1.1) belonging to the goose/Guangdong 2.3.4.4b hemagglutinin phylogenetic clade. This clade has caused mass mortality events in avian and mammalian species across the globe since 2022.[2,3,5,15] The transcontinental circulation of clade 2.3.4.4b viruses within bird populations allows reassortment with low pathogenicity avian influenza (LPAI) viruses resulting in numerous genotypes of different phenotypes across species and the globe, some of which caused neurologic disease in mammals, a manifestation not observed in our study.[6,7,20] Nonetheless, the presence of NP (nucleoprotein) antigen in endothelial cells of pigs infected with A/bald eagle/FL/22 suggest this strain may spread systemically.

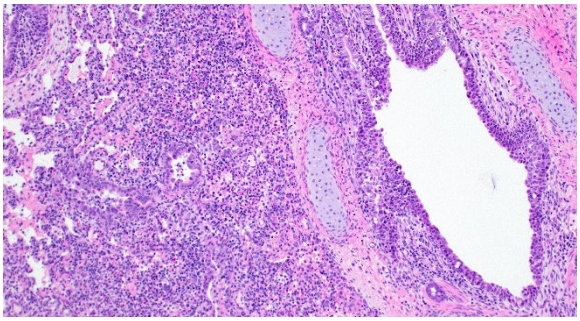

Figure 3-3. Lung, pig. Inflammation is present both within airways (the traditional target of influenza viruses) as well as the surrounding alveolar septa, which are profoundly expanded by a mixed cellular infiltrate o macrophages, lymphocytes, and fewer plasma cells, as well as type II pneumocyte hyperplasia. (HE, 400X) (*Photo courtesy of*: USDA/ARS-National Animal Disease Center, 1920 Dayton Avenue, Ames Iowa 50010)

A subset of mammalian isolates of HPAI H5N1 clade 2.3.4.4b acquired mammalian adaptation markers (E627K, D701N, or T271A) in the polymerase basic (PB) 2 protein that pose a public health risk should these viruses gain efficient transmission in mammals.[4,10] Mammalian adaption of HPAI is multigenic, and the genetic changes necessary for H5N1 strains to adapt to and transmit in swine are not well understood. Although uncommon, incursion of LPAI into commercial swine herds in North America occurs often due to unidentified sources.[8,9,11] Swine-adapted IAV (influenza A viruses) have a propensity for evolution through polymerase errors and reassortment followed by transmission through populations of densely housed commercial pigs and live pig transport. If an avian IAV strain such as H5Nx 2.3.4.4b were to infect domestic swine, there is potential for interspecies transmission, reassortment with endemic swine IAV, and/or acquisition of adaptive mutations that may allow an avian-to-mammalian switch.[10] Increased viral fitness leading to transmission of LPAI strains following reassortment with swine adapted IAV in pigs was demonstrated both in commercial swine herds and experimentally.[1,11] Furthermore, on farm transmission between pigs of a HPAI H5N1 virus and identification of a purified clone that recognizes α2,6 sialic acid receptors was reported.[14] More recently, infection of domestic pigs with clade 2.3.4.4b was demonstrated.[16]

Swine are commonly identified as a 'mixing vessel' supporting reassortment that could lead to antigenic shift.[10] Yet, at a receptor level, it has been suggested that swine are no more susceptible to infection by avian IAVs than humans.[10] The HA (hemagglutinin) of HPAI 2.3.4.4b H5N1 preferentially bind to α2,3-linked sialic acids on host cells,[7] which are at low abundance in the upper respiratory

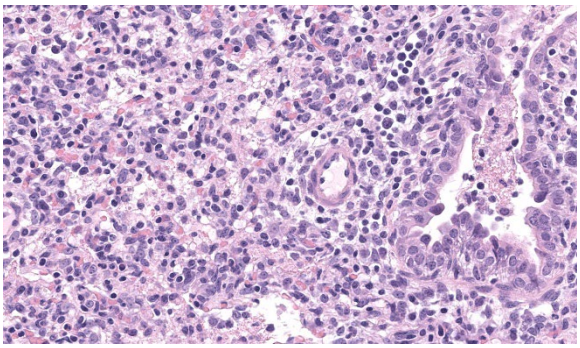

Figure 3-4. Lung, pig. High magnification of inflammatory changes within the airways and septa There is mixed inflammation, polymerized fibrin and edema within alveoli and bronchiolar lumina, and type II pneumocyte hyperplasia in these areas. (HE, 400X)

tract of swine.[12] This low abundance and historic lack of PB2 mammalian adaption mutations may explain why avian IAV does not readily transmit among swine.

The quantity of α2,3-linked sialic acids is relatively higher in the lower respiratory tract of pigs and humans and localizes to pneumocytes and non-ciliated bronchiolar cells.[13,17,19] This distribution is consistent with the extent and distribution of IAV nucleoprotein and RNA labeling in the lung of pigs inoculated with A/bald eagle/FL/22. Labeling is not commonly observed in swine-adapted IAV infection which is most commonly restricted to the conducting airway epithelium. Host-adapted IAV most consistently infect airway epithelium lining conducting airways, resulting in epithelial cell degeneration and necrosis that leads to loss of the epithelial integrity, triggering airway and alveolar inflammation that alters function and interferes with gaseous exchange. Bronchitis and bronchiolitis were a feature of this case due to replication of A/bald eagle/FL/22 in the respiratory epithelium. However, the prominent accumulation of alveolar luminal exudate because of pneumocyte infection and necrosis is not commonly observed in swine-adapted IAV

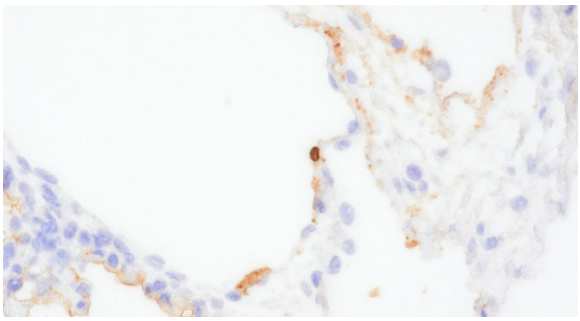

Figure 3-5. Lung, pig. Endothelial cells are immunopositive for HPAI A/bald eagle/Florida/W22-134-OP/2022; Genotype B1.1 (anti-HPAI, 400X) (*Photo courtesy of*: USDA/ARS-National Animal Disease Center, 1920 Dayton Avenue, Ames Iowa 50010)

infection and is likely in part due to the distribution of α2,3-linked sialic acids in swine and propensity of 2.3.4.4b H5N1 to bind α2,3-linked sialic acids.

There is a risk of reassortment of the HPAI H5N1 2.3.4.4b lineage with endemic swine IAV based on the susceptibility of swine to this lineage, the prevalence of IAV infection and co-morbidities in swine herds, and animal husbandry practices.[18,21] Continued phenotypic assessment of HPAI H5NX 2.3.4.4b strains will facilitate awareness and detection capabilities in the swine sector and inform risk assessments and warning systems to safeguard human health.

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JPC Diagnosis:
Lung: Pneumonia, bronchointerstitial, lymphohistiocytic, subacute, multifocal to coalescing, moderate.

JPC Comment:
The contributor provides a detailed summary of HPAI that remains quite relevant to ongoing events several years after this experimental case was first sent to us. Dr. Brown discussed the current paradigm of dairy cattle-associated HPAI with the group. Recent research on the distribution of α2,3-linked sialic acids has demonstrated that the bovine mammary gland (in addition the respiratory tract) contains abundant receptors for avian influenza viruses.[22] Replication of the virus within the mammary gland epithelium is characterized by attenuation of secretory alveoli and ducts with intraluminal neutrophilic exudate, though the degree of mastitis is moderate.[22] HPAI-positive milk has been to be infectious when given orally to mice;[23] replication competent wild rodents may also play a role in the continued spread of HPAI on cattle farms.[24]

The survival and subsequent infectivity of HPAI in milk presents an important public health question.[22,23,24] Simple assays injecting virus into milk samples ('spiking') have demonstrated the hardiness of influenza A virus to heat treatment; the virus also remains infective in raw milk samples stored at refrig-

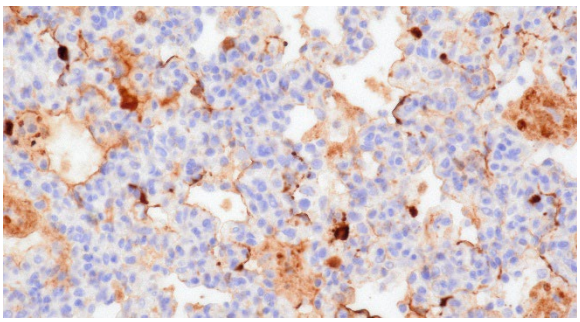

Figure 3-6. Lung, pig. HPAI A/bald eagle/Florida/W22-134-OP/2022; Genotype B1.1 (anti-HPAI, 400X) immunoprotein is distributed throughout pneumocytes and bronchiolar cells, which are high in sialic acid residues. (*Photo courtesy of*: USDA/ARS-National Animal Disease Center, 1920 Dayton Avenue, Ames Iowa 50010)

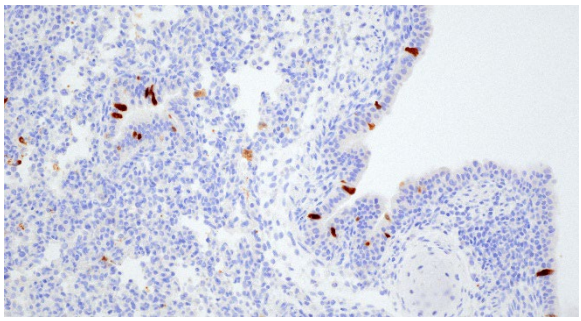

Figure 3-7. Lung, pig. HPAI A/bald eagle/Florida/W22-134-OP/2022; Genotype B1.1 (anti-HPAI, 400X) immunoprotein is within airway epithelium. (*Photo courtesy of*: USDA/ARS-National Animal Disease Center, 1920 Dayton Avenue, Ames Iowa 50010)

eration temperatures for 5 weeks.[23] Natural infection with replication in the mammary gland likely makes inactivation of the virus more difficult due to partial incorporation with fat globules and casein protein which act as an additional barrier to heat treatment.[23] That stated, the effectiveness of conventional commercial pasteurization of milk in addressing influenza A is still being characterized. In our experience (US Army Veterinary Services), many commercial facilities have adapted additional time and/or temperature controls for milk, yogurt, and other dairy products to achieve a desired product composition and provide a wide safety buffer. The recommendation to avoid raw milk nonetheless remains prudent.

We differ slightly in our interpretation of the predominant inflammatory cell type for this pneumonia and felt that the degree of necrosis was overshadowed by predominantly lymphocytes and histiocytes. Several conference participants also noted bronchial and glandular epithelial regeneration which hinted at a slightly longer time course as well. Select alveolar macrophages contained irregular eosinophilic material that some participants interpreted as botryoid inclusions (i.e. from porcine circovirus) while others interpreted necrotic macrophages as a potential correlate of PRRSV which Dr. Brown acknowledged. Absent the history provided in this case, these two viruses are important differentials in porcine granulomatous pneumonias.

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CASE IV:

Signalment:
Six-month-old Brangus, bovine (*Bos taurus taurus*)

History:
In the period between December 2018 and

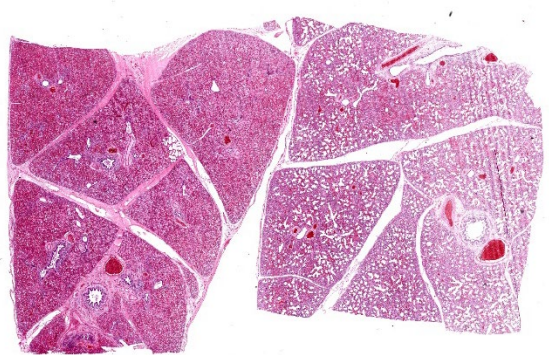

Figure 4-1. Lung, calf. Two sections of lung are submitted for examination with varying degrees of consolidation and expansion of interlobular septa. (HE, 5X)

February 2019, a farm owner recorded the death of five six-month-old calves, including nursing and weaned calves, from a group of 117. Cattle were vaccinated against foot-and-mouth disease, rabies, and clostridiosis, and the herd was kept in native pastures. Affected calves presented similar clinical signs that included fever, apathy, severe respiratory distress, nasal discharge, and diarrhea. Clinical course lasted approximately three days, and calves died spontaneously. All calves that presented clinical signs were treated with enrofloxacin, dipyrone, imizole, and levamisole; however, no significant clinical improvement was observed. One of the deceased calves was submitted for postmortem examination.

Gross Pathology:
The calf showed good body condition. Gross findings included mild jaundice in the subcutaneous tissue, oral mucosa, and ocular conjunctiva. At the opening of the thoracic cavity, the lungs were diffusely pink-red, non-collapsed, with a rubbery texture, and presented interlobular edema on the cut surface. In the cranioventral areas of the lung and in the ventral regions of the caudal lobes, multifocal red-tan and firm areas (2-7 cm in diameter) were observed. At the inspection of the abdominal cavity, the liver was markedly enlarged, with round edges, and diffuse orange discoloration. The gallbladder was distended and filled with grumous bile. The spleen was also severely enlarged. No alterations were observed in other organs.

Laboratory Results:
Culture and isolation of *Salmonella* spp. were attempted using fresh samples of lung. These samples were added in buffered peptone water and incubated overnight (37°C). Following the pre-enrichment, they were inoculated in selective enrichment broth (Rappaport-Vassiliadis broth) and incubated at 42°C overnight. Bacterial isolates were submitted for serotyping using a slide micro-agglutination test. Fresh lung samples were tested through RT-PCR for bovine respiratory complex agents (BoHV/PI-3/BRSV) and Influenza D. Lung sections were submitted for immunohistochemistry (IHC) using a commercial polyclonal antibody against *Salmonella* spp. (Biogenesis®), as previously described by JUFFO et al. (2017).[4]

Salmonella spp. was isolated from the lungs, and the serotype identified was *Salmonella* Dublin. Mild, multifocal,cytoplasmic labeling (IHC) of the bacteria was detected in lung macrophages and freely in the pulmonary interstitial space. RT-PCR for bovine respiratory complex agents (BoHV/PI-3/BRSV) and Influenza D were negative.

Microscopic Description:
Each submitted slide presents a section of lung. Diffusely, alveolar septa are markedly thickened and expanded by severe inflammatory infiltrate of macrophages, lymphocytes, and neutrophils, as well as moderate to marked type II pneumocyte hyperplasia. Similar inflammatory infiltrate composed of large foamy macrophages, lymphocytes, neutrophils, and rare multinucleated syncytial cells, is observed filling alveolar spaces, and less

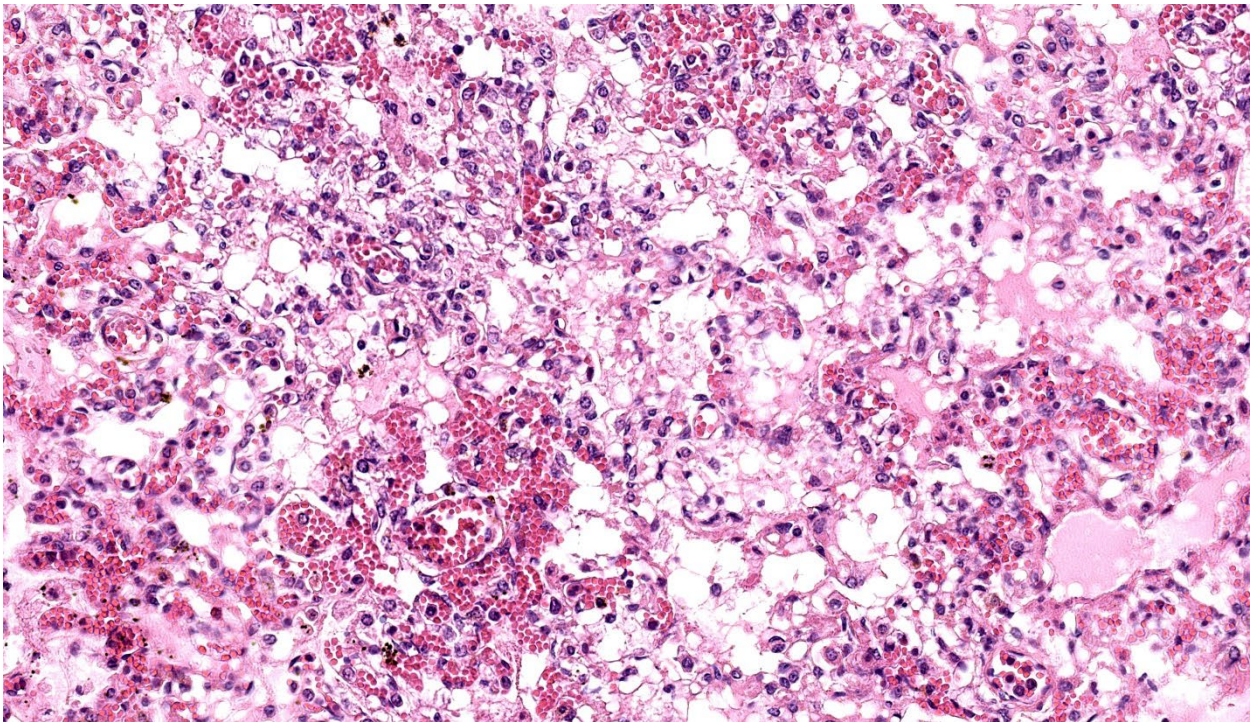

Figure 4-2. Lung, calf. Diffusely, alveolar septa are markedly congested and contain numerous macrophages and lymphocytes as well as scattered Type II pneumocyte hyperplasia. Alveolar lumina contain abundant hemorrhage, edema, polymerized fibrin, alveolar macrophages, and neutrophils. (HE, 450X)

frequently the lumen of bronchioles and bronchi. Multifocal, moderate areas of fibrin deposition, intra-alveolar hemorrhage and mild necrosis are also observed. Additionally, multifocal areas of mild thrombosis, and marked expansion of interlobular septa by eosinophilic homogenous material (edema) are observed.

Contributor's Morphologic Diagnosis:
Lung: Pneumonia, interstitial, lymphohistiocytic, and neutrophilic, multifocal to coalescing, severe, with type II pneumocyte hyperplasia, and interlobular edema, Brangus, bovine.

Contributor's Comment:
Pathological, immunohistochemical and microbiological findings supported the diagnosis of *Salmonella*-associated pneumonia in this case. In cattle, salmonellosis is predominantly caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *S. enterica* subsp. *enterica* serovar Dublin.[1,9,11] *Salmonella* Typhimurium may cause septicemia and is commonly detected in outbreaks of enteric disease in calves. In contrast, *S.* Dublin, a host adapted serovar in cattle, is predominantly associated with septicemia across all age groups in cattle.[11] Salmonellosis in cattle is often characterized by watery or mucoid diarrhea containing fibrin and blood, septicemia, respiratory disease, weight loss, and abortions.[8,1] Lesions include enterocolitis, pneumonia, paratyphoid nodules in the liver, necrotic foci in the kidney, and splenomegaly.[2]

In cases of *Salmonella*-associated pneumonia, the most representative changes include interstitial pneumonia, and therefore, the main differential diagnosis should include

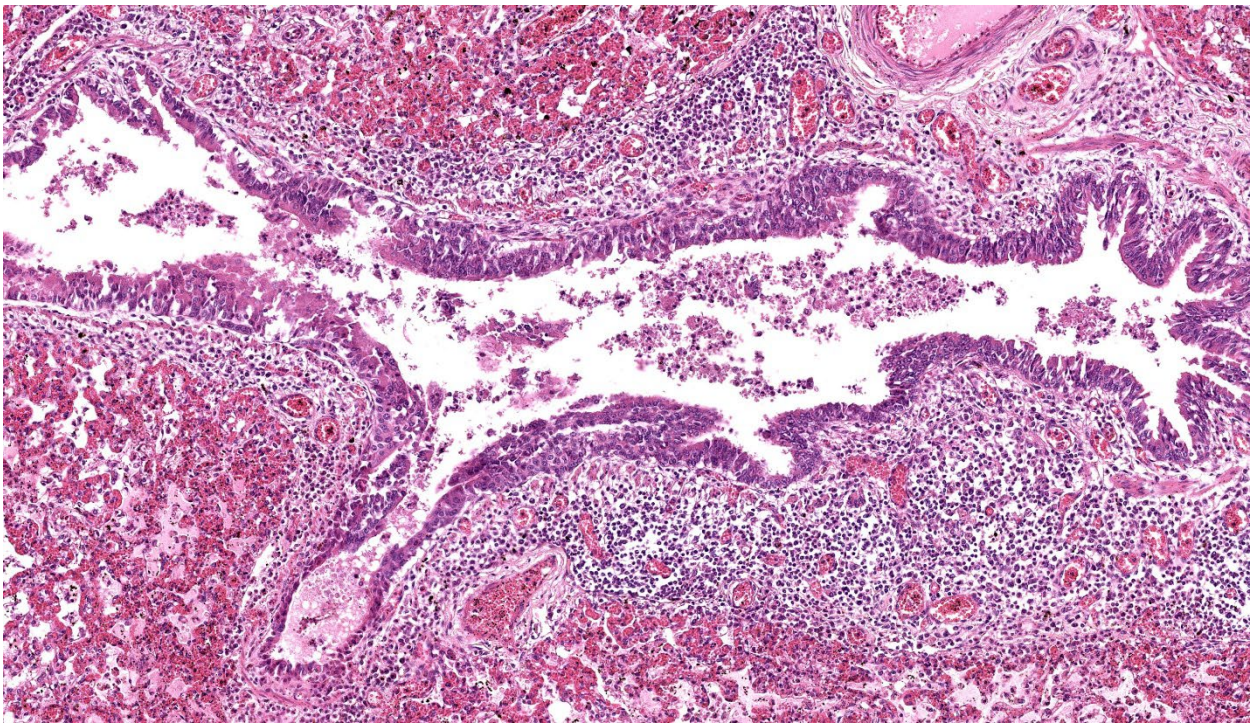

Figure 4-3. Lung, calf. Airways contain refluxed alveolar contains admixed with sloughed airway epithelium. Airway epithelium is hyperplastic, and there are numerous lymphocytes and plasma cells within the peribronchiolar tissue. Alveolar lumina contain abundant hemorrhage, edema, polymerized fibrin, alveolar macrophages, and neutrophils. (HE, 181X)

other important causes of bronchointerstitial pneumonia in calves, such as BoHV (bovine herpesvirus), PI-3 (parainfluenza virus) and BRSV (bovine respiratory syncytial virus), which were ruled out by molecular assays in this case. An outbreak of septicemic salmonellosis with lung involvement has been described in the North region of Brazil associated with serovar Dublin.[5] Salmonellosis with primary lung involvement, in the absence of enteric lesions, has been reported in the Central-West region of Brazil,[3] and in Southern Brazil, by our research group.[7] Likewise, a study conducted in the United States of America reported similar lung lesions in young cattle.[9]

The fecal-oral route represents the main route of transmission, and disease development is associated with bacterial efficiency in invading the intestinal mucosa, colonizing lymphoid tissues, and evading host immune response. Affected animals or asymptomatic carriers may spread the disease in the herd.[8] Young calves (< 6 months of age) are more vulnerable to the disease and may be infected a few hours after calving; nonetheless, adult cattle may also develop the clinical disease.[6, 8] Potential predisposing factors for the development of clinical salmonellosis include concomitant diseases, stressors and immunosuppression, which may favor the development of clinical disease or may prompt cattle to become asymptomatic carriers.[8] Infection in cattle may also be favoured by age and physiological stage.[8] In this case, we believe that weaning-associated stressors may have contributed to the development of clinical salmonellosis.

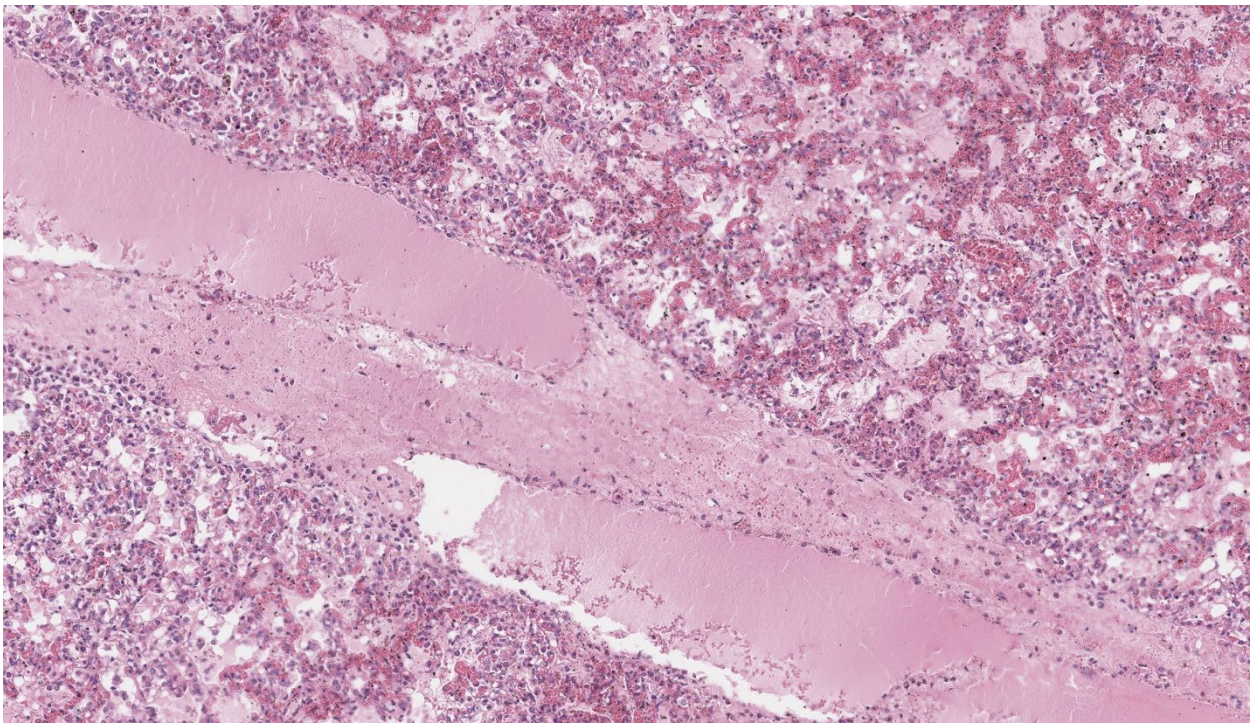

Figure 4-4. Lung, calf. Interlobular septa are markedly edematous with dilated lymphatics. (HE, 205X)

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Faculdade de Veterinária
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Setor de Patologia Veterinária
http://www.ufrgs.br/patologia

JPC Diagnosis:
Lung: Pneumonia, interstitial, lymphohistiocytic and neutrophilic, subacute, multifocal to coalescing, severe, with type II pneumocyte hyperplasia, thrombosis, and edema.

JPC Comment:
The final case of this conference is also a pneumonia which allows for a thoughtful comparison with Case 3. Dr. Brown emphasized several subtle aspects that hint at the underlying pathogenesis. There is hypoplasia of bronchus-associated lymphoid tissue (BALT) as well as a large thrombus in section which are suggestive of a septicemia.

The expansion of the alveolar septa is partially lymphohistiocytic like the previous case, though the distribution is very different. In the present case, alveolar septal expansion is diffuse whereas in the previous case, inflammation was multifocal and more prominent at the bronchoalveolar junction. This case fits better with a hematogenous portal of entry whereas Case 3 is more consistent with an aerogenous agent.

The conference concluded with a brief review of the role of bacteria in pneumonias. In bacterial bronchopneumonia, aerogenous entry of the agent and direct infection of the airway leads to exudation of the alveoli and bronchioles, largely with neutrophils and originating at the bronchiolar-alveolar junction. Conversely, bacterial interstitial pneumonias are the result of indirect action through cytokine effects and endotoxemia. Pulmonary intravascular macrophages (PIMs) have received increased attention for

their role in a variety of diseases.[12] In particular, these macrophages secrete proinflammatory cytokines (e.g. TNF- α) in response to both intracellular and extracellular pathogens and or related markers (e.g. LPS). These cytokines subsequently contribute to capillary dysfunction through a variety of changes such as disruption of endothelial tight junctions.[12] In concert with the action of LPS on endothelial cells (i.e. activation), the large degree of edema and fibrin in this case is hardly surprising. Macrophage activation (MAS) has also been identified as a contributor to COVID-19 related lung pathology,[13] though delineating whether cytokine-induced changes are local to the lung or systemic vasculature has spawned a related term of MAS-like immunopathology. In classic MAS, macrophages across tissue lines (e.g. Kupffer cells and alveolar macrophages) are activated concurrently, leading to hemophagocytosis and an eventual consumption coagulopathy with secondary liver dysfunction.[13] In COVID-19 however, hemophagocytosis is localized as part of a response to diffuse alveolar damage.[13] Common shared outcomes underscore similarities in molecular mechanisms of disease, particularly in the role of macrophage activation and cytokine production.

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