Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #1 17 August 2022

CASE I:

Signalment:
6 year-old. Female. Roya Bilbilitana. Ovine (*Ovis orientalis aries*)

History:
The animal presented with respiratory distress to the clinical service for ruminants (*Servicio Clínico de Rumiantes* – SCRUM) of the University of Zaragoza. The animal came from a herd of 1300 sheep and seven of them presented the same clinical signs. At clinical examination, the animal showed tachypnea and abundant foamy fluid flowing from the nostrils.

Gross Pathology:
Prior to post-mortem examination, educational computed tomography (CT) scans were recorded of the thorax and evinced multiple nodules throughout the cranioventral area of the pulmonary parenchyma. At necropsy, the sheep was in poor body condition. Lungs presented a bilateral consolidation of the anterior lobes and the ventral area of the posterior ones. Lung weight was three times higher in comparison with a normal lung. There was abundant fluid in the trachea and bronchi.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Lung. Expanding and substituting up to 70% of the pulmonary parenchyma there is a multilobulated, non-encapsulated, well-demarcated, moderately cellular and expansive neoplasm that compress adjacent airways and alveoli. Neoplastic cells form acini, tubuli and rare papillae, supported by a moderate amount of fibrovascular stroma that are multifocally expanded by dense mature collagen and fibrocytes (scirrhous reaction). Cells are polygonal to columnar, 10-15 µm; show distinct cell borders, occasional apical cilia, a moderate amount of finely granular eosinophilic cytoplasm, a round to oval 7-12 µm basally located nuclei with coarsely

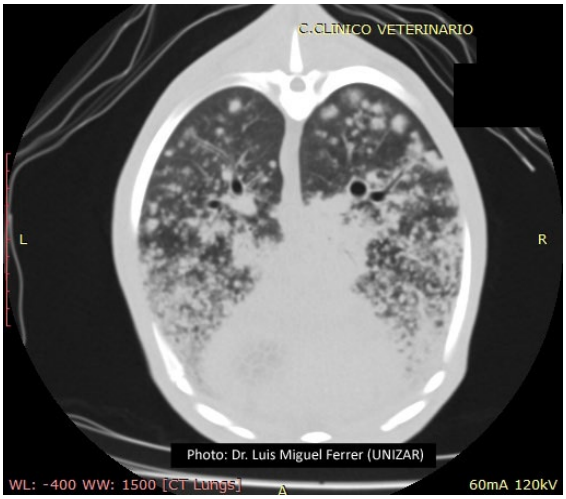

Figure 1-1. Lung, sheep. A CT scan of the anterior lung field demonstrates numerous coalescing nodular opacities. (Photo courtesy of: Universidad de Zaragoza Departamento de Pathologia Animal, https://patologiaanimal.unizar.es.)

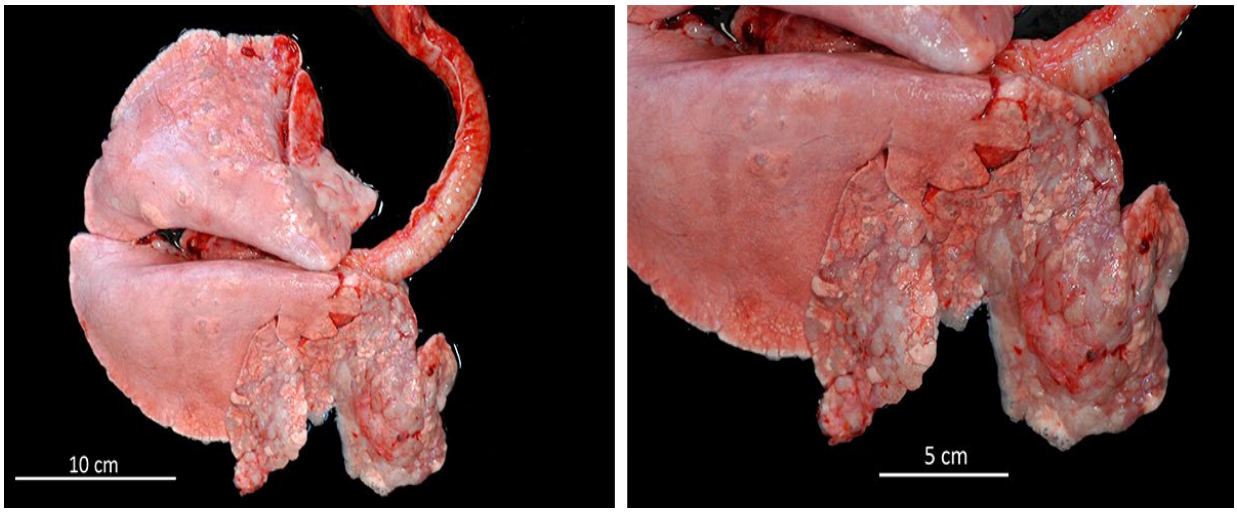

Figure 1-2. Lung, sheep. There is bilateral cranioventral lung lobe consolidation. (Photo courtesy of: Universidad de Zaragoza Departamento de Pathologia Animal, https://patologiaanimal.unizar.es.)

stippled chromatin and 1-2 visible nucleoli. There is moderate anisocytosis and anisokaryosis and less than one mitotic figure per 1 HPF (0.237 mm^2). Multifocally, within the peritumoral non-affected parenchyma, there are intra-alveolar sheets of foamy alveolar macrophages (alveolar histiocytosis). There are intra and peritumoral aggregates of lymphocytes, plasma cells and less histiocytes as well as dense aggregates of viable and degenerated neutrophils within some tumoral acini. There are multifocal areas of alveolar edema, emphysema and prominent peribronchial lymphoid follicles (BALT hyperplasia).

Contributor's Morphologic Diagnoses:
Lung. Adenocarcinoma

Condition: Ovine Pulmonary Adenocarcinoma / Jaagsiekte / Pulmonary adenomatosis

Cause: Ovine betaretrovirus

Contributor's Comment:
This is the classical form of the Ovine Pulmonary Adenocarcinoma (OPA), also called Sheep Pulmonary Adenomatosis (SPA). This contagious pulmonary tumor is caused by an exogenous betaretrovirus, Jaagsiekte sheep retrovirus (JSRV), that target respiratory epithelial cells, mainly bronchiolar club cells and alveolar type II pneumocytes.[8]

There are two forms of presentation of the tumor. Classical OPA usually affects cranioventral pulmonary lobes that become solid, grey or light purple and which bronchiole are filled by abundant alveolar fluid.[5] The tumor usually progress leading to respiratory embarrassment of the animal, that is evinced by dyspnea, tachypnea, exercise intolerance and, in severe cases, marked movement of the abdominal wall (abdominal lift). On the other hand, the Atypical OPA is compound of solitary or aggregated white hard nodules. There is no production of alveolar fluid and lesion do not progress so it usually remains subclinical and is a necropsy finding.

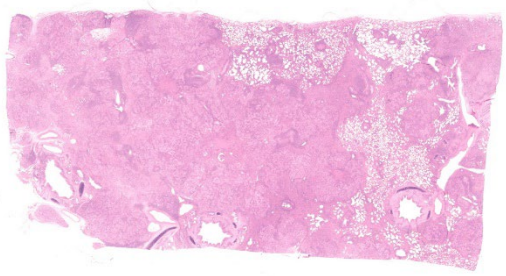

Figure 1-3. Lung, sheep. At subgross magnification, 90% of the section is effaced by coalescing neoplastic nodules. (HE, 7X)

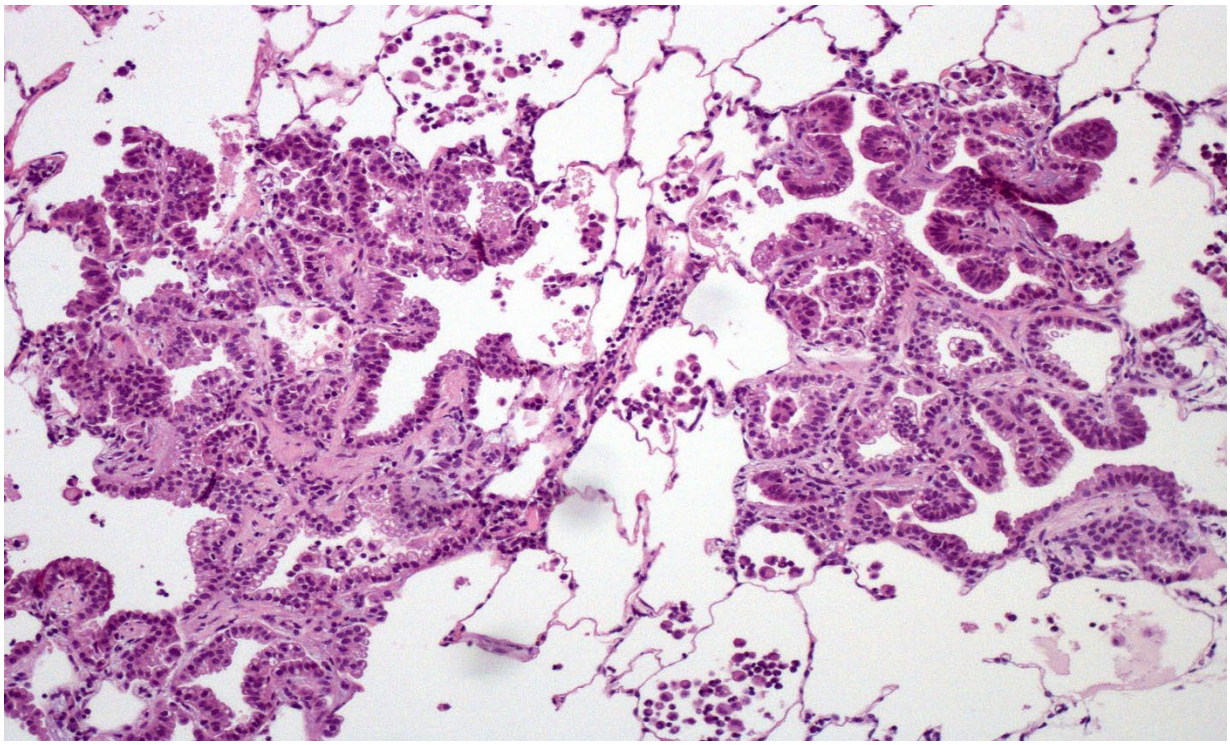

Figure 1-4. Lung, sheep. The lepidic form of growth, in which neoplastic cells extend outward along alveolar septa is best seen in less affected areas of the section. (Photo courtesy of: Universidad de Zaragoza Departamento de Pathologia Animal, https://patologiaanimal.unizar.es.) (HE, 100X)

Microscopically, both forms are characterized by a lepidic cell pattern that can become papillary or acinar in certain areas. Classical form is usually more invasive whereas atypical form is well demarcated and surrounded by prominent infiltrate of lymphocytes and plasma cells.[6,12] Metastasis to regional lymph nodes may occur in 10% of animals affected by the classical form but dissemination to other organs is extremely rare.

Pathogenesis of the tumor is not fully understood but envelope protein has been shown to induce tumor in mice.[8] The development of the classical or the atypical form seems to stem from the host immune response. Atypical forms contain more intratumoral CD4 and CD8 T-cell subsets as well as higher MHC Class II receptor expression in the tumor cells.[11] It has been thought that JSRV could be lined with the induction of human lung adenocarcinoma, however new studies discard this hypothesis.[6]

Transformed epithelial respiratory cells are the major sites for viral replication but JSRV can also be found in lung fluid and lymphoid tissues.[4] The virus is mainly transmitted by the respiratory route and close contact between animals plays a crucial role.[12]

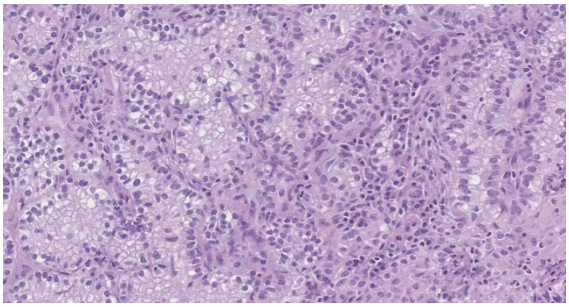

Figure 1-5. Lung, sheep. Most of the section is solidly cellular with alveoli lined by neoplastic columnar epithelium which fills the lumen and effaces normal alveolar architecture. (HE, 314X)

Retroviruses that Induce Tumors in Animals – Adapted from Fenner's Veterinary Virology.[10]		
Genus	**Species**	**Syndrome**
Alpharetrovirus	Avian leukosis virus	Leukosis (lymphoma, leukemia), nephroblastoma in fowl
	Rous sarcoma virus	Sarcoma in fowl
	Avian myeloblastosis virus	Myeloblastosis in fowl
Betaretrovirus	Mouse mammary tumor virus	Mammary carcinoma in fowl
	Mason-Pfizer monkey virus	Sarcoma and immunodeficiency disease in monkeys
	Ovine pulmonary adenocarcinoma virus (Jaagsiekte virus)	Pulmonary adenocarcinoma in sheep
	Enzootic nasal tumor virus	Enzootic nasal adenocarcinoma in sheep and goats
Gammaretrovirus	Feline leukemia virus	Leukemia in cats
	Feline sarcoma virus	Sarcoma in cats
	Murine leukemia and sarcoma viruses	Leukemia, lymphoma, and sarcoma in mice
Deltaretrovirus	Avian reticuloendotheliosis virus	Reticuloendotheliosis in fowl
	Bovine leukemia virus	Leukemia (B cell lymphoma) in cattle

Table 1-1

Perinatal transmission via colostrum and milk is also possible.[1] Most lambs become infected at very early age but only a minority (5-20%) develop OPA and they usually do between 2 and 4 years of age, evincing a long incubation period.[5] Resistance to infection increases with age due to the decrease of the proliferation rate of type II pneumocytes in adults.

The main differential diagnosis of the classic form of OPA is Maedi or ovine progressive pneumonia. This disease also presents as chronic progressive respiratory problems. However, there is no fluid production and the gross appearance is that of uncollapsed lungs and diffuse interstitial pneumonia. Gross appearance of classical OPA could be easily confused by chronic bronchopneumonia.
Goats can also suffer from OPA but the incidence is lower.[8] The transmission of the tumor between both species is possible although rare. Cattle and other animals are resistant.

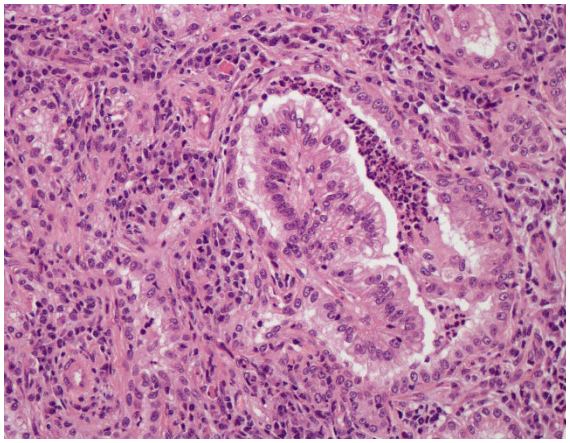

Figure 1-6. Lung, sheep. Occasionally, neoplastic epithelium extends outward into ectatic alveoli, forming papillary projections into the lumen, which is partially filled by a neutrophilic exudate. (Photo courtesy of: Universidad de Zaragoza Departamento de Pathologia Animal, https://patologiaanimal.unizar.es.) (HE, 400X)

Another betaretrovirus infection in sheep is Enzootic Nasal Tumor; however, both tumors are rarely seen in the same animal.

Contributing Institution:
Universidad de Zaragoza. Departamento de Patología Animal
https://patologiaanimal.unizar.es/

JPC Diagnosis:
Lung: Pulmonary adenocarcinoma.

JPC Comment:
The contributor provides an excellent overview of both the classical and atypical forms of ovine pulmonary adenocarcinoma.

Jaagsiekte is an Afrikaans term meaning "chase sickness", and refers to the exacerbation of clinical signs from the stresses and physical demands of herding.[9] The disease was originally described in South Africa in the 1800's and is now seen regularly in South America, Africa, Europe, Africa, and Asia.[2,9] The disease has not been documented in Australia.[2] In Iceland, a major outbreak in the 1930's affected up to 30% of the nation's sheep population and was eradicated by widespread culling and strict isolation measures.[6]

Antemortem diagnosis of OPA can be challenging. Sheep do not produce a humoral response to viral infection, so no serological tests are available. [8] PCR for pro-viral DNA in peripheral blood has low sensitivity for an individual animal (11%) but may be useful in monitoring at the flock level.[6,8] Bronchoalveolar lavage fluid is more sensitive for PCR testing, however it is an impractical test to employ in the field.[6] Thoracic ultrasound has also been described but has low sensitivity for lesions found deeper within the lungs and a high false positive rate. In a recent survey of sheep in the UK, only 24% of animals diagnosed with OPA via ultrasound were positive on histologic and IHC examination.[3]

As the contributor stated, the gross appearance of OPA can mimic that of chronic bronchopneumonia, and in one study of OPA in abattoirs in Ireland, there was a 89% false-positive rate for diagnosis of OPA based on gross lesions alone.[6] Histologic examination improves diagnostic rates, however confirmatory IHC or RT-PCR may be needed to rule out the less common non-JSRV associated pulmonary adenocarcinoma or to diagnose OPA masked by concurrent disease (i.e. bronchopneumonia).[6]

Table 1-1, adapted from Fenner's Veterinary Virology, summarizes the major retroviruses

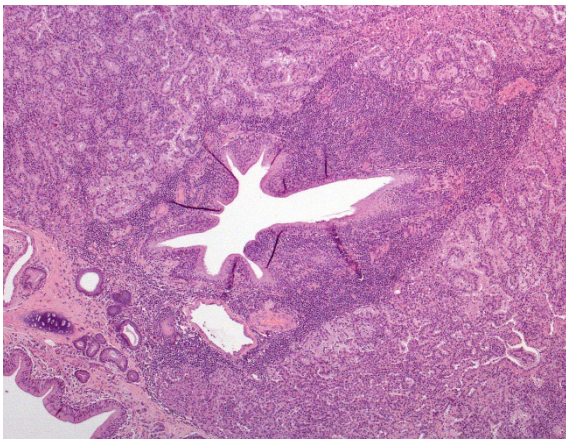

Figure 1-7. Lung, sheep. There is marked BALT hyperplasia. (Photo courtesy of: Universidad de Zaragoza Departamento de Pathologia Animal, https://patologia-animal.unizar.es.) (HE, 200X)

that induce neoplasia in animals. Both the enzootic nasal tumor retroviruses and JSRV are classified as a *trans*-activating retroviruses as neoplastic transformation stems from expression of the viral *env* gene, compared to *cis*-acting retroviruses that alter expression of the host proto-oncogenes. [10]

During the conference, the moderator, Dr. Corrie Brown from the University of Georgia, described clinical and pathologic features of the cases she has seen during her work overseas, including the wheelbarrow test. Lifting an affected animal's hindlimbs like a wheelbarrow will cause frothy fluid pour from the nostrils; this is secondary to agonal reflux of high-protein pulmonary edema. Dr. Brown also reminded conference participants of the impact this diagnosis can have on small farmers in low-income countries: if one animal is affected, the farmer will invariably lose more of their herd – and a significant portion of their livelihood.

References:

1. Borobia M, De Las Heras M, Ramos JJ, et al. Jaagsiekte Sheep Retrovirus Can Reach Peyer's Patches and Mesenteric

Lymph Nodes of Lambs Nursed by Infected Mothers. Vet Pathol.; 2016; 53(6):1172-1179.
2. Caswell JL, Williams KJ. The respiratory system. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. Vol 2. Philadelphia, PA: Elsevier Saunders; 2016: 559-562.
3. Davies P, Strugnell B, Waine K, et al. To scan or not to scan? Efficacy of transthoracic ultrasonography for ovine pulmonary adenocarcinoma screening in a large commercial UK sheep flock. Vet Rec. 2022; e1578.
4. De Las Heras M, De Martino A, Borobia M, et al. Solitary Tumours Associated with Jaagsiekte Retrovirus in Sheep are Heterogeneous and Contain Cells Expressing Markers Identifying Progenitor Cells in Lung Repair. J Comp Pathol; 2014; 150:138–147.
5. Griffiths DJ, Martineau HM, Cousens C. Pathology and Pathogenesis of Ovine Pulmonary Adenocarcinoma. J Comp Pathol. 2014; 142:260–283.
6. Lee AM, Wolfe A, Casside JP, et al. First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter. Ir Vet J. 2017;70(33): 1-12.
7. Miller AD, De Las Heras M, Yu J, et al. Evidence against a role for jaagsiekte sheep retrovirus in human lung cancer. Retrovirology. 2017; 14:3
8. Monot M, Archer F, Gomes M, Mornex J-F, Leroux C. Advances in the study of transmissible respiratory tumours in small ruminants. Vet Microbiol. 2015; 181:170–177.
9. Murphy B. Chapter 14 – Retroviruses. In: MacLachlan NJ, Dubovi EJ eds. Fenner's Veterinary Virology. 5th ed. Boston, MA: Academic Press, 2017: 269-298.
10. Niewiesk S, Oglesbee M. Chapter 3 – Pathogenesis of Viral Infections and Diseases. In: MacLachlan NJ, Dubovi EJ eds. Fenner's Veterinary Virology. 5th ed. Boston, MA: Academic Press, 2017: 47 – 78.
11. Sharp JM, De Las Heras M. Contagious respiratory tumours. In: Aitken ID, editor. Diseases of Sheep. Fourth Edition. Oxford (UK): Blackwell Publishing; 2007. 211–217.
12. Summers C, Benito A, Ortin A, et al. The distribution of immune cells in the lungs of classical and atypical ovine pulmonary adenocarcinoma. Vet Immunol Immunopathol. 2012; 146:1–7.

CASE II:

Signalment:
32-month-old, female, cross- corriedale sheep (*Ovis aries*)

History:
This ewe showed depression, dysphagia, nasal discharge, coughing, and drooling. Two days prior to death, she developed a high fever, constant salivation and head drooping. There were no vesicular formation or ulcers in the oral cavity. Subsequently, the sheep died, and was autopsied in the next morning. No animals had been introduced on this farm for three years.

Gross Pathology:
The lung presented bilaterally dark red in color with a firm consistency and did not collapse after the thorax was opened. The respiratory tract was filled with yellowish-white foamy materials. The gastrointestinal tract was dark red in color throughout.

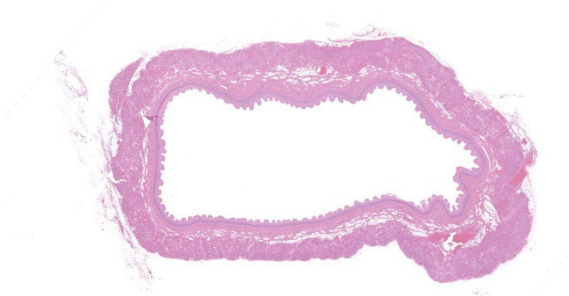

Figure 2-1. Esophagus, sheep. There is diffuse thinning of the esophageal muscle, and the section appears flaccid and partially collapsed. (HE, 8X)

Laboratory Results:
The gene of Bluetongue virus (serotype 21) was detected from the blood samples by reverse transcription-polymerase chain reaction (RT-PCR).

Any bacterial pathogens were not detected from major organs examined. Postmortem testing of hepatic selenium and Vitamin E concentrations reveal levels as adequate.

Microscopic Description:
Upper esophagus: The histologic lesion is mainly in the muscle layer as multifocal muscle fiber degeneration and necrosis, and the epithelial layer is intact. Degenerate and necrotic muscle fibers are fragmented with loss of cross striations, and hyalinized homogenous sarcoplasm. There is infiltration of prominent macrophages, a few lymphocytes and plasma cells between affected muscle fibers. The necrotic areas are replaced by a variable amount of collagens and fibroblasts. The regenerative change of myofibers characterized by a row of nuclei, centralized nuclei and basophilic cytoplasm frequently appears adjacent to affected region. Small blood vessels are occasionally lined by hypertrophied endothelial cells or disrupted endothelium with scattered pyknotic or karyorrhectic nuclei.

Contributor's Morphologic Diagnosis:
Esophagus: Multifocal myofiber degeneration and necrosis, moderate, with mild fibrosis and regeneration.

Contributor's Comment:
This slide presents a typical case of Bluetongue (BT) with muscle fiber degeneration and necrosis, and a vascular lesion. The muscle lesion also showed regenerative changes

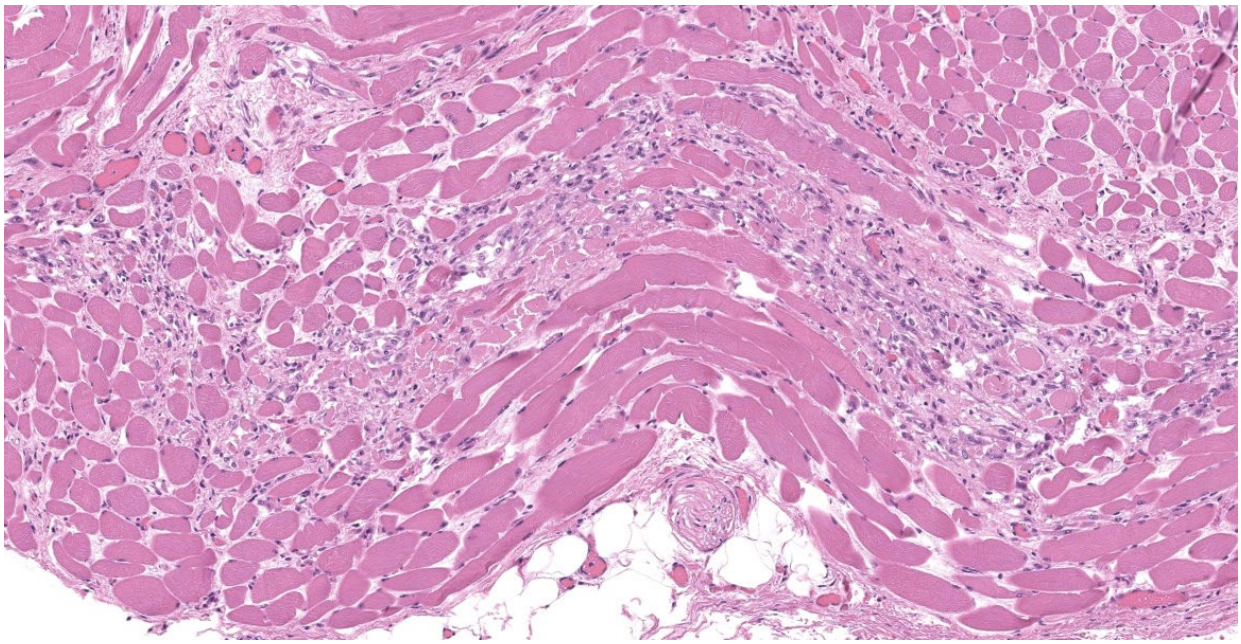

Figure 2-2. Esophagus, sheep. There are multifocal areas of myofiber degeneration and necrosis and replacement fibrosis. (HE, 314X)

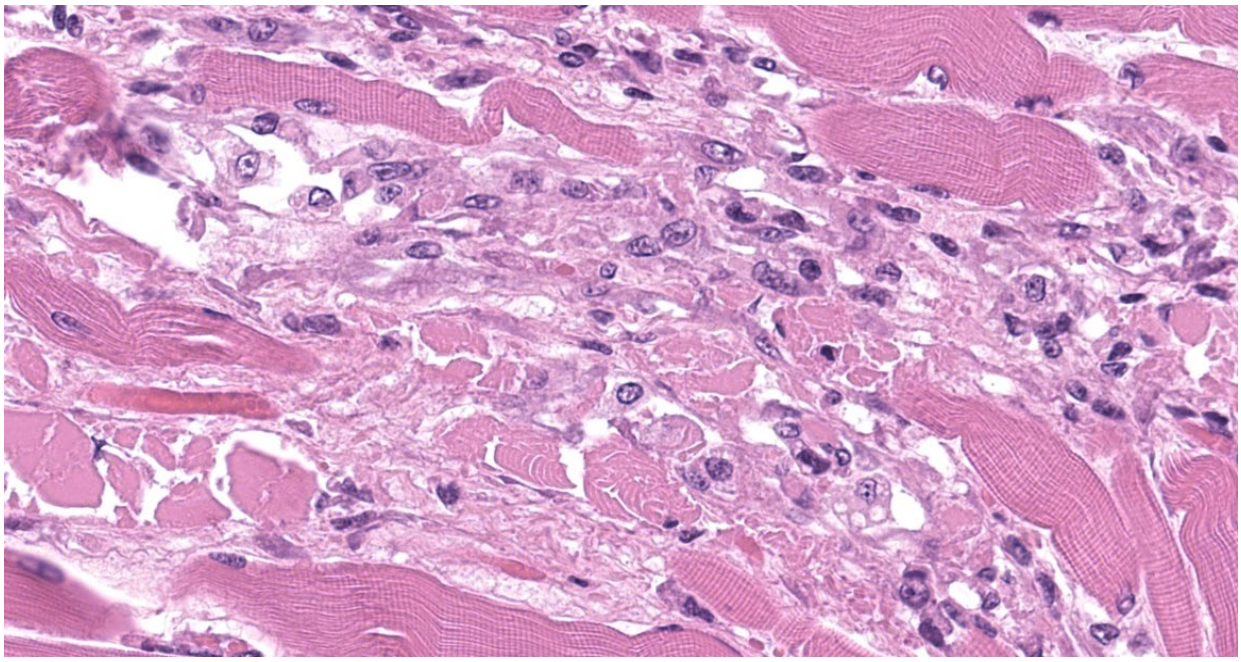

Figure 2-3. Esophagus, sheep. Higher magnification of an area of myofiber degeneration and necrosis with infiltration by macrophages and replacement fibrosis. (HE, 614X

including basophilic myoblasts, myotubes and fibrovascular response. These findings indicate that 1-2 weeks have elapsed since lesion formation. This is consistent with that the ewe died about a week after the onset of clinical symptoms.

BT is an infectious, non-contagious, vector-borne viral disease that affects wild and domestic ruminants.[14] The disease is caused by Bluetongue virus (BTV), family Reoviridae, the prototype virus for genus Orbivirus.[1] At least 27 or more distinct BTV serotypes were defined.[4,12] The virus is essentially transmitted by *Culicoides* biting midges among susceptible animals, however vertical transmission and horizontal transmission have also been reported. [2,13,16,21] BTV infection has been reported from worldwide including tropical, subtropical and some temperate regions, and the global distribution of the virus is consistent with the distribution of competent insect vectors and appropriate climatic conditions.[15]

Among domestic ruminants, clinical BT cases mainly occur in sheep. Clinical manifestations of the disease vary widely depending on the type and strain of infected virus, rearing factors and breed. Other species including cattle and goat are typically asymptomatic or subclinical, although specific BTV strains such as serotype 1 and 8, currently circulating in Europe, can induce severe disease in these animals. [5,10,23] BT in sheep causes severe hemorrhagic syndrome characterized by fever, edema, hemorrhages, dyspnea, mucosal erosions and ulcerations, muscle necrosis, and coronitis.[6,20]These symptoms are generally reflect viral kinetics.

After *Culicoides* insect bites, BTV first replicates in the regional lymph nodes, then it spreads to other organs including lung, lymph nodes and spleen. BTV demonstrates a tropism for a variety of cell types, including dendritic cells, mononuclear phagocytic cells, activated lymphocytes and endothelial cells. [9,11,22] The virus replication in endothelial cells directory induce degenerate, necrotic, and hyperplasic changes in the endo-

thelium, which result in increased of vascular permeability and causing edema, congestion, hemorrhage, thrombosis and necrosis. Additionally, the inflammatory and vasoactive mediators released by BTV infected cells have been proposed as responsible for worsening of endothelial dysfunction and vascular damage.[14]

Pulmonary lesions such as edema and hemorrhage are important change observed in almost all BT in sheep.[14] Greater susceptibility to BTV replication is reported in endothelial cells of respiratory micro-vesicular system.[7,8] Moreover, aspiration pneumonia is frequently observed as a result of aspiration of regurgitated food content followed by paralysis of the esophagus due to muscle necrosis induced by virus induced vascular damage.[3]

The differential diagnosis of BT in sheep should be done in conjunction with other diseases that cause edema, hemorrhage and epithelial damage.[3] The type of lesion and its distribution in affected animals can support development of BT diagnosis. The differential diagnosis includes foot-and-mouth disease, vesicular stomatitis, peste des petits ruminants, contagious ecthyma, sheep pox and photosentisation.[19,25] The initial lesion of BT is similar to foot-and-mouth disease. However, foot-and-mouth disease lesions are vesicular, whereas BT lesions are hemorrhagic and edematous. Vesicular stomatitis is also a disease that can be mistaken for BT. Since the lesion findings are similar to those of foot-and-mouth disease, the same criteria can be used to make a diagnosis. In the case of peste des petits ruminants, extensive gastrointestinal mucosal lesions are impressive, and may be concentrated in lymphoid tissues, such as Peyer's patches. In the case of contagious ecthyma and sheep pox, histopathology revealed proliferative lesion and intracytoplasmic inclusion body in epithelial layers. Photosensitization is also included in differential diagnosis. The absence of hemorrhage and erosions in the oral cavity, in addition to the generalized nature of the skin lesions, and their association with non-pigmented areas of the skin may be helpful for diagnosis. In addition, lesions such as myonecrosis in BT may mimic nutritional muscular dystrophy due to vitamin E and selenium deficiency. It is important that the clinical history and lesions be confined to the muscle for the differential diagnosis.

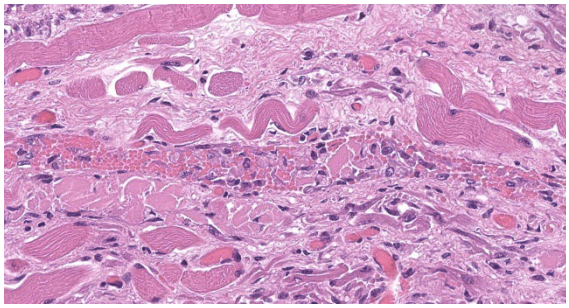

Figure 2-4. Esophagus, sheep. There is segmental loss of endothelium, multiple non-occlusive thrombi, and perivascular hemorrhage within rare vessels in the muscular layers. (HE, 670X)

Contributing Institution:
National Institute of Animal Health,
National Agriculture and Food Research Organization (NARO)
3-1-5 Kannondai, Tsukuba, Ibaraki 3050856, Japan

JPC Diagnosis:
Esophagus: Myocyte degeneration, necrosis, and regeneration, circumferential, multifocal to coalescing, moderate, with replacement fibrosis and multifocal vasculitis.

JPC Comment:
The contributor provides an excellent overview of the pathogenesis, clinical signs, and differential diagnoses for bluetongue virus (BTV) infections.

While phylodynamic models estimate that BTV has been circulating in ruminants for over a millennium, the first documented

cases occurred in South Africa in the nineteenth century, and in 1902, the term malarial catarrhal fever was used to describe the disease. Once a viral etiology was uncovered in 1905, the disease became known by its current and similarly descriptive name, which denotes the cyanosis most visible on the swollen tongue. The disease slowly spread through tropical climates during the first half of the twentieth century, and has now also been reported in Australia, the Americas, Asia, and Europe with serious economic consequences.[4] Ongoing geographic spread of this arbovirus can be partially attributed to changing environmental conditions and subsequent expansion of the *Culicoides* vector habitat.[17,18]

BTV is a segmented dsRNA virus that encodes seven structural (VP 1-7) and five nonstructural (NS 1-4) proteins.[17] VP7 is the most conserved protein and the basis for most seroconversion assays.[18] As the contributor describes, there are at least 27 serotypes of BTV, and this diversity is due to high variability of VP2, segment re-assortment during infection with multiple strains, and genetic drift.[17,18] There is little cross-protection between serotypes.[18] Recently, several atypical serotypes of BTV have been identified which are associated with asymptomatic to mild infections in small ruminants only and at least one of these serotypes (BTV-25) is associated with persistent infection.[18]

After initial infection, the BTV causes a biphasic viremia, with the initial spike in viral replication reduced by an interferon response.[18] BTV affects multiple lymphoid populations early during viremia, producing transient immunosuppression and increased susceptibility to secondary infections.[18] During the second and third week, adaptive immune responses begin effectively controlling infection with expansion of CD8+ T-cells and CD21+ B cells inducing seroconversion against VP7. In some cases, the virus may persist by evading these adaptive immune responses. BTV can disrupt the function of follicular dendritic cells, delaying antibody production by B cells and decreasing T cell responsiveness through unknown mechanisms.[18] Ongoing research into BTV dynamics and immune evasion will be critical in understanding and controlling this important disease.

Conference participants discussed esophageal hyperkeratosis and potential causes in this case. Orthokeratotic hyperkeratosis in the esophagus can occur in an anorectic animal as keratinized squamous epithelial cells are not removed by passing ingesta.[24] Alternatively, parakeratotic hyperkeratosis may occur with reactive epithelial hyperplasia from epithelial injury.[24]

References:

1. Attoui H, Maan S, Anthony SJ, Mertens PPC. Bluetongue virus, other orbiviruses and other reoviruses: Their relationships and taxonomy. In: *Bluetongue*. 1st ed. London, UK: Academic Press; 2009:23–52.
2. Backx A, Heutink R, van Rooij E, van Rijn P. Transplacental and oral transmission of wild-type bluetongue virus serotype 8 in cattle after experimental infection. *Vet Microbiol*. 2009;138:235–243.
3. Bianchi RM, Panziera W, Faccin TC, et al. Clinical, pathological and epidemiological aspects of outbreaks of bluetongue disease in sheep in the central region of Rio Grande do Sul. *Pesqui Vet Bras*. 2017;37:1443–1452.
4. Bumbarov V, Golender N, Jenckel M, et al. Characterization of bluetongue virus serotype 28. *Transbound Emerg Dis*. 2020;67:171–182.
5. Dal Pozzo F, De Clercq K, Guyot H, et al. Experimental reproduction of bluetongue virus serotype 8 clinical disease

in calves. *Vet Microbiol*. 2009;136:352–358.
6. Darpel KE, Batten CA, Veronesi E, et al. Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. *Vet Rec*. 2007;161:253–261.
7. DeMaula CD, Jutila MA, Wilson DW, MacLachlan NJ. Infection kinetics, prostacyclin release and cytokine-mediated modulation of the mechanism of cell death during bluetongue virus infection of cultured ovine and bovine pulmonary artery and lung microvascular endothelial cells. *J Gen Virol*. 2001;82:787–794.
8. DeMaula CD, Leutenegger CM, Jutila MA, MacLachlan NJ. Bluetongue virus-induced activation of primary bovine lung microvascular endothelial cells. *Vet Immunol Immunopathol*. 2002;86:147–157.
9. Drew CP, Heller MC, Mayo C, Watson JL, MacLachlan NJ. Bluetongue virus infection activates bovine monocyte-derived macrophages and pulmonary artery endothelial cells. *Vet Immunol Immunopathol*. 2010;136:292–296.
10. Fabiana DP, Claude S, Etienne T. Bovine infection with bluetongue virus with special emphasis on European serotype 8. *Vet J*. 2009;182:142–151.
11. Hemati B, Contreras V, Urien C, et al. Bluetongue Virus Targets Conventional Dendritic Cells in Skin Lymph. *J Virol*. 2009;83:8789–8799.
12. Jenckel M, Emmanuel B, Schulz C, et al. Complete coding genome sequence of putative novel bluetongue virus serotype 27. *Genome Announc*. 2015;3(2):e00016-15.
13. Koenraadt CJM, Balenghien T, Carpenter S, et al. Bluetongue, Schmallenberg - what is next? Culicoides-borne viral diseases in the 21st century. Vol. 10, *BMC Vet Res*. 2014;10:77.
14. Maclachlan NJ, Drew CP, Darpel KE, Worwa G. The Pathology and Pathogenesis of Bluetongue. *J Comp Pathol*. 2009;141:1–16.
15. Maclachlan NJ, Mayo CE, Daniels PW, Savini G, Zientara S, Gibbs EPJ. Bluetongue. *OIE Rev Sci Tech*. 2015;34:329–340.
16. Menzies FD, McCullough SJ, McKeown IM, et al. Evidence for transplacental and contact transmission of bluetongue virus in cattle. *Vet Rec*. 2008;163:203–209.
17. Rivera NA, Varga C, Ruder MG, et al. Bluetongue and Epizootic Hemorrhagic Disease in the United States of America at the Wildlife-Livestock Interface. *Pathogens*. 2021;10: 1-21.
18. Rodriguez-Martin D, Louloudes-Lazaro A, Avia M, Martin V, Rojas JM, Sevilla N. The Interplay between Bluetongue Virus Infections and Adaptive Immunity. *Viruses*. 2021;13: 1-17.
19. Rojas JM, Rodríguez-Martín D, Martín V, Sevilla N. Diagnosing bluetongue virus in domestic ruminants: current perspectives. *Vet Med Res Reports*. 2019;10:17–27.
20. Schulz C, Sailleau C, Bréard E, et al. Experimental infection of sheep, goats and cattle with a bluetongue virus serotype 4 field strain from Bulgaria, 2014. *Transbound Emerg Dis*. 2018;65:e243–e250.
21. Sluijs MTW van der, Schroer-Joosten DPH, Fid-Fourkour A, et al. Transplacental transmission of bluetongue virus serotype 1 and serotype 8 in sheep: *Virological and pathological findings*. PLoS One. 2013; 8(12): e81429.
22. Stott JL, Blanchard-Channell M, Scibienski RJ, Stott ML. Interaction of bluetongue virus with bovine lymphocytes. *J Gen Virol*. 1990;71:363–368.

23. Toussaint JF, Vandenbussche F, Mast J, et al. Bluetongue in northern Europe. *Vet Rec*. 2006;159:327.
24. Uzal FA, Plattner BL, Hostetter JM. Alimentary System. In: Maxie GM, ed. Vol. 2, *Jubb, Kennedy, and Palmer's pathology of domestic animals*. St. Louis, Missouri: Elsevier, Inc; 2016:31.
25. Williamson S, Woodger N, Darpel K. Differential diagnosis of bluetongue in cattle and sheep. *In Pract*. 2008;30:242–251.

CASE III:

Signalment:
10-month-old calf, female, Limousine, Bovine (*Bos taurus taurus).*

History:
A flock of 70 calves enters the feedlot at the beginning of August 2020. Soon after, these animals began to suffer severe problems (such as conjunctivitis, dyspnea and sudden deaths) that lasts until November. Before death, the animal suffered severe dyspnea for a week.

Gross Pathology:
Lung: cranioventral to caudal chronic, severe, pulmonary consolidation with multifocal to coalescing, well-demarcated and variably sized pale yellow and slightly raised nodules (caseonecrotic bronchopneumonia). On cut, these lesions with caseous material extend through parenchyma and appear within distended airways, such in small bronchioles and alveoli, accompanied by areas of hemorrhage. Along the dorsal aspect of the lung, there was a severe emphysematous distension and thickening of lobular septae (interstitial emphysema). On cut surfaces, these septae appeared thickened and edematous with small gas bubbles admixed.

Laboratory Results:
PCR positive for *Mycoplasma bovis*.

See Table 3-1

Microscopic Description:
Lung: a lesion of inflammatory origin is diffusely obliterating respiratory spaces throughout the whole section of the lung, with extensive multifocally necrotizing coalescing foci centered mainly in bronchioles and alveoli. These foci are composed by an eosinophilic material with a mild admixed cellular debris delimited by a variable number of devitalized leukocytes that maintain cellular figures (ghost-like remnants of leukocytes) and a small number of active macrophages with scattered lymphocytes and some fibroblasts (caseonecrotic centers). Other bronchioles and alveoli are obliterated by high number of viable neutrophils (initial lesions). Sometimes they have multifocal mineralized areas and have multiple distributed clusters of basophilic cocci to coccobacilli bacterial colonies mainly at the periphery, but also ad mixed with the caseonecrotic debris. Occasionally, there are also inside, foreign bodies most compatible with inhaled vegetable structures or hair.

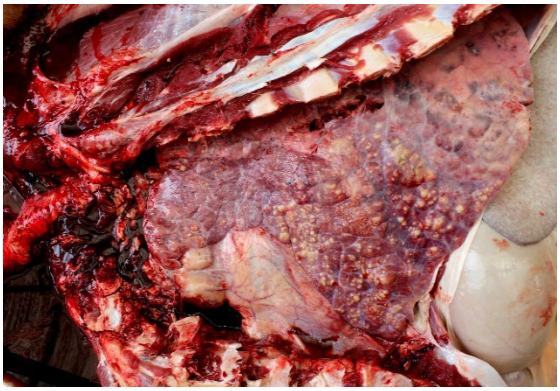

Figure 3-1. Lung, ox. There is marked consolidation and scattered caseonecrotic nodules within all but caudal lung fields. (Photo courtesy of: Universidad de Zaragoza. Departamento de Patología Animal Web: https://patologiaanimal.unizar.es)

Sometimes, these exogenous particles contain bacterial colonies or are mineralized. There are small, scattered foci with leukocytes with streaming hyperchromatic to smudgy nuclei fill the alveoli (oat cells) with fibrin, edema and necrotic karyorrhectic debris with no centers of caseous necrosis. Main bronchi epithelium is hyperplasic with some degenerative changes, individual apoptotic cells and mild submucosal lymphoplasmacytic infiltrate and reactive BALT. In the rest of pulmonary parenchyma alveolar septa are thickened by a marked hyperplasia of pneumocytes type II, macrophages, neutrophils, red blood cells and some fibrin thrombi. Alveolar spaces have areas containing eosinophilic and fibrillar agglomerated substance (polymerized fibrin) and unsettled areas of homogenous eosinophilic clear fluid (edema). Others have severely number of macrophages sometimes phagocytizing fibrin and occasional multinucleated cell of 2-7 nuclei. Some arterioles and small arteries within the parenchymal tissue have extended walls with muscular layer hypertrophy and fibrin deposition. Other major arteries present intraluminal thrombi and some mononuclear inflammatory cells. Pleura and pulmonary septa around main arteries are distended by

SAMPLE	TEST	AGENT	RESULT
Fresh lung	Culture and mass spectrometry technology (MALDI-TOF)	*Mannheimia haemolytica*	Positive ++
Fresh lung	Culture and mass spectrometry technology (MALDI-TOF)	*Trueperella pyogenes*	Positive +++
Fresh lung	Culture and mass spectrometry technology (MALDI-TOF)	*Escherichia coli*	Positive +++
Pool of 2 samples of fresh lung	Real time PCR	*Pestivirus*	Positive
Pool of 2 samples of fresh lung	Real time PCR	*IBR*	Positive
Pool of 2 samples of fresh lung	Real time PCR	*Parainfluenza 3*	Positive
Pool of 2 samples of fresh lung	Real time PCR	*BRSV*	Negative
Pool of 2 samples of fresh lung	Real time PCR	*Bovine coronavirus*	Negative
Pool of 2 samples of fresh lung	**Real time PCR**	***Mycoplasma bovis***	**Positive**
Pool of 2 samples of fresh lung	Real time PCR	*Pasteurella multocida*	Positive
Pool of 2 samples of fresh lung	Real time PCR	*Mannheimia haemolytica*	Positive
Pool of 2 samples of fresh lung	Real time PCR	*Histophilus somni*	Negative
Pool of 2 samples of fresh lung	Real time PCR	*Birbestenia trehalosi*	Negative

Table 3-1

edema and some mononuclear cells that also distend some lymphatic vessels.

Contributor's Morphologic Diagnoses:
Lung: Severe chronic multifocal to coalescing caseonecrotic and necrotic bronchopneumonia with intralesional colonies of bacterial cocci.

Lung: Diffuse interstitial pneumonia with pneumocytes type II hyperplasia, fibrin and interlobular edema.

Contributor's Comment:
Mycoplasmas were discovered for the first time by the team of Pasteur, Nocard and Roux in 1898, when the first report of mycoplasmosis was released [35]. This mycoplasma was isolated from a cattle and was compatible with the current *Mycoplasma mycoides* subsp *mycoides* (formerly known as *M. mycoides* subsp. *mycoides* ¨small colony¨). At that time mycoplasma organisms were known as *pleuropneumonia like-organisms (PPLO)* for being the causative agent of contagious bovine pleuropneumonia (CBPP) [33,35]. Nowadays these microbes belong to the genus *Mycoplasma spp.* and the class Mollicutes, which

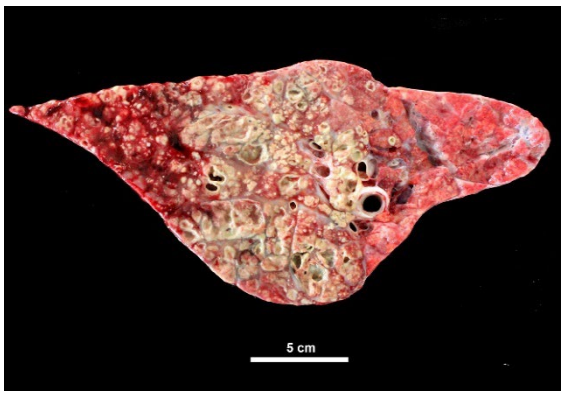

Figure 3-2. Lung, ox. On cut section, there is consolidation (left), áreas of caseating and cavitating necrosis (center), and some partially aerated, less affected lung (right). (Photo courtesy of: Universidad de Zaragoza. Departamento de Patología Animal
Web: https://patologiaanimal.unizar.es)

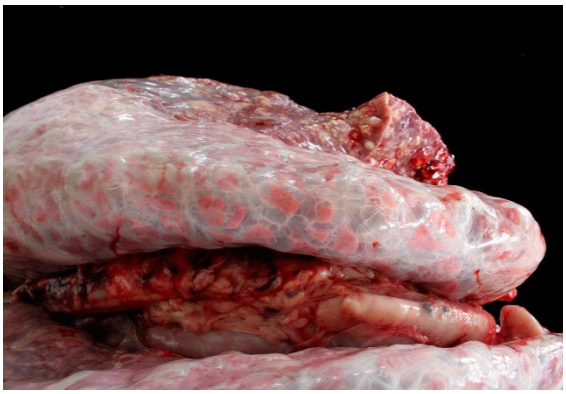

Figure 3-3. Lung, ox. On gross inspection of caudal lung lobes, there is marked interlobular emphysema. (Photo courtesy of: Universidad de Zaragoza. Departamento de Patología Animal
Web: https://patologiaanimal.unizar.es)

differ from other bacteria by their small genomes (580-2220 Kb) and no cell wall [44]. Mycoplasmas are highly contagious organisms and their virulent spread throughout Europe and the world during middle 19th century by cattle trade is well documented. The infection was controlled by "stamping out" policies, persisting in African countries during 20th century [14,36].

Currently *Mycoplasma bovis* is one of the major causative agents of bovine mycoplasmosis [7,8] and is also consider a major player in bovine respiratory disease complex (BRD) [3]. It was first isolated in 1961 in the United States [13], since then, has been turned to be the one of the more pathogenic species of the genus *Mycoplasma* [9]. This has not been enough to implement restrictions on cattle movement related to *M. bovis* [37]. *M. bovis* has a well-known economic, productivity, welfare and health impact in dairy and beef cattle worldwide [32], particularly on the North America and more recently in continental Europe [1]. This impact is due to the frequent chronicity of the infection [10,12,32], the increasingly unresponsiveness to most treatments due to antibiotic resistance and this chronicity [28,31] and the variety of clinical manifestations includ-

ing most commonly bronchopneumonia[2,8,11,16,18,19,42], otitis media[16,27], mastitis[1,6,8,15,20,32,36,43] and arthritis/tenosynovitis[19,42]. Other less consistent manifestations include urogenital disorders (metritis, abortion, infertility, endometritis, salpingitis, reduction of conception rate, seminal vesiculitis, epididymitis and orchitis in bulls) [6,11,23], meningitis[4], keratoconjunctivitis [2,29], decubital abscesses [26] and endocarditis[25].

The incubation period is difficult to define because it varies with the age and these clinical and pathological effects [9]. The clinical expression of these manifestations is highly variable and there are individuals without clinical symptoms in which the organism is isolated from the upper respiratory tract (URT). Thus, the presence *M. bovis* not always result in disease [21,32,43]. As "Trojan horses", these asymptomatic individuals are the leading cause of introduction of *M. bovis* into disease-free herds and the maintenance of infection. These animals can shed the bacteria intermittently from a few months to several months or even years [11,32]. Probably, this particular case and the flock were naïve at the moment of entry to the chronic infected feedlot. In fact, stressors related to immunosuppression such as transportation, entry of a new animal, overcrowding and weaning increase the shedding of the bacteria [9,12,32,37].

The mammary gland and the mucosa of the upper respiratory tract (URT) have been

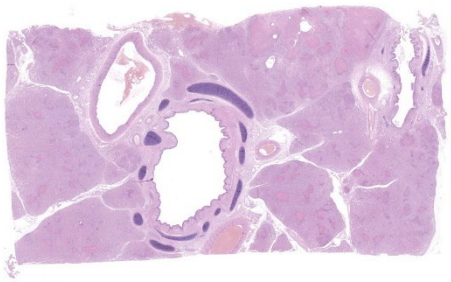

Figure 3-4. Lung, ox. There is diffuse consolidation of the entire section with loss of normal architecture. (HE, 5X).

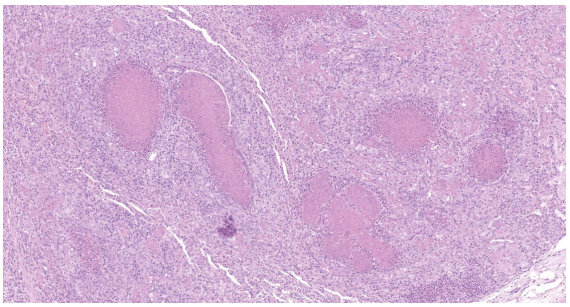

Figure 3-5. Lung, ox. Airways are filled and effaced by abundant brightly eosinophilic cellular debris which extends through the walls and is contained with multiple layers of fibrous connective tissue. (HE, 86X)

shown to be the most serious sites of persistence and transmission. It is transmitted through secretions of the UPR, through milk, from udder to udder, by ingestion and inhalation of aerosols in the case of the calf and through close contact between animals. Infrequently, colostrum, genital secretions, and fomites are a source of infection [11,13,21,32,37]. The possible intrauterine route has also been discussed [22].

Endowed with some proposed virulent mechanisms, *M. bovis* evades the host immune response resulting in chronic infections [9]. However, the pathogenesis is not fully understood and this and other molecular mechanisms involved are still under study[7]. Great advances have been made in this regard with the improvement of genetic techniques, such as the complete sequencing of the *M.bovis* genome [34,45]. Certain high-frequency rearrangements of its genome give it an ability to variably express the surface Vsp lipoprotein and avoid the host immune response. Other mechanisms are adhesion to host cells with adhesins, through surface Vsp, or even metabolic enzymes such as a-enolase, NADH oxidase, TrmFO protein and the in vitro discovered fructose 1,6-biphosphate aldolase and methylenetetrahydrofolate-tRNA-(uracil-5-)-methyltransferase. *M. bovis* has nucleases (such as MBOVPG45, MnuA and MBOV_RS02825) that can destroy NETs and also cause macrophage apoptosis. Other

form of immunologic evasion would be by intracellular infection of epithelial cells, red blood cells and circulating immune cells [9,37]. The formation of a protective biofilm continue to be studied [9,32,37] and Vsp not only mediates adherence, but strongly induces immune response through activation of TLR2 and IL-1β production [9]. The production of H2O2 as part of its virulence is suspected [9,37].

The most common gross lesions described for *M. bovis* are those observed in this case characterized by cranioventral lung consolidation and multifocal to coalescing caseonecrotic nodules that varies from pinpoint to several centimeters. They are well-demarcated, coalescing, circular, white, dry, crumbly, bulging from the pleural or cut surfaces of the lung. In addition, these nodules are delimited by areas of reddened, collapsed and consolidated lung, and the majority of nodules can be better observed with a cut through the parenchyma. Normally, these lesions are bilateral and can affect the 20% to 90% of the lung in severe cases, extending from cranioventral lobes to medial and even with relative sparing of the caudal and dorsal aspects of the caudal lobes. The key feature of this diagnosis is the friable caseous nature of the lesions, but sometimes can appeared liquefied with a suppurative consistence because secondary contamination of nodules with other bacteria (such as *Trueperella pyogenes*) [11,12]. Occasionally we can also observe foci of coagulative necrosis that differ from caseonecrotic nodules, because they are irregular in shape, red tan, non-friable and not raised [11]. The secondary interstitial emphysema observed in the dorsal aspect of the lung may be associated with the "*one-way valve effect*" of pulmonary exudates and/or the high pressure of air within the lung caused by the severe dyspnea [30]. Infection with *M. bovis* by itself could be involved as a neutrophils elastase has been related with emphysema [17,30] and *M. bovis* can induce greater secretion of theses proteases by neutrophils [24].

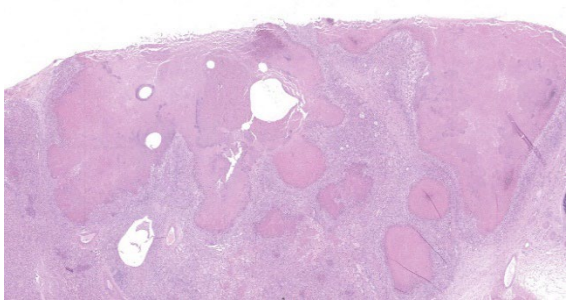

Figure 3-6. Lung, ox. In some lobules, effaced airways have coalesced in to large foci of necrosis, some of which have cavitations within the necrotic debris. (HE, 21X)

Histologic lesions include four main patterns of lesions: caseonecrotic bronchopneumonia (most common), bronchopneumonia with foci of coagulation necrosis, suppurative bronchopneumonia without necrosis and chronic bronchopneumonia with abscessation. Caseonecrotic nodules are distinctive lesions with caseous necrosis that fill small bronchioles, alveoli or interlobular septa. As we could observed on tissue section, in the earliest lesions, leukocytes are within the airways, but undergo a distinctive form of necrosis maintaining their ghost-like cellular outlines, having hypereosinophilic cytoplasm with inapparent or fragmented nuclei. The respiratory epithelium appeared eroded and this nodules are delimited by layers of necrotic cells with pyknotic nuclei, macrophages, lymphocytes and fibroblasts [11,12].

Coagulative necrosis could coexist and complicate lesions. We could observe scattered patterns of this necrosis in which bronchiolar or alveolar structure remains visible. *M. bovis* could be related with those patterns sometimes, but they are indistinguishable from *Mannheimia haemolytica*. The suspicion of *Mannheimia haemolytica* coinfection was confirmed by PCR and its presence could be related with those areas of the so called "*oat*

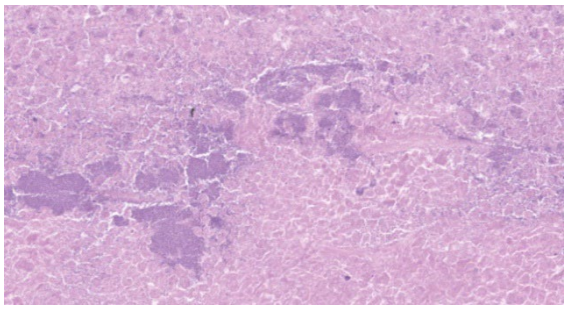

Figure 3-7. Lung, ox. In larger areas of suppuration, there are large colonies of bacilli consistent with Trueperella. (HE, 670X)

cells", that are not expected in mycoplasmosis [11]; elongated hyperchromatic cells, with the appearance of being perforated and smudged. This is a result of the activity of the typical ruminant specific *Mannheimia haemolytica* leukotoxin (Lkt), which induces multiple transmembrane pores in the macrophages membrane at a high concentration, and induces bovine cells to undergo respiratory burst and release of inflammatory mediators and cytokines at a sublytic concentrations [5,40]. In fact, bacterial coinfections with mycoplasma are common in cases of pneumonia [18,19,32,42] and even otitis [27,32]. On one occasion, *M. bovis* was isolated from 82% of feedlot calves with fibrinosuppurative pneumonia from which *Mannheimia haemolytica* was isolated. [19,32]. Other bacteria such as *Pasteurella multocida* (isolated in this case) or *Histophilus somni* have been described [9,13,18,37], so bacterial culture should be done in order to identify coinfective organisms. Mycoplasma exhibits a very slow growth up to 10 days in culture, which is why PCR is the preferred method for confirming the pathogen [36]. Concomitant infections with other viruses such as bovine viral diarrhea virus (BVDV), Infectious bovine rhinothracheitis virus (IBRV) and Bovine parainfluenza 3 virus (bPI-(3)V) were detected. The relationships with viral coinfections and Mycoplasma is less clear [34], but these and other viruses including BRSV (Bovine respiratory syncytial virus), bovine adenoviruses (BdVs) or bovine coronavirus (BCV) have been isolated. These viruses have been frequently detected with *M. bovis* and their possible synergistic role in the disease has been extensively discussed [9,10,18,32,37,42]. However, it exists some discrepancies about it [38].

Recently, *M. bovis*, which had been anecdotally described in North American bison from 2000, was finally detected in an outbreak affecting bisons of all ages. In this case, *M. bovis* bison strain (most probably a host-adapted variant) showed different genetics to that isolated from cattle and, unlike bovines, the organism was the primary agent of a process that had a mortality of up to 45% [37,39].

Contributing Institution:
Universidad de Zaragoza. Departamento de Patología Animal
https://patologiaanimal.unizar.es

JPC Diagnosis:
Lung: Pneumonia, fibrinosuppurative, caseating and necrotizing, diffuse, severe, with bronchiectasis and colonies of coccobacilli.

JPC Comment:
The contributor provides a thorough report on the pulmonary manifestation of *Mycoplasma bovis* as well as the history, virulence factors, control, and ongoing research for this economically important disease. During the conference, the moderator emphasized the importance of the mucociliary apparatus in protecting the lung and described a list of pathogens which can cause dysfunction, including *Mycoplasma, Bordetella*, viruses, and dehydration. Mucociliary apparatus dysfunction is an important part of the pathogenesis of bovine respiratory disease complex, a disease which costs $1B USD annually in the United States alone.

As the contributor mentions, *M. bovis* can cause a spectrum of diseases, and a brief review of the other most common manifestations is worthwhile. Within the host, *Mycoplasma bovis* bacteremia results in hematogenous spread to other sites and can lead to mastitis, otitis, and arthritis.[13,32] Mastitis may be the result of udder-to-udder transmission or spread from other primary sites of infection and can affect dairy cows in all life stages, including dry cows.[32] The infection varies from subclinical to severe with multiple quarters affected and results in fibrinosuppurative to caseonecrotic mastitis.

Otitis media due to *M. bovis* tends to affect dairy and beef calves two to four months of age.[27,32] Infection may be unilateral or bilateral, and most affected calves have concurrent *M. bovis* pneumonia. Initial stages are characterized by ear pain and head shaking. As the disease progresses, animals may become febrile, and involvement of the facial nerve can cause drooping of the ear and ptosis. Severe infections may progress to the inner ear and possible meninges, resulting in dysfunction of the vestibulocochlear nerve and glossopharyngeal nerve.[32] Histologically, the otitis is characterized by suppurative inflammation with extensive bony lysis.[27]

Arthritis due to *M. bovis* frequently occurs in concert with pneumonia or mastitis and is a feature of chronic pneumonia and polyarthritis syndrome (CPPS) described in feedlot cattle.[32] Clinical signs are typical of a septic arthritis, and large high-movement joints such as the hips and stifle tend to be affected. Severe cases are characterized by fibrinosuppurative arthritis with synovial hyperplasia, erosion of articular cartilage, and extension of edema, necrosis, and fibrosis into the periarticular tissues.[19,32]

References:

1. Aebi M, Bodmer M, Frey J, Pilo P. Herd-specific strains of Mycoplasma bovis in outbreaks of mycoplasmal mastitis and pneumonia. *Vet Microbiol*. 2012;157:363–368.
2. Alberti A, Addis MF, Chessa B, et al. Molecular and antigenic characterization of a Mycoplasma bovis strain causing an outbreak of infectious keratoconjunctivitis. *J Vet Diagnostic Investig*. 2006;18:41–51.
3. Arcangioli MA, Duet A, Meyer G, et al. The role of Mycoplasma bovis in bovine respiratory disease outbreaks in veal calf feedlots. *Vet J*. 2008;177:89–93.
4. Ayling R, Nicholas R, Hogg R, et al. Mycoplasma bovis isolated from brain tissue of calves. *Vet Rec*. 2005;156:391–392.
5. Benz R, Piselli C, Potter AA. Channel formation by LktA of Mannheimia (Pasteurella) haemolytica in lipid bilayer membranes and comparison of channel properties with other RTX-Cytolysins. *Toxins (Basel)*. 2019;11:16.
6. Biddle MK, Fox LK, Evans MA, Gay CC. Pulsed-field gel electrophoresis patterns of Mycoplasma isolates from various body sites in dairy cattle with Mycoplasma mastitis. *J Am Vet Med Assoc*. 2005;227:455–459.
7. Bürgi N, Josi C, Bürki S, Schweizer M, Pilo P. Mycoplasma bovis co-infection with bovine viral diarrhea virus in bovine macrophages. *Vet Res*. 2018;49.
8. Bürki S, Frey J, Pilo P. Virulence, persistence and dissemination of Mycoplasma bovis. Vol. 179, *Veterinary Microbiology*. 2015:8.
9. Calcutt MJ, Lysnyansky I, Sachse K, Fox LK, Nicholas RAJ, Ayling RD. Gap analysis of Mycoplasma bovis disease, diagnosis and control: An aid to identify future development requirements. *Transbound Emerg Dis*. 2018;65.

10. Caswell JL, Williams WJ. Respiratory System. In: Maxie GM, ed. Vol. 2, *Jubb, Kennedy, and Palmer's pathology of domestic animals*. St. Louis, Missouri: Elsevier, Inc; 2016:465–591.
11. Caswell JL, Archambault M. Mycoplasma bovis pneumonia in cattle. *Anim Heal Res Rev*. 2007;8:161–186.
12. Caswell JL, Bateman KG, Cai HY, Castillo-Alcala F. Mycoplasma bovis in respiratory disease of feedlot cattle. Vol. 26, *Veterinary Clinics of North America - Food Animal Practice*. 2010:
13. Dudek K, Szacawa E. Mycoplasma bovis infections: Occurrence, diagnosis and control,. Vol. 9, *Pathogens*. 2020:640.
14. Dupuy V, Manso-Silván L, Barbe V, et al. Evolutionary history of contagious bovine pleuropneumonia using next generation sequencing of Mycoplasma mycoides Subsp. mycoides 'Small Colony'. *PLoS One*. 2012;7:9.
15. Fox LK. Prevalence, incidence and risk factors of heifer mastitis. *Vet Microbiol*. 2009;134:82–88.
16. Francoz D, Fecteau G, Desrochers A, Fortin M. Otitis media in dairy calves: A retrospective study of 15 cases (1987 to 2002). *Can Vet J*. 2004;45:661–666.
17. Fujie K, Shinguh Y, Yamazaki A, Hatanaka H, Okamoto M, Okuhara M. Inhibition of elastase-induced acute inflammation and pulmonary emphysema in hamsters by a novel neutrophil elastase inhibitor FR901277. *Inflamm Res*. 1999;48:160–167.
18. Fulton RW, Blood KS, Panciera RJ, et al. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *J Vet Diagnostic Investig*. 2009;21:464–477.
19. Gagea MI, Bateman KG, Shanahan RA, et al. Naturally occurring Mycoplasma bovis-associated pneumonia and polyarthritis in feedlot beef calves. *J Vet Diagnostic Investig*. 2006;18:29–40.
20. Hale HH, Helmboldt CF, Plastridge WN, Stula EF. Bovine mastitis caused by a Mycoplasma species. *Cornell Vet*. 1962 Oct;52:582–591.
21. Hazelton MS, Sheehy PA, Bosward KL, et al. Short communication: Shedding of Mycoplasma bovis and antibody responses in cows recently diagnosed with clinical infection. *J Dairy Sci*. 2018;101:584–589.
22. Hermeyer K, Peters M, Brügmann M, Jacobsen B, Hewicker-Trautwein M. Demonstration of Mycoplasma bovis by immunohistochemistry and in situ hybridization in an aborted bovine fetus and neonatal calf. *J Vet Diagnostic Investig*. 2012;24:364–369.
23. Hirth RS, Nielsen SW, Plastridge WN. Bovine Salpingo-oophoritis Produced with Semen Containing a Mycoplasma. *Vet Pathol*. 1966;3:616–632.
24. Jimbo S, Suleman M, Maina T, Prysliak T, Mulongo M, Perez-Casal J. Effect of Mycoplasma bovis on bovine neutrophils. *Vet Immunol Immunopathol*. 2017;188:27–33.
25. Kanda T, Tanaka S, Suwanruengsri M, et al. Bovine Endocarditis Associated with Mycoplasma bovis. *J Comp Pathol*. 2019;171.
26. Kinde H, Daft BM, Walker RL, Charlton BR, Petty R. Mycoplasma bovis associated with decubital abscesses in Holstein calves. *J Vet Diagnostic Investig*. 1993;5:194–197.
27. Lamm CG, Munson L, Thurmond MC, Barr BC, George LW. Mycoplasma otitis in California calves. *J Vet Diagnostic Investig*. 2004;16:397–402.
28. Ledger L, Eidt J, Cai HY. Identification of antimicrobial resistance-associated genes through whole genome sequencing of mycoplasma bovis isolates with

different antimicrobial resistances. *Pathogens*. 2020;9:588.
29. Levisohn S, Garazi S, Gerchman I, Brenner J. Diagnosis of a mixed mycoplasma infection associated with a severe outbreak of bovine pinkeye in young calves. *J Vet Diagnostic Investig*. 2004;16:579–581.
30. López A, Martinson SA. Respiratory System, Mediastinum, and Pleurae. In: Zachary FJ, ed. *Pathologic Basis of Veterinary Disease Expert Consult*. St. Louis, Missouri: Elsevier Inc.; 2017:471–560.
31. Lysnyansky I, Ayling RD. Mycoplasma bovis: Mechanisms of resistance and trends in antimicrobial susceptibility. *Front Microbiol*. 2016;7.
32. Maunsell FP, Woolums AR, Francoz D, et al. Mycoplasma bovis infections in cattle. *J Vet Intern Med*. 2011;25:772–783.
33. Morowitz HJ. When PPLO became mycoplasma. *American Scientist*. 2011;99:102–105.
34. Nicholas RAJ. Bovine mycoplasmosis: Silent and deadly. *Vet Rec*. 2011;168:459–462.
35. Nocard, Roux. The microbe of pleuropneumonia. *Clin Infect Dis*. 1990;12:354–358.
36. Parker AM, Sheehy PA, Hazelton MS, Bosward KL, House JK. A review of mycoplasma diagnostics in cattle. Vol. 32, *Journal of Veterinary Internal Medicine*. 2018:
37. Perez-Casal J. Pathogenesis and Virulence of Mycoplasma bovis. *Vet Clin North Am - Food Anim Pract*. 2020;36:269–278.
38. Prysliak T, Van Der Merwe J, Lawman Z, et al. Respiratory disease caused by Mycoplasma bovis is enhanced by exposure to bovine herpes virus 1 (BHV-1) but not to bovine viral diarrhea virus (BVDV) type 2. *Can Vet J*. 2011;52.
39. Register KB, Olsen SC, Sacco RE, et al. Relative virulence in bison and cattle of bison-associated genotypes of Mycoplasma bovis. *Vet Microbiol*. 2018;222:55–63.
40. Singh K, Ritchey JW, Confer AW. Mannheimia haemolytica: Bacterial-host interactions in bovine Pneumonia. *Vet Pathol*. 2011;48:338–348.
41. Stalheim OHV, Page LA. Naturally occurring and experimentally induced mycoplasmal arthritis of cattle. *J Clin Microbiol*. 1975;2:165–168.
42. Stipkovits L, Ripley P, Varga J, Pálfi V. Clinical study of the disease of calves associated with Mycoplasma bovis infection. *Acta Vet Hung*. 2000;48.
43. Thomas A, Ball H, Dizier I, et al. Isolation of Mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. *Vet Rec*. 2002;151:472–476.
44. Weisburg WG, Tully JG, Rose DL, et al. A phylogenetic analysis of the mycoplasmas: Basis for their classification. *J Bacteriol*. 1989;171:6455–6467.
45. Wise KS, Calcutt MJ, Foecking MF, et al. Complete genome sequence of Mycoplasma bovis type strain PG45 (ATCC 25523). Vol. 79, *Infection and Immunity*. 2011:982–983.

CASE IV:

Signalment:
6-month-old, Aberdeen-Angus bull calf (*Bos taurus or domesticus*)

History:
The clinical signs prior to death included diarrhea, snotty nose, and a low body temperature (99-100 F˚). The calf had been vaccinated 2 weeks prior with a vaccine containing a modified-live Bovine Viral Diarrhea Virus (BVDV).

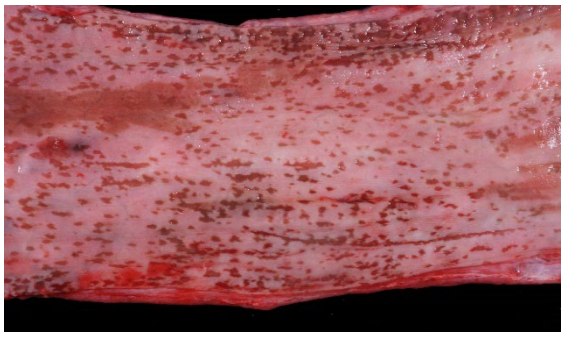

Figure 4-1. Esophagus, ox. There are multifocal to coalescing, approximately linear erosions/ulcers widely disseminated on the esophageal mucosa. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

Gross Pathology:
Alimentary system – At the rostral aspect of the hard palate and at the margins of the buccal mucosa, there were multifocal to coalescing, 5-10mm diameter, dark red, slightly depressed regions with partial to complete loss of the mucosa (erosions and ulcers) as well as multifocal pinpoint erosions/ulcers at the junction of the hard and soft palate. There were numerous, multifocal to coalescing, approximately linear, 1-3mm x 5-10mm, slightly depressed erosions/ulcers that were widely disseminated along the entire length of the mucosal aspect of the esophagus. The abomasal mucosa was diffusely red with numerous, 2-5mm dark red to black erosions/ulcers widely disseminated throughout the mucosal surface affecting approximately 20-30% of the surface area. The small intestinal mucosa was reddened. Multifocally and widely disseminated throughout the mucosal surface of the small intestine, there were small numbers of 3mm diameter, black foci. The colonic and rectal mucosa had multiple red approximately linear regions. There was a large amount of soft to watery intestinal contents.

Laboratory Results:
Molecular Diagnostics: Tissue homogenate was positive for BVDV by PCR.

Microscopic Description:
Esophagus – Multifocally, there is partial to complete loss and sloughing of the epithelium (erosion and ulceration) with replacement by necrotic cellular debris, few neutrophils, a small amount of fibrin, few erythrocytes, and occasional bacterial colonies. The adjacent epithelial cells are often swollen and pale (ballooning degeneration) or are shrunken, deeply eosinophilic with pyknotic to karyorrhectic nuclei (necrosis). Multifocally in other regions, the epithelium is degenerate to necrotic. There are small numbers of neutrophils infiltrating the remaining epithelium (transmigration) and occasionally extending into the underlying submucosa forming microabscesses. The mucosal and submucosal blood vessels are congested.

Contributor's Morphologic Diagnosis:
Esophagus – necroulcerative esophagitis, multifocal to coalescing, marked, acute with few neutrophils and rare microabscesses.

Contributor's Comment:
Bovine viral diarrhea virus (BVDV) is an RNA virus that most commonly causes disease in cattle, but it, or closely related viruses, can infect most even-toed ungulates including swine and camelids. [14] BVDV (BVDV-1 and BVDV-2) is in the genus *Pestivirus* (fam-

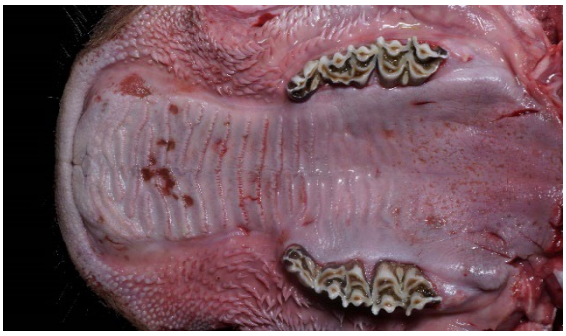

Figure 4-2. Hard palate, ox. Within the oral cavity, there were multifocal to coalescing erosions/ulcers on the hard palate, buccal mucosa, and extending into the soft palate. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

ily Flaviviridae) which also includes Classical swine fever virus and Border disease virus.[14] BVDV has two biotypes including both a noncytopathic (NCP) and cytopathic (CP) form.[14] Additionally, there is a more recently identified species of pestivirus which has been classified as BVDV-3, also known as HoBi-like or atypical pestivirus.[14]

There are multiple varying clinical presentations of BVDV in juvenile to adult cattle ranging from mild clinical signs (fever, anorexia, lethargy) to severe clinical signs and death.[14] A severe acute form of BVDV is termed BVD type 2 (often caused by BVDV-2) and presents with a fever, sudden death, diarrhea, or pneumonia.[14] A thrombocytopenic form of BVDV has been described.[14] These varying forms are not mutually exclusive and often present with overlap. Fetal infections, which vary clinically by time of gestation, can occur when an immunocompetent, seronegative dam is infected. If the dam is infected during approximately the first 4 months of gestation by a NCP form of BVDV that crosses the placenta, the fetus may die leading to resorption, mummification, abortion, develop congenital abnormalities, or survive leading to birth of a persistently infected (PI) calf (typically 42-125 days gestation).[8,14,16]

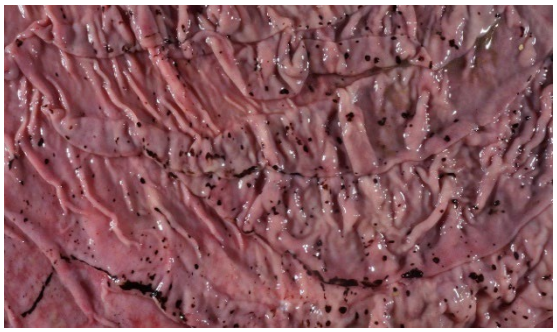

Figure 4-3. Abomasum, ox. There were multifocal dark red to black erosions/ulcers widely disseminated throughout the abomasal mucosa. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

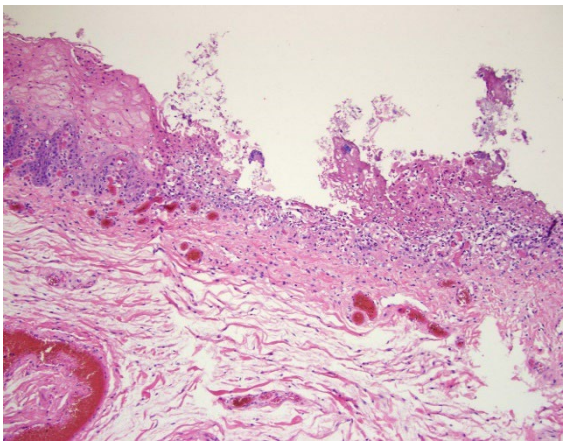

Figure 4-4. Esophagus, ox. Esophagus – Focally, there is loss of the epithelium (ulceration) with replacement by a small amount of cellular debris and few bacteria. There is degeneration and necrosis of the adjacent epithelial cells with infiltration by few neutrophils. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu) (HE 200X)

At birth PI calves can appear completely normal or have non-specific clinical signs (poor doing, rough hair coat), or are undersized. PI calves remain viremic throughout life and shed large amounts of virus.[14] Due to the timeframe of fetal infection and immune system development, these calves are seronegative but antigen positive, and viral antigen can be identified with immunohistochemistry in a variety of tissues.[14] PI calves must be differentiated from acutely infected calves by the absence of lesions in the presence of antigenic positivity.[14] PI calves are vulnerable to mucosal disease.[14]

Mucosal disease is a syndrome whereby PI calves become infected with a CP biotype that is similar to the NCP biotype of original fetal infection or when the NCP biotype of the persistent infection mutates.[14] Evidence has shown that vaccination with a modified-live BVDV can also lead to mucosal disease in PI calves.[14] Due to the immunotolerance created by the timing of the fetal infection, the calf is unable to elicit an immune response to the CP BVDV leading to an overwhelming infection and destruction of mucosal epithelial cells.[8,14,16]

Histopathologic features of mucosal disease and acute BVDV include epithelial necrosis with erosions and ulcerations throughout the alimentary tract often including esophagus, rumen, reticulum, abomasum, and intestine.[14] The most characteristic histopathologic features of BVDV are found in the intestine and include destruction of the crypts of Lieberkühn with luminal dilation by mucus along with lysis of the Peyer's patches and associated overlying mucosal inflammation.[14] Differential etiologies for the histologic findings of epithelial necrosis with erosions and ulceration in the alimentary tract include malignant catarrhal fever, rinderpest, and vesicular diseases; however, the intestinal changes are only similar in rinderpest.[8,14] Osteopetrosis has also been identified in PI calves.[15]

Due to the economic importance of BVDV, there have been a few recent studies completed to streamline diagnosis and understand pathogenesis and lesions. As PI animals are the main concern for spread of disease, better detection strategies for rapid identification were warranted. According to Brodersen, the use of ear notches with immunohistochemistry has enabled quick identification of PI animals to assist with on-farm control strategies.[4] As previously described, mucosal disease typically affects the entire alimentary tract; however, a recent report describes a mucosal disease outbreak with lesions restricted to the upper alimentary tract and skin (interdigital) with no lesions in the intestine, which may pose a challenge when differentiating from vesicular diseases.[3] In order to more fully understand the pathogenesis of mucosal disease, varying apoptotic pathways were evaluated by Hilbe et al.[10] It was found that caspase-3 and caspase-9 (intrinsic pathway) are more strongly expressed in mucosal disease lesions while caspase-8 (extrinsic pathway) was not.[10]

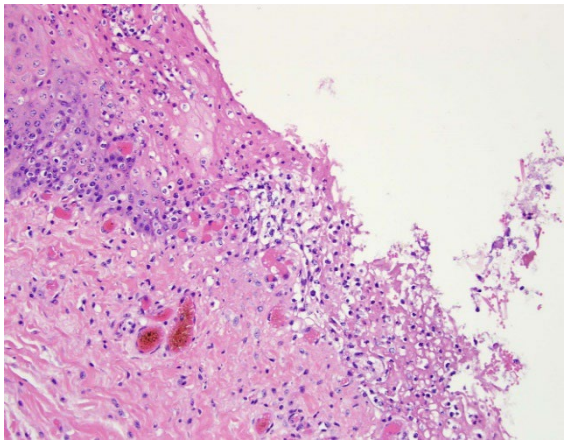

Figure 4-5. Esophagus, ox. Esophagus – The epithelial cells are degenerate to necrotic with infiltration and transmigration of few neutrophils. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu) (HE 400X)

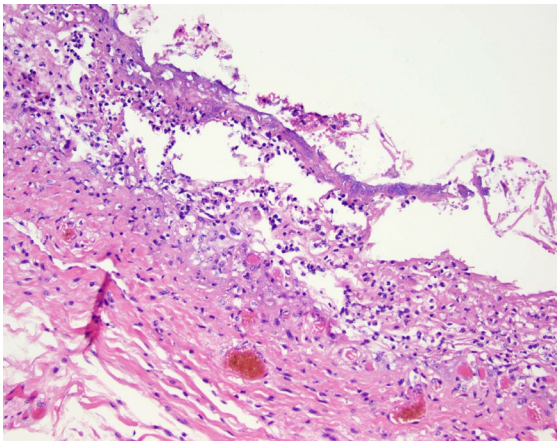

Figure 4-6. Esophagus, ox. There is sloughing of the epithelium. There is epithelial infiltration by few neutrophils and superficial bacterial colonies. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu) (HE 400X)

Contributing Institution:
Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu

JPC Diagnosis:
Esophagus: Esophagitis, ulcerative, acute, multifocal.

JPC Comment:
Bovine viral diarrhea was first described as a new disease of cattle in 1946 by researchers

in New York. Subsequent work in cell cultures allowed for isolation of the virus and vaccine development in the 1960s.[6] The proclivity of the virus to survive in culture and the spread of the infection in pregnant cows lead to contamination of numerous ruminant cell cultures uncovered in the 1980s. Viral contamination of cell cultures can occur through the addition of infected fetal bovine serum or, less commonly, by using infected bovine fetal tissue for the initial culture.[2,9] Such viral contamination compromises research studies and in several instances has led to contamination of vaccine stocks in both veterinary patients and humans. Early poliovirus vaccines produced on rhesus monkey kidney cell cultures were contaminated with simian polyomavirus SV40, a virus endemic to rhesus macaques and carcinogenic in humans.[5] This led to widespread exposure of the human population between 1955 and 1963 and even later in some countries. Strict controls on international trade have been implemented and new improved protocols require irradiation of serum prior to addition to cell cultures.[9] Additionally, technological advances now allow for detection and sequencing of all nucleic acids within a culture.[9] It was through these detection methods that HoBi-like pestivirus (HoBiPeV) was first identified in 2004 when it was isolated from contaminated bovine fetal serum from Brazil.[11]

As the contributor described, bovine viral diarrhea virus has two traditional genotypes, BVDV-1 and BVDV-2. HoBiPeV can cause the produce the same spectrum of disease as the typical BVDV genotypes and has now been reported in South America, Europe, and Asia.[1,11] In a recent report of HoBi-like pestivirus in feedlot steer in Argentina, the virus was associated with bronchointerstitial pneumonia in one animal and fibrinosuppurative bronchopneumonia in another animal coinfected with *Mannheimia hemolytica*.[11] This report is suggestive of HoBiPeV's role in development of bovine respiratory disease complex, similar to that of BVDV. This study also demonstrated HoBiPeV's potential cross-reactivity against BVDV immunohistochemical stains, which is attributed to the highly-conserved viral glycoprotein GP48 present in all three species. Current antigenic and serologic tests are less or ineffective at detecting HoBiPeV, and there is limited cross protection between strains in commercially available BVDV vaccines.[7,11]

Since 2000, the list of known pestiviruses has grown to include several additional pathogens of cloven-hoofed animals, including atypical porcine pestivirus and Bungowannah virus in pigs, Aydin-like pestivirus in sheep and goats, and pronghorn antelope vi-

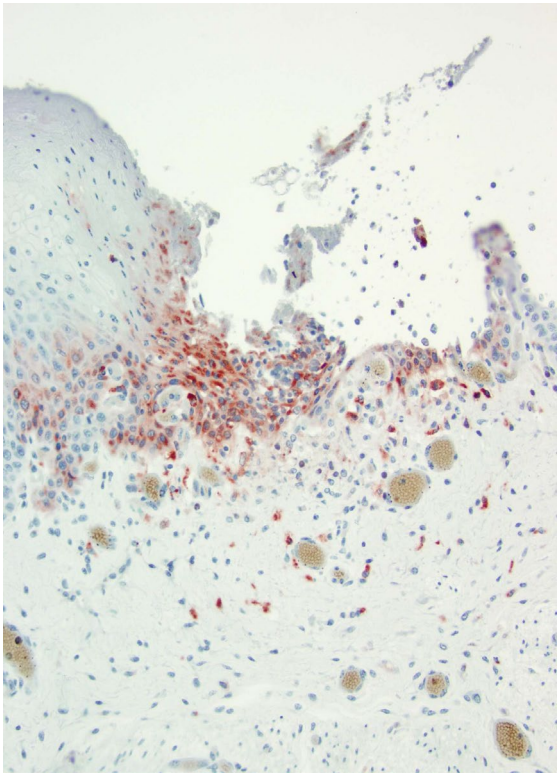

Figure 4-7. Esophagus, ox There is strong intracytoplasmic BVDV immunoreactivity of numerous epithelial cells and few inflammatory cells within an affected region of esophagus (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu) (anti-BVDV, 400X)

rus. Additionally, a number novel pestiviruses of questionable pathogenicity have been isolated from non-ungulate species, including pangolins, rodents, bats, and harbor porpoises.[12] With this growing list, there is a proposed revision of Pestivirus taxonomy, with each species denoted by a single letter. Under the new taxonomy, BVDV-1 is designated Pestivirus A, BVDV-2 is Pestivirus B, and HoBiPeV is Pestivirus H.[13]

References:

1. Balasuriya UBR, Reisen W. Chapter 29 – Flaviviruses. In: MacLachlan NJ, Dubovi EJ eds. *Fenner's Veterinary Virology*. 5th ed. Boston, MA: Academic Press, 2017: 525-546.
2. Barrat-Boyes S. Golde WT. Chapter 4 – Antiviral Immunity and Virus Vaccines. In: MacLachlan NJ, Dubovi EJ eds. *Fenner's Veterinary Virology*. 5th ed. Boston, MA: Academic Press, 2017: 79-104.
3. Bianchi MV, Konradt G, de Souza SO, et al. Natural Outbreak of BVDV-1d-Induced Mucosal Disease Lacking Intestinal Lesions. *Vet Pathol*. 2017;54:242-248.
4. Brodersen BW. Bovine Viral Diarrhea Virus Infections: Manifestations of Infection and Recent Advances in Understanding Pathogenesis and Control. *Vet Pathol*. 2014;51:453-464.
5. Carbone M, Gazdar A, Butel JS. SV40 and human mesothelioma. *Transl Lung Cancer Res*. 2020 Feb;9(Suppl 1):S47-S59.
6. Coffey LL. Chapter 1 – The Nature of Viruses. In: MacLachlan NJ, Dubovi EJ eds. *Fenner's Veterinary Virology*. 5th ed. Boston, MA: Academic Press, 2017:1-16.
7. Decaro N. HoBi-Like Pestivirus and Reproductive Disorders. *Frontiers in Vet Sci.* 2020;7:1-5.
8. Gelberg HB. Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity. In: Zachary JF. *Pathologic Basis of Veterinary Disease. 6*th ed. St. Louis, MO: Elsevier; 2017: 395-397.
9. Heidner H. Chapter 2 – Virus Replication. In: MacLachlan NJ, Dubovi EJ eds. *Fenner's Veterinary Virology*. 5th ed. Boston, MA: Academic Press, 2017:17-46.
10. Hilbe M, Girao V, Bachofen C, et al. Apoptosis in Bovine Viral Diarrhea Virus (BVDV)-Induced Mucosal Disease Lesions: A Histological, Immunohistological, and Virological Investigation. *Vet Pathol*. 2012;50:46-55.
11. Margineda CA, Ferreyra FM, Masnyj F, et al. HoBi-like pestivirus in 2 cases of fatal respiratory disease of feedlot cattle in Argentina. *J Vet Diagn Invest.* 2022; 34(4):693-698.
12. Postel A, Smith DB, Becher P. Proposed Update to the Taxonomy of Pestiviruses: Eight Additional Species within the Genus *Pestivirus*, Family *Flaviviridae*. *Viruses*. 2021 Aug 4;13(8):1542.
13. Smith DB, Meyers G, Bukh J, et al. Proposed revision to the taxonomy of the genus *Pestivirus*, family *Flaviviridae*. *J. Gen. Virol.* 2017;**98**:2106–2112.
14. Uzal FA, Plattner BL, and Hostetter JM. Alimentary System. In: Maxie MG. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Missouri: Elsevier, 2016: Vol 2: 122-139.
15. Webb BT, Norrdin RW, Smirnova NP, et al. Bovine Viral Diarrhea Virus Cyclically Impairs Long Bone Trabecular Modeling in Experimental Persistently Infected Fetuses. *Vet Pathol*. 2012;49:930-940.
16. Zachary JF. Mechanisms of Microbial infections. In: Zachary JF. *Pathologic Basis of Veterinary Disease.* 6th ed. St. Louis, MO: Elsevier; 2017: 200-201.