Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 23

20 April 2022

CASE I: S 962-17 (JPC 4116730)

Signalment:
Seven-month-old, male sheep (*Ovis aries*)

History:
The sheep was presented due to persistent chronic stridor. Endoscopically, the larynx was severely swollen. Finally, the animal was sacrificed due to acute onset of severe respiratory distress.

Gross Pathology:
Macroscopically, the larynx exhibited a severe, diffuse edema with obstruction of the laryngeal cavity. Bilaterally, the mucosa of the dorsal parts of the arytenoid cartilages showed approximately 0.5 cm in diameter sized, dark brown, focal defects with extension into the cartilage. Within the cartilage, a 0.5 x 1 x 1 cm large cavity filled with a yellowish, viscous material was present.
Additionally, the right cranial and medial pulmonary lobes displayed a moderate, multifocal, catarrhal and suppurative bronchopneumonia.

Laboratory Results:
Microbiologically, *Fusobacterium necrophorum*, *Streptococcus ovis* and *Clostridium septicum* were isolated from laryngeal tissue.

Microscopic Description:
Larynx: Within the arytenoid cartilage a 1 x 0.5 cm sized cavity communicating with the laryngeal cavity was found. This cavity and the surrounding tissue including cartilage, submucosa, submucosal glands and skeletal musculature were infiltrated by numerous inflammatory cells. These infiltrates consisted of high numbers of viable and degenerated neutrophils and fewer macrophages, lymphocytes and plasma cells. Furthermore moderate amounts of cellular debris were found, mainly within the cavitation. The matrix of the adjacent cartilage exhibited a loss of basophilia and numerous shrunken, hypereosinophilic chondrocytes and empty lacunae (necrosis). Additionally, the cartilage surface was irregularly

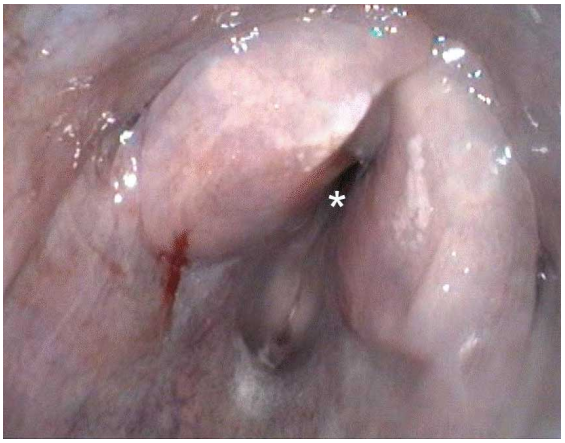

Figure 1-1. Larynx, sheep. Endoscopically, the larynx was severely swollen with obstruction of laryngeal cavitiy (asterisk). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, aD-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

shaped with clefts and fragmentation of the cartilaginous matrix. The luminal surface was lined by high numbers of 0.2 x 7-10 µm sized basophilic filamentous bacteria and fewer basophilic round cocci of approximately 1 µm in diameter. Filamentous bacteria were also found within underlying parts of cartilaginous matrix. Furthermore, the intracartilaginous inflammatory infiltrate was multifocally accompanied by moderate numbers of fibroblasts embedded in a collagenous matrix with perpendicularly arranged blood vessels (granulation tissue).

Histochemical analysis revealed a decreased, cartilaginous matrix staining intensity by Alcian blue and Safranin-O in the arytenoid lesions. The Gram stain highlighted gram-positive, coccoid bacteria adjacent to cartilaginous ulcerations. In addition, abundant PAS-positive, gram-negative bacteria arranged in chains were demonstrated at the surface of cartilaginous ulcerations and within the underlying parts of arytenoid cartilage. High amounts of collagenous fibers were detected within the granulation tissue using the Azan stain.

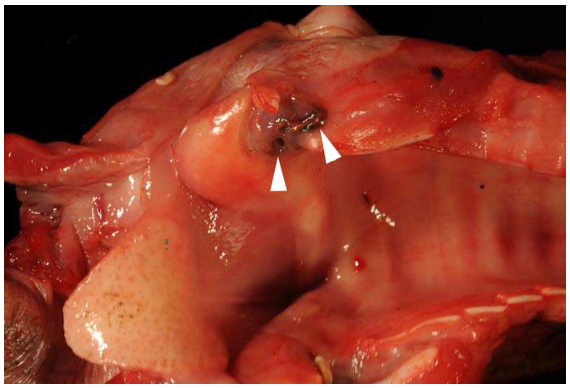

Figure 1-2. Larynx, sheep. The mucosa at the dorsal parts of the arytenoid cartilages showed approximately 0.5 cm in diameter sized, dark brown, focal defects (arrowheads) with extension into the cartilage. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, aD-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

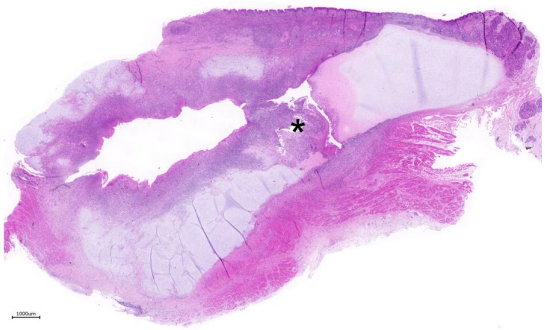

Figure 1-3. Larynx, sheep. Arytenoid cartilage revealed severe cavitation (asterisk) and focal fracture. (HE, 5X). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, aD-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

The laryngeal mucosa exhibited multifocal thinning and loss of epithelium with infrequent karyopyknosis, karyorrhexis and karyolysis as well as sloughing of epithelial cells (ulceration and necrosis). Occasionally, the cytoplasm of macrophages contained a coarse yellowish pigment (hemosiderin) or single erythrocytes (erythrophagocytosis).

Contributor's Morphologic Diagnosis:

1. Larynx: Laryngitis, severe, focally extensive, chronic, erosive to ulcerative and suppurative.

2. Arytenoid cartilage: Chondritis, severe, focally extensive, chronic, necrosuppurative.

Contributor's Comment:

Inflammatory changes of the larynx often accompany lesions of the upper and lower respiratory tract but they also appear solely.[3] Ovine laryngeal chondritis is a rare entity reported in different sheep breeds in California,[1,2] Great Britain,[10] Iceland,[14] and New Zealand.[4,7,13] The disease is characterized by low morbidity and high mortalitiy.[14] Clinical examination often indicates chronic inflammatory processes in the upper respiratory tract. Lambs and yearlings of both genders can be affected and disease often occurs within the winter term.[1,2,14]

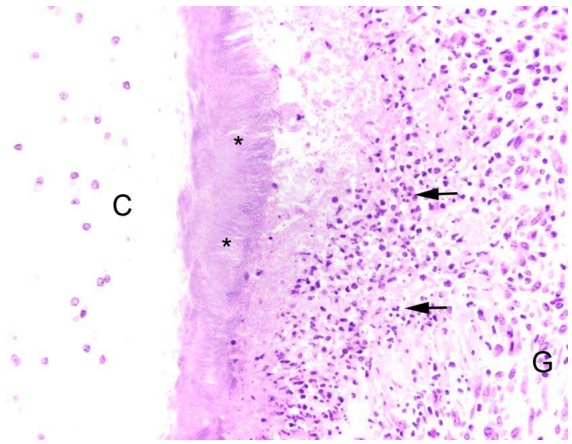

Figure 1-4. Larynx, sheep. adjacent to the cartilage (C) there is a band-like accumulation of filamentous bacteria (asterisks) and an infiltration of neutrophilic granulocytes (arrows) accompanied by immature granulation tissue (G). (HE, 400X)(Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, aD-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Microbiological analysis of affected larynges revealed a variable involvement of *Fusobacterium necrophorum, Bacteroides* spp., *Trueperella pyogenes, Streptococcus* spp., *Pasteurella* spp. and *Escherichia coli.* Most often a mixed growth of gram-positive and gram-negative bacteria was determined.[14] Laryngeal lesions are also reported in ovine systemic pasteurellosis.[5]

The pathogenesis of this entity remains unclear. Primary traumatization of laryngeal mucosa[1,2,13] as well as breed predisposition are discussed.[10,14] Irritating agents, toxins, coughing as well as aspirated grains may cause primary mucosal damage,[2,3,13,14] thereby disrupting the mucosal barrier.

Similar lesions occur in calves,[3,9] thoroughbreds,[3,6] and humans.[11] The lesions are a part of oral cavity necrobacillosis in calves which is caused by *Fusobacterium necrophorum,* or they are detected in arytenoid chondropathy of racing horses. In both diseases the pathogenesis remains undetermined although mucosal traumatization due to forced respiration and secondary bacterial colonization is discussed.[3] In humans, an autoimmune pathogenesis is discussed. Rarely lesions induced by *Mycobacterium tuberculosis*,[11] or those caused by *Corynebacterium diphtheriae* are observed.[8]

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JPC Diagnosis:
Larynx: Chondritis, pyogranulomatous, multifocal to coalescent, with necrosis and chronic-active perilaryngeal myositis.

JPC Comment:
In 1943, Cameron and Britton described a condition affecting stud yearling lambs in California that they identified as “chronic ovine laryngitis”. Common features included chronic arytenoid cartilage abscesses and laryngeal edema, which often resulted in the suffocation of affected animals.[13] As noted by the contributor, the condition now known as ovine laryngeal chondritis has since been described in multiple geographic locations and was recently described for the first time on the European mainland in two German rams in 2020.[12]

Risk factors for ovine laryngeal chondritis include season, age of the animal, and breed.

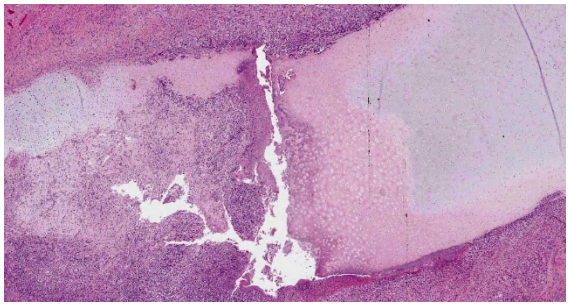

Figure 1-5. Larynx, sheep. There is a focal and complete fracture of the arytenoid cartilage. The depletion of mucopolysaccharides in the chondroid matrix results in a pale pink coloration rather than blue. (HE, 30X)

Although any sex and age may be affected, lambs and yearlings are overrepresented, with the majority of cases occurring during winter. A theory proposed in regard to younger animals being overrepresented is due to the smaller size of their rima glottidis in contrast to adults. In addition, breeds such as the Texel and Southdown are thought to be predisposed, which may be due to variation in the structure of the upper airway in comparison to other breeds. For example, laryngeal narrowing and shortening have been found to be a feature of Texel rams, which also have disproportional enlargement of arytenoid cartilage and epiglottis when compared to bluefaced Leicesters. Laryngeal edema influenced by male sexual hormones may also contribute toward development of the disease in rams.[12]

Affected animals may present with variable range of clinical signs depending on severity, including tachypnea with an increased abdominal component, inspiratory and expiratory laryngeal stridor, extension of the head and neck, tachycardia, and cyanotic mucous membranes.[12] External palpation may reveal laryngeal enlargement and may also incite transient worsening of dyspnea and/or coughing.[12] Cases are often submitted for post-mortem examination with suspicion of pneumonia.[13]

The mucosa overlying the arytenoid cartilages may either be thickened by diffuse uni- or bilateral asymmetric edematous swelling or thickened with a pale and undulant surface; either may result in nearly complete airway obstruction. On cut surface, the tissue surrounding the arytenoid cartilage is typically edematous, discolored, and often associated with a purulent exudate.[13]

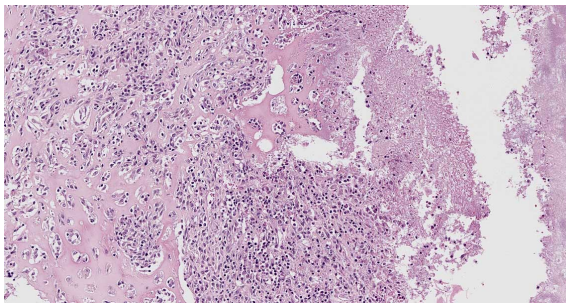

Figure 1-6. Larynx, sheep. There is a focal and complete fracture of the arytenoid cartilage. The depletion of mucopolysacchrides in the chondroid matrix results in a pale pink coloration rather than blue. (HE, 30X)

Common histopathologic features include extensive chronic-active inflammation with granulation tissue formation within the sub-mucosa, which often extends around cartilage and into adjacent striated muscle. The cartilage is often degenerative and eroded by adjacent inflammation, with purulent or fibrinopurulent exudate concentrated at the periphery, frequently with numerous bacteria admixed.[13]

As noted by the contributor, mixed populations of bacteria are often isolated. One study[13] found >80% of cases had mixed gram-positive and gram-negative bacteria, with the most common isolates (each in approximately 50% of cases) being *Fusobacterium* spp., *Trueperella pyogenes* and *Streptococcus* spp.[13]

Although early cases of ovine laryngeal chondritis have been successfully treated with antibiotics and corticosteroids, these therapies often fail. As a last resort, tracheostomy may be performed as a life-saving measure. However, surviving animals are of limited value given their utilization as breeding stock is not recommended due to the potential heritable component of this disease.[12]

Conference participants engaged in spirited discussion regarding appropriate morphologic diagnosis terminology. Specific attention was directed toward appropriate utilization of "coalescing" versus "coalescent". Since key components of the morphologic diagnosis (i.e. chronicity, distribution, and severity) describe a noun (e.g. "chondritis"), use of the adjective "coalescent" may be more appropriate than the historically ubiquitous verb "coalescing".

References:

1. Britton J. Further observations on chronic ovine laryngitis. *Cornell Vet*. 1945;35:210–213.
2. Cameron H, Britton J. Chronic ovine laryngitis. *Cornell Vet*. 1943;33:265–268.
3. Caswell JL, Williams KJ. Respiratory System. In: Maxie G, ed. *Jubb, Kennedy, and*

Palmer's pathology of domestic animals. 6th ed., St. Louis: Elsevier, 2016:481–482.
4. Davis G. A sheep mortality survey in Hawke's Bay. *N Z Vet J*. 1974;22:39–42.
5. Dyson DA, Gilmour NJ, Angus KW. Ovine systemic pasteurellosis caused by *Pasteurella haemolytica* biotype T. *J Med Microbiol*. 1981;14:89–95.
6. Garrett K, Embertson R, Woodie J, Cheetham J. Ultrasound features of arytenoid chondritis in Thoroughbred horses. *Equine Vet J*. 2013;45:598–603.
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9. Jensen R, Lauerman L, England J, et al. Laryngeal diphtheria and papillomatosis in feedlot cattle. *Vet Pathol*. 1981;18:143–150.
10. Lane JG, Brown PJ, Lancaster ML, Todd JN. Laryngeal chondritis in Texel sheep. *Vet Rec*. 1987;121:81–84.
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12. Reineking W, Punsmann TM, Wagener MG, et al. Laryngeal chondritis as a differential for upper airway diseases in German sheep. *Acta Vet Scand*. 2020;62(1):12.
13. Salisbury R. Chronic ovine laryngitis. *N Z Vet J*. 1956;4:144–146.
14. Sigurðardóttir Ó, Jörundsson E, Friðriksdóttir V. Laryngeal chondritis in sheep in Iceland. *J Comp Pathol*. 2016;155:310-313.

CASE II: H20-1916-5 (JPC 4159332)

Signalment:
A 1-year-old ram lamb (O*vis aries*)

History:
Animal was presented for necropsy following a brief clinical history of 2 to 3 days of scours, and a 4-day history of anorexia and hyperdipsia. The producer noted that the lamb often used a provided salt lick.

Gross Pathology:
The lamb was in good body condition. The right cranial and middle lung lobes were consolidated, with yellow-tinged foam exuding from small airways on the cut surface. The abomasal mucosa was grey, with few scattered petechiae.

Laboratory Results:
- Fecal flotation (sugar): *Trichostrongylus* sp., light parasite load. Coccidian parasite of unknown identity, light parasite load.
- Ileocecal valve culture: *E. coli*, coagulase negative *Staphylococcus* sp.
- Lung culture: *E. coli*, coagulase negative *Staphylococcus* sp., Gram negative bacilli of unknown identity.

Microscopic Description:
Abomasum: Multifocally within the mucosa, there are multiple cysts of apicomplexan parasites that measure 20-250 µm diameter, have a thick capsule (schizonts) and contain numerous merozoites frequently arranged in blastophores. Some of these cysts are ruptured, with focal areas of necrosis of the mucosa plus infiltrates of large numbers of neutrophils, macrophages, and occasional multinucleated giant cells, which are phagocytizing merozoites. The lamina propria and the submucosa are infiltrated with moderate numbers of lymphocytes, plasma cells and macrophages. In the mucosa, there are a few fibrin thrombi.

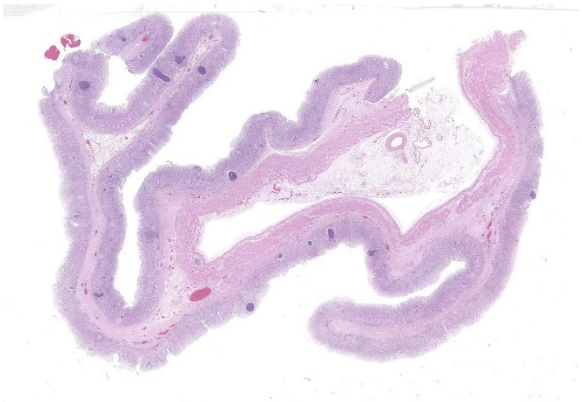

Figure 2-1. **Abomasum, sheep: Multifocally, large basophilic apicomplexan schizonts are scattered throughout the mucosa. (HE, 5X).**

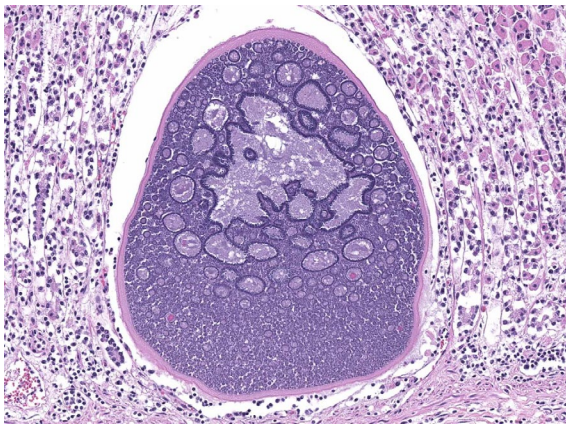

Figure 2-2. Abomasum, sheep. Megaloschizonts are composed of a thick bilayered hyaline wall enclosing innumerable zoites which often surround round to serpiginous blastopores. (HE, 181X)

Contributor's Morphologic Diagnosis:
Abomasum: Abomasitis, neutrophilic and histiocytic to granulomatous, multifocal, with mucosal necrosis and numerous intralesional apicomplexan parasites.

Contributor's Comment:
The morphology of the abomasal parasite in this case is consistent with the apicomplexan parasite *Eimeria gilruthi*, an incompletely characterized coccidian parasite of the gastrointestinal system in sheep, which was most recently described in the *Journal of Veterinary Diagnostic Investigation* in 2019.[1] These coccidia have a characteristic microscopic morphology, with large protozoal schizonts ("megaloschizonts") with a thick, eosinophilic wall surrounding thousands of elongate merozoites, often arranged in circular blastophores. Megaloschizonts can be so large that they form miliary, pale, pinpoint foci on the mucosal surface of the abomasum visible on postmortem examination. [4]

Although many species of *Eimeria* can be found within the alimentary tract of sheep, few are considered pathogenic. *E. crandallis* and *E. ovinoidalis* are the two paramount pathogenic species, both of which typically affect lambs up to 6 months old, especially those reared under crowded or stressful conditions. A recent diagnosis of *E. gilruthi* in a group of 15-month-old ewes demonstrated its pathogenicity of this coccidian parasite even in older animals.[1] Clinical signs are typical of many intestinal parasites and usually consist of progressive anemia, diarrhea, anorexia, and weakness. Rapid weight loss in production animals often leads to euthanasia, although the protozoan itself can cause lethal disease in severe cases. Damage to host cells is typically through traumatic rupture following the growth and expulsion of merozoites and, for many *Eimeria* species, the severity of clinical disease correlates with the parasite burden.[2]

This diagnostic exercise would be remiss if it did not mention the life cycle of this parasite. Although the life cycle of *E. gilruthi* is not completely elucidated, it very likely mirrors that of other *Eimeria* species.[2] A typical life cycle of these species includes both a sexual and an asexual form of reproduction. The infectious stage is the sporozoite, which is capable of penetrating and infecting host intestinal cells, and which typically enters the gastrointestinal system via ingestion by the host of sporulated oocysts from the environment. Once intracellular, the sporozoites undergo a morphological change into trophozoites, which are located within a membrane-bound parasitophorous vacuole within the host cell cytoplasm.[2] The trophozoites further expand into structures known as schizonts, which produce merozoites through asexual reproduction. In *E. gilruthi* in particular, these schizonts develop to such a massive size that they are called megaloschizonts.[2,4]

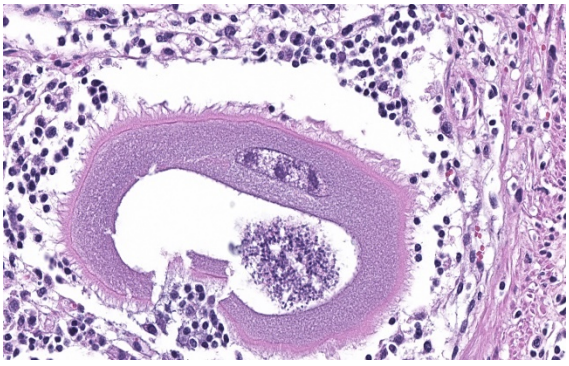

Figure 2-3. Abomasum, sheep. Megaloschizont are surrounded by a thick bilayered hyaline wall with cilia-like laminar protrusions, a markedly hypertrophic host cell nucleus, and abundant vacuolated cytoplasm (which will in time, be replaced by proliferating zoites. (HE, 483X)

Animal	Coccidia	Organ affected/Clinical signs
Birds Chickens Turkey Geese & ducks Sandhill/whooping cranes Parrots	*E. acervulina* *E. necatrix/maxima* *E. brunette* *E. tenella* *E. meleagridis* *E. adenoeides* *E. meleagrimitis* *E. gallopavonis* *E. truncata* *E. anseris/nocens* *E. reichenowi* *E. psittaculae*	Duodenum/enteritis Jejunum/enteritis Ileum/enteritis Ceca/typhylitis Cecum Cecum, ileum Upper intestine Ileum, large intestine Kidney/anorexia, depression Intestine Disseminated Intestine
Cattle	*E. bovis/zuernii* *E. alabamensis*	Cecum and colon/diarrhea Small intestine
Sheep	*E. ahsata/christenseni* *E. brakuensis* *E. crandallis* *E. ovinoidalis*	SI SI SI Cecum, colon
Goats	*E. christenseni* *E. arloingi* *E. hirici* *E. ninakohlyakimovae*	SI SI SI LI
Equine	*E. leukarti* *Klossiella equi*	SI
Swine	*E. debliecki*	SI (in 1-3 week old piglets)
Canine	*I. canis*	Ileum, colon occasionally
Feline	*I. felis*	SI, colon occasionally
Mice	*Klossiella muris* *E. falciformis* *E. vermiformis* *E. papillata* *E. ferrisi*	kidney Colon Intestine Intestine Intestine
Rabbit	*E. stiedae* *E. intestinalis* *E. flavescens*	Bile ducts Ileum & cecum Ileum & cecum
Ferret	*E. furonis*	SI

Table 1

Merozoites exit the cell (typically in a manner that damages the host cells) and infect neighboring host cells. There is a finite number of asexual reproductive cycles possessed by each *Eimeria* species, which differs between the species; the number is not yet elucidated for *E. gilruthi*. After the final asexual reproductive cycle, the resulting merozoites develop into either a microgamont (male sex cell) or macrogamont (female sex cell). The macrogamont further develops by growing in size and storing energy; the fully mature female sex cell is a macrogamete.[2] Microgamonts undergo repeated nuclear division, eventually splitting into multiple, biflagellate, uninuclear microgametes. A zygote forms when a microgamete penetrates and fertilizes a macrogamete (sexual reproduction); it is referred to as an oocyst when it develops hyaline granules and a wall. The oocyst exits the host cell via rupture of the cell and trafficking through the feces, and sporulates within the environment.[2]

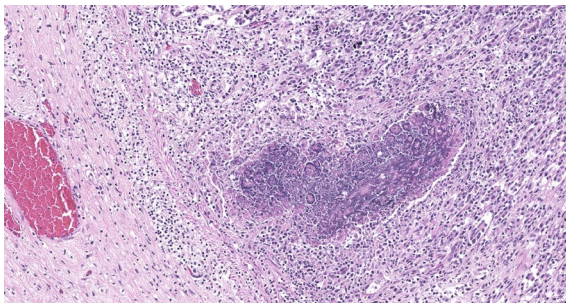

Figure 2-4. Abomasum, sheep: occasionally megaloschizonts are ruptured, inciting a pyogranulomatous response within the mucosa. (HE, 168X)

Contributing Institution:
Department of biomedical Sciences and Pathobiology, Virginia Maryland Regional College of Veterinary medicine, Virginia Tech https://www.vetmed.vt.edu/departments/dbsp/

JPC Diagnosis: Abomasum: Apicomplexan megalo-schizonts, with mild lymphoplasmacytic and neutrophilic abomasitis.

JPC Comment:
The contributor provides an excellent review of *Eimeria gilruthi*, an entity making its inaugural appearance in the WSC.

Abomasal lesions consistent with *E. gilruthi* were first described by Maske in 1893, who erroneously attributed them to gregarines, a group of apicomplexan parasites now known to only infect invertebrates. Similar lesions were later observed by Gilruth and studied in greater detail by Chatton in 1910, who named the organism *Gastrocystis gilruthi*, although multiple early reports identified it as *Globidium giltruthi*. *E. gilruthi* underwent multiple scientific name changes over the ensuing decades with the current nomenclature initially being favored by Levine and Soulsby during the 1960s.[3]

Interestingly, sheep and goats may not be definitive hosts for *E. gilruthi.* Despite the apicomplexan's ability to develop one or more generations of schizonts in small ruminants, reports are conflicting in regard to its ability to undergo gametogony. Attempts to close such knowledge gaps are confounded by the parasite's sporadic occurrence and attempts to replicate its life cycle in vitro have so far been unsuccessful.[1]

Common post-mortem findings associated with *E. gilruthi* include edema and multifocal raised white foci within the mucosa of the abomasum, which histologically correlate to megalo-schizonts. However, *Teladorsagia circumcincta* (previously known as *Ostertagia circumcincta),* a trichostrongyle nematode, may cause similar gross lesions in the abomasum of sheep and goats. Microscopic examination is required for differentiation between these entities.[1]

The majority of coccidian parasites, including *Eimeria* spp., are host specific. Notable exceptions include *E. pallida, E. caprovina*, *E. punctate*, and *E. gilruthi,* which have been reported in both sheep and goats.[4]

The table 1 identifies hosts and organs affected by coccidian species.[4]

References:

1. Ammar SI, Watson AM, Craig LE, et al. Eimeria gilruthi-associated abomasitis in a group of ewes. *J Vet Diagn Invest*. 2019;31(1):128-132.
2. Bowman DD. *Georgis' Parasitology for Veterinarians*. 9th ed. St. Louis, MO: Saunders Elsevier; 2009: 93-94.
3. Hilali M. Studies on globidial schizonts in the abomasum of Norwegian sheep. The fine structure of one of the four merozoite forms investigated. *Acta Vet Scand*. 1973;14(1):22-43.
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CASE III: P5952-17 (JPC 4118012)

Signalment:
Adult (exact age not specified but assessed as 2-3 year-old by veterinarian), female, crossbred, domestic goat (*Capra aegagrus hircus*)

History:
This goat was culled for undisclosed reasons and sent to the abattoir. Although the body condition

was suboptimal, this goat was assessed as fit for slaughter at the ante-mortem examination.

Gross Pathology:
The submitted post-mortem examination report only mentioned abnormal lungs. They were described by the veterinarian as diffusely mottled with numerous firm, gray to pale red consolidated areas. On cut section, there was a thick mucopurulent exudate in the bronchi of the anterior portions of both lungs. Fresh and formalin-fixed samples from all lobes were sent to our laboratory.

Laboratory Results:
Immunohistochemistry was multifocally strongly positive for small ruminant lentivirus (SRLV → MMV and CAEV).

Microscopic Description:
The lesions are similar in all pulmonary lobes (only one section submitted). The lesions are lobular, with normal lobules adjacent to pneumonic ones. In affected lobules, there is a lymphoplasmacytic infiltrate variably thickening the airway and vascular adventitia, and to a lesser degree the alveolar septa (interstitial pneumonia). Multifocally, the alveoli are lined by usually plump type II pneumocytes (proliferative pneumonia). The alveoli are filled by dense amorphous eosinophilic material (alveolar proteinosis) with a few macrophages and desquamated type II pneumocytes and occasional neutrophils; occasionally, clumps of necrotic cells (neutrophils) are also present. Some bronchi and bronchioles are filled with a mucopurulent exudate that sometimes extends into adjacent alveoli and/or submucosal glands; their propria-submucosa is infiltrated by lymphocytes and plasma cells and there is mild to moderate BALT hyperplasia.

Contributor's Morphologic Diagnosis:
1. Lobular, moderate to marked, extensive lymphoplasmacytic interstitial and proliferative pneumonia with alveolar proteinosis, consistent with SRLV pneumonia (caprine arthritis-encephalitis/CAE).
2. Marked, subacute mucopurulent bronchitis and bronchiolitis.

Note: the samples were originally submitted as ovine tissues (ewe), but based on the typical CAE lesions and small size of red blood cells (goats have the smallest RBCs of domestic animals) we called the abattoir which confirmed the mistake.

Contributor's Comment:
Microscopic features and IHC results are consistent with small ruminant lentivirus (SRLV) pneumonia in goats. Diseases caused by SRLV are known as caprine arthritis-encephalitis (CAE) in goats and maedi-visna (MV) in sheep; MV is also known as ovine progressive pneumonia (OPP) in the USA. The mucopurulent bronchitis and bronchiolitis are likely due to a secondary bacterial infection, although routine aerobic culture did not yield any significant bacteria (contaminants).

Lentiviruses are a genus (*Lentivirus*) of non-oncogenic viruses in the Retroviridae family (single-stranded RNA). This genus includes immunodeficiency viruses (HIV, SIV, FIV and BIV), the equine infectious anemia virus (EIAV in Equidae → hematological, neurological and immunological) and small ruminant lentiviruses (SRLV).[6] Lentiviruses share many similarities with other retroviruses. Their proviral RNA genome includes three structural genes (*gag*, *pol* and *env*) and long terminal repeats (LTRs) at each end. Two proteins encoded by the *pol* gene are the enzymes reverse transcriptase, which allows transcription of viral RNA into double-stranded DNA, and integrase which enables its integration

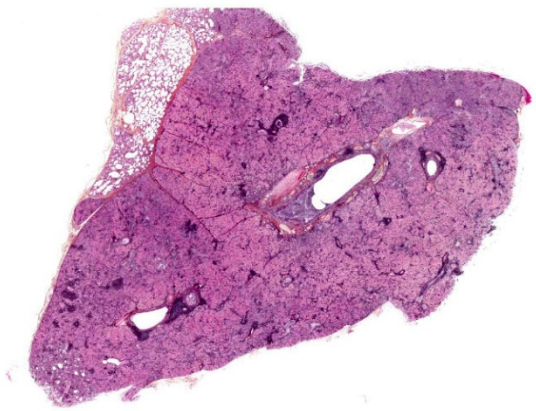

Figure 3-1. Lung, goat. There is profound lobular consolidation and cellular infiltration. (HE, 5X)

into the host cells' genome.[2,3,6] All lentiviruses infect and replicate in histiocytic cells; HIV, SIV, and BIV are also highly lymphotropic, in contrast to SRLV, EIAV, and BIV (the latter is only mildly immunosuppressive).[2,6] Although often referred to as caprine arthritis-encephalitis virus (CAEV) in goats and maedi-visna virus (MVV) in sheep, these viruses are now considered a spectrum of SRLV variants with some detected only in sheep (classical MMV strains), some only in goats (classical CAEV strains) and some in both species; furthermore, there is now also evidence for potential dual infection.[2,3,6] SRLV has also been demonstrated (PCR) in wild ibexes' (*Capra ibex*) monocytes, experimentally-infected Mouflon-domestic sheep hybrids, and naturally-infected Rocky Mountain goats (*Oreamnos americanus*) with typical CAEV lesions. This is unusual for lentiviruses which are, with a few exceptions (e.g. SIV in human and non-human primates; FIV in multiple felid species), species-specific.[2]

Caprine arthritis-encephalitis (CAE) in goats (*Capra aegagrus hircus*) and maedi-visna (MV) in domestic sheep are similar with regard to pathogenesis, pathology and clinical signs. Infection by SRLV is mainly through ingestion of colostrum/milk; inhalation of infected nasal secretions, directly or through aerosols, is another possible mode of transmission.[2,3] The main targets of SRLV are histiocytic cells (monocytes/macrophages and dendritic cells) in which they establish lifelong infection because the virus is able to evade the immune system by several mechanisms.[2,3,6] Among others,

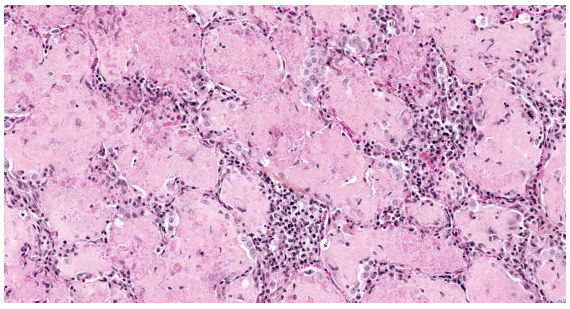

Figure 3-2. Lung, goat. Alveolar septa are markedly expanded by variable combinations and concentrations of lymphocytes, histocytes and plasma cells and they are rarely discontinuous (necrosis) Alveolar lumina are flooded with abundant proteinaceous fluid. (HE, 254X)

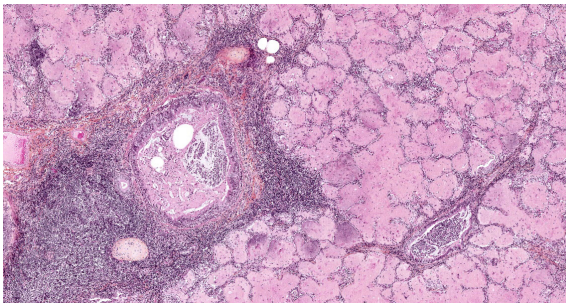

Figure 3-3. Lung, goat. There is marked hyperplasia of peribronchiolar and perivascular lymphoid tissue. (HE, 53X)

lentiviruses (and retroviruses in general) tend to mutate at a relatively high rate due to the error-prone reverse transcription mechanism, thus creating several variants within a cell (quasi-species); this is followed by recombination, thus creating relatively high genetic variability in viruses within an individual animal.[2,6] Also, there are relatively low levels of circulating SRLV-infected cells (and free virus is rare) and the virus remains latent in monocytes, thus allowing widespread dissemination mainly to CNS, lung, mammary gland and joints.[2] When monocytes become macrophages in tissues, viral transcription and protein production increases to high levels and cytokines are produced; this leads to a chronic immune-mediated reaction which involves CD4+ and CD8+ T cells, B cells/plasma cells and macrophages.[2,6] SRLV also infects epithelial cells of the mammary gland and thus, as mentioned above, colostrum/milk is an important source of virus.[2]

Both CAE and MV cause encephalomyelitis, pneumonia, arthritis and mastitis that can occur singly or in different combinations in susceptible hosts.[3,6,7] The pathologic picture depends on viral and host factors (e.g. certain breeds of sheep are more susceptible).[2,3,6] They are progressive wasting diseases mainly seen in adult animals, with the exception of the neurological form of CAE which is mostly seen in 2-4 month-old goat kids; the respiratory and neurological forms are invariably fatal. Arthritis and encephalomyelitis are the most frequent pathological manifestations of CAE, while they are uncommon to rare in MV.[3] Pneumonia is the most common manifestation in MV, but is relatively uncommon in CAE; maedi is the name of the respiratory form of MV.[3] Mastitis may be a more frequent

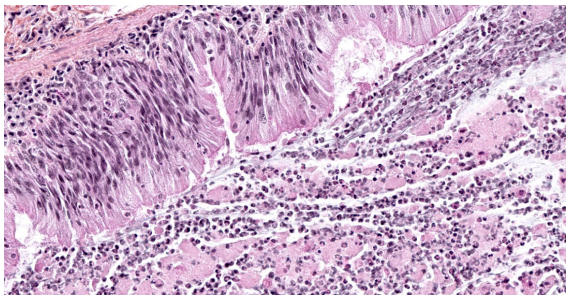

Figure 3-4. Lung, goat. Regionally, the character of the inflammation, especially within airways, is suppurative. (HE, 310X)

occurrence than generally reported in MV as a study in the province of Quebec found more mammary than pulmonary lesions in MV-seropositive sheep.[1] In MV, the lungs fail to collapse when the thorax is opened, and they are diffusely pale gray to tan with a rubbery to firm texture (interstitial pneumonia). Gross lesions are similar in CAE, but they vary from patchy to diffuse.[3,8] Three inflammatory patterns have been described in the CNS, lung and mammary gland of MV-affected sheep: lymphocytic (mainly CD8+ T cells), histiocytic (mainly macrophages and B cells) and mixed;[4] we did not find a similar study for CAE. Pulmonary lesions in CAEV, MV and other lentiviral diseases share a common pattern of lymphocytic interstitial pneumonia (LIP). In maedi, but not in CAE, lesions are also characterized mainly by lymphoid follicular formation and smooth muscle hypertrophy; mild interstitial fibrosis is also described. The alveolar proteinosis is typical of CAE; it has been suggested this phenomenon may be more host-related than disease-related.[3,8] Ultrastructurally, the intra-alveolar proteinaceous material consists of myelin figures, consistent with surfactant.[8] Perivascular and peribronchiolar lymphoid infiltration with follicular formation has been described.[8]

Contributing Institution:
Faculty of veterinary medicine, Université de Montréal.
http://fmv.umontreal.ca/faculte/departements/pathologie-et-microbiologie

JPC Diagnosis:
1. Lung: Pneumonia, interstitial, lobular, lymphohistiocytic, moderate with abundant proteinaceous alveolar edema and type II pneumocyte hyperplasia.
2. Lung: Peribronchiolar and perivascular lymphoid hyperplasia and bronchiolar epithelial hyperplasia.
3. Lung: Bronchopneumonia, suppurative, focally extensive, mild to moderate.

JPC Comment:
The contributor provides an outstanding review of the pathogenesis and various clinical manifestations of small ruminant lentiviruses (SRLVs). In addition, lentiviral mastitis and SRLVs in general were recently discussed during WSC 14, case 1.

As noted by the contributor, SRLVs were historically believed to be species specific with maedi-visna virus infecting sheep while the aptly named caprine arthritis and encephalitis virus (CAEV) infected goats.[9] SRLVs have since been divided into five genetically diverse genotypes (A-E), with most genotypes and associated subtypes reported to infect both sheep and goats. However, exceptions include the E genotype, which has only been reported in sheep, while six A subtypes have only been reported in goats. While it is possible these variants are species specific, it is also possible interspecies transmission has not yet occurred or simply have not yet been detected.[5]

SRLVs have been recognized throughout the world for decades, with maedi first being described in South Africa in 1915. Ovine progressive pneumonia virus (OPPV) was identified less than decade later in Montana in 1923, with the affected sheep being described as "lungers" as they tended to lag behind the flock. In contrast, goats infected with SRLV were initially believed to be affected by a hereditary disease due to a perceived relationship between the clinical disease and certain family lines. The virus now known as CAEV was first identified as a cause of leukoencephalitis in the in 1974; researchers from the same laboratory later isolated the same virus from goats with chronic arthritis. Historically, SRLVs had a worldwide distribution. However, several countries are now SRLV free, including Iceland, New Zealand, and Australia.[9]

For reasons previously discussed by the contributor, SRLVs are associated with lifelong infection. However, only approximately 30% of infected animals develop clinical illness. The remaining animals with subclinical infections are particularly problematic as they serve as carriers, introducing the disease to offspring as well as naïve herds and flocks. In addition, subclinical infections result in significant economic loss that is not often readily apparent on an individual basis unless metrics are closely monitored. Examples of such losses include reductions in market weight of lambs reared by infected ewes (3.86kg) compared to those of unaffected ewes and seropositive goats have been associated with significantly lower length of lactation, milk yield, milk fat, and lactose levels.[9]

Interestingly, many producers are unaware of the risk posed by SRLVs. A 2011 study conducted by the US Department of Agriculture on operations representing over 70% of US farms with ewes and over 85% of the total ewe inventory found nearly half (46.5%) of producers were not familiar with ovine progressive pneumonia virus (i.e. SRLV). Of the 53.5% of producers aware of SRLV, over 72% did not know their current status and only 16.2% had a flock health management program implemented for the control or eradication of SRLV. Although this study did not assess seroprevalence, another 2011 study conducted by the USDA in Wyoming assessed sera from 1415 sheep and 54 flocks of various size ranges and grazing types and found 18% of sheep and 47.5% of flocks were seropositive for OPPV.[9]

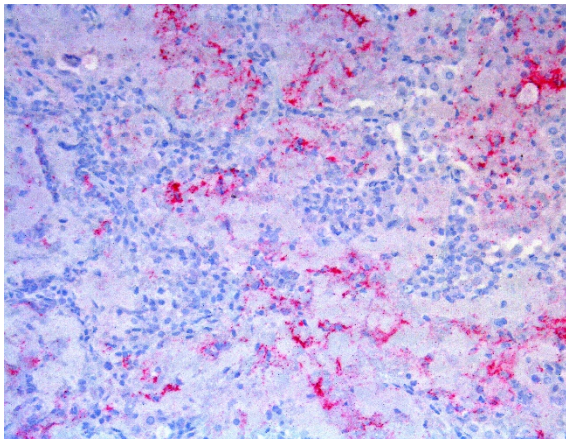

Figure 3-5. Lung, goat. Infiltrating macrophages are positive for small ruminant lentivirus antigen. (Photo courtesy of: Faculty of veterinary medicine, Université de Montréal. http://fmv.umontreal.ca/faculte/departements/pathologie-et-microbiologie)

As demonstrated by this case, characteristic features of caprine SRLV pneumonia include dense eosinophilic alveolar fluid with foamy alveolar macrophages, type II pneumocyte hyperplasia, and expansion of alveolar septa by lymphocytes and fibrosis. In contrast, type II pneumocyte hyperplasia is not a prominent feature of maedi, which is histologically characterized by lymphoplasmacytic infiltrates expanding septa, perivascular tissue, and airways which often form characteristic lymphoid follicles.[3]

Participants were in agreement with the contributor in regard to the likelihood of a secondary bacterial component in this case. The moderator strongly suspected this goat had concurrent bronchopneumonia due to *Mycoplasma* spp. such as *M. ovipneumoniae* due to the presence of characteristic peribronchiolar and perivascular lymphoid hyperplasia, bronchiolar epithelial hyperplasia, and negative aerobic bacterial culture. However, given both SRLV and *Mycoplasma* spp. cause lymphohistiocytic interstitial pneumonia it is not possible to definitively ascertain the cause of the secondary bronchopneumonia without additional diagnostics such as PCR.

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CASE IV: N-190/20 (JPC 4165839)

Signalment:
6 years old, female, Breed Rasa Aragonesa, Ovine (*Ovis aries)*

History:
Animal with history of chronic rhinitis, non-responsive to antibiotic treatment.
Physical findings revealed marked dyspnea, and weakness.

Gross Pathology:
The ventral conchae in the left nasal cavity was severely swollen, with a roughed, thickened mucosa compound of multiple, small, whitish or yellowish polypoid proliferations covered by abundant mucus. The proliferative mucosa was obliterating the meatus and protruding through the nostrils.

Laboratory Results:
Nasal swabs were obtained during the necropsy.

Pure cultures of *Salmonella enterica subsp. diarizonae serovar 61:K:1, 5, 7 w*ere obtained from the swabs.

Microscopic Description:
Nasal Mucosa: Up to 100% of the tissue is affected by a proliferative and inflammatory process. Diffusely the mucosa is thickened up to 5-20 times the normal, frequently forming multiple polypoid projections compound of abundant hyperplastic disorganized respiratory epithelium that contain abundant intracytoplasmic 1-2 µm eosinophilic bacilli/cocobacilli. Between the epithelial cells are moderate amounts of neutrophils, lymphocytes, plasma cells, Mott cells, and macrophages, the same type of inflammatory cells are severely infiltrating and expanding the adjacent submucosa, admixing with moderate amount of edema, some areas of mild fibrosis and capillary congestion. Multifocally there is seromucous gland hyperplasia that contain abundant eosinophilic amorphous material, cellular debris and the same inflammatory cells, and intracytoplasmic bacteria previously described. Covering the mucosa is abundant eosinophilic amorphous material and few cell debris.

Contributor's Morphologic Diagnosis:
Nasal mucosa: Diffuse proliferative, lymphoplasmacytic and neutrophilic rhinitis with abundant intracytoplasmic bacilli, chronic, severe.

Condition: Chronic proliferative rhinitis.

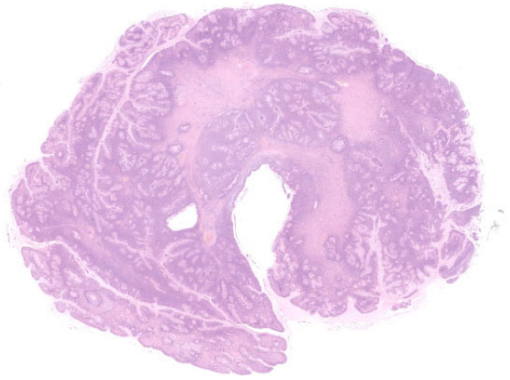

Figure 4-1. Nasal mucosa, sheep. A section of hyperplastic nasal mucosal with widely spaced glands is submitted for examination. (HE, 5X)

Etiology: *Salmonella enterica subsp. diarizonae serovar 61:K:1, 5, 7.*

Contributor's Comment:
The bacteria *Salmonella enterica subsp. diarizonae serovar 61: K:1,5,7* is host adapted in sheep, can colonize and persist in the nasal mucosa. Infected animals may occasionally develop chronic nasal inflammation, particularly after being stressed.[4]

Clinical signs started with unilateral or bilateral nasal discharge of thick mucus together with wheezing and snoring. These signs persist and progress for several weeks, with almost complete nasal obstruction caused by the presence of proliferating tissue, often visible at the nares.[4]

Gross findings include thickened mucosa with multifocal proliferations composed of multiple small white or yellow polypoid structures covered by mucus. The ventral turbinates appear to be more affected.[4]

Histological findings reveal a thickened nasal mucosa with multiple polypoid projections or layers of disorganized epithelial cells covered by hyperplasic respiratory epithelium. These cells have elongated eosinophilic cytoplasm or vacuoles filled with gram-negative bacilli. Nuclei are generally rounded with peripherally condensed chromatin and some nuclei showing degenerative changes. Groups of neutrophils invade basal or apical portions of the epithelium. The proliferative epithelium may be covered by

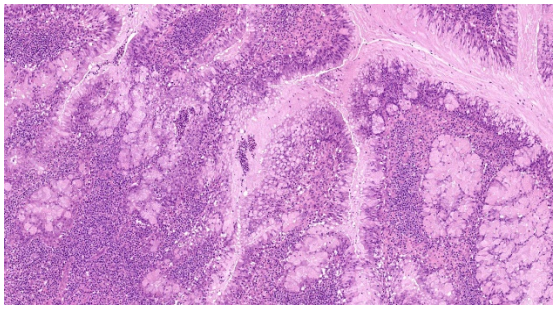

Figure 4-2. Nasal mucosa, sheep. Glands are surrounded and widely separated by an infiltrate of large numbers of plasma cells, lymphocytes and macrophages. Glandular lumina contain moderate numbers of neutrophils and eosinophils admixed with mucin and cellular debris. (HE, 83X)

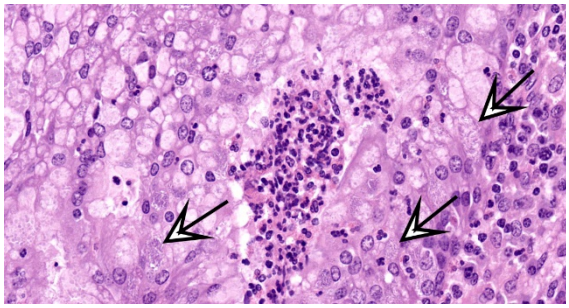

Figure 4-3. Nasal mucosa, sheep. Glandular epithelium often contains a vacuole replete with numeous 2-3 µm bacilli (arrows). The glandular lumen contains moderate number of neutrophils and fewer eosinophils. (HE, 538X)

eosinophilic amorphous material mixed with cell debris. The submucosa is expanded and densely infiltrated by plasma cells, macrophages, and neutrophils. Nasal glands are hyperplasic with variable degree by secretion.[4]

Investigation of affected flocks indicate direct animal to animal transmission and possible shedding of *Salmonella* by nasal discharge.[6]

There are many different diseases affecting the upper respiratory tract in sheep that could confuse the diagnosis, such as oestrosis, enzootic nasal adenocarcinoma, or fungal rhinitis.[5]

Salmonella enterica subsp. diarizonae has been implicated in infections of other organs as an opportunistic gastrointestinal pathogen in lambs and suppurative epididymitis and orchitis in rams.[2,3]

The presence of *S. enterica subsp. diarizonae serovar 61:k:1,5,(7)* in sheep could have public health significance, since human infections through the consumption of uncooked meat or contaminated animal products has been described.[2] The prevalence of Salmonella was high in sheep and low in goats at slaughter. The tonsils allow a better estimation of the prevalence of Salmonella in asymptomatic sheep than fecal samples.[1]

Generally, human pathologies associated with these bacteria occur in individuals with underlying diseases. However, the kinetics of the

infection are not completely understood. Further studies may clarify the prevalence of SED 61:k:1,5,(7) in different flocks and the persistence in the nasal mucosa of sheep.[5]

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

JPC Diagnosis:
Nasal mucosa: Rhinitis, proliferative and lymphoplasmacytic**,** diffuse, chronic, severe, with numerous intraepithelial and intra-histiocytic bacilli.

JPC Comment:
The contributor provides an excellent overview of ovine proliferative rhinitis, a unique condition first described in the United States by Meehen et al. in 1992 and subsequently in Spain (2012) and Switzerland (2017).

Salmonella enterica is a facultative anaerobic, gram-negative, rod-shaped, flagellated bacterium divided into six subspecies composed of *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, and *houtenae.* The most common subtype isolated from infected warm blooded animals and humans is *S. enterica* subsp. *enterica* whereas *S. enterica* subsp. *diarizonae* is most commonly isolated from reptiles. A notable exception to the latter is *S. enterica* subsp. *diarizonae* serovar 61:k:1,5,(7), which has been identified as being host-adapted for sheep (also known as "sheep associated *S. diarizonae*" or "SASd"). Two distinct lineages of this subtype are ST432 and ST439. The ST432 linage is most prevalent, with the majority of isolates historically being of ovine origin whereas all ST439 isolates have been obtained from human clinical samples. Thus, it has been proposed that the ST432 lineage of SASd is host adapted for sheep whereas STS439 is not. Despite these differences, both subtypes are highly similar, which is in turn facilitates scientific discovery as to which genetic variances favor ST432's host adaptation to sheep.[7]

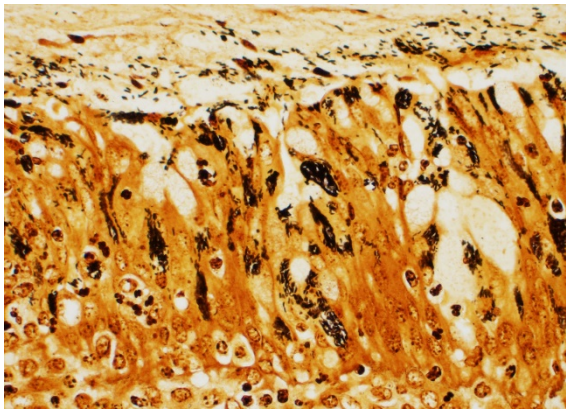

Figure 4-4. Nasal mucosa, sheep. A silver stain demonstrates the presence of numerous 2-3 um bacilli within glandular epithelial cytoplasm. (Warthin Starry 4.0, 400X)

Pseudogenetization is a phenomenon thought to facilitate the process of host-adaptation. This process occurs when previously functional and full-length genes are inactivated, disrupted, eroded, and eventually removed from the genome and is thought to occur when those genes no longer necessary for survival. Therefore, the number of pseudogenes possessed by an entity may correlate to its level of host adaption. Although STS432 and STS439 both possess an increased numbers of pseudogenes, ST432 has a significantly higher share. Examples of ST432 pseudogenes include *acrD,* a gene encoding a multidrug efflux transporter involved in aminoglycoside efflux, *narX* and *phoX*, two genes involved in substrate sensing and signaling, and *cas3*, a gene that when knocked-out has been found to result in decreased virulence and increased cellular survival, features that would in turn favor the survival of an intracellular organism.[7]

ST432 also possess additional unique virulence factors absent in ST432, such as five genes considered to encode fimbria with a high similarity to the P/Pap pilus gene cluster of uropathogenic *E. coli*, which mediate attachment to uroepithelial cells. These fimbriae are thought to facilitate intestinal colonization in sheep. An additional unique virulence factor of ST432 compared to ST439 is *asr,* which encodes an acid shock protein, which may facilitate intrahistio-cytic survival.[7]

Chronic proliferative rhinitis due to SASd is rarely reported. However, SASd infection is quite common in sheep, though with variable

geographical prevalence. For example, the pathogen is considered endemic in Sweden where 72% of tested farms in a 2015 report had at least one positive fecal sample, of which 94% were positive for SASd whereas only 1.1% of slaughtered sheep within the United Kingdom were positive for *Salmonella*, with SASd being the most common isolate.[6]

A 2017 Swiss report provides additional insight in regard to host factors associated with SASd infection. Three ewes from a herd of 34 ewes and 28 lambs less than 6 months of age developed chronic proliferative rhinitis and eventually died or were euthanized due to respiratory distress. Bacteriological analysis using nasal swabs collected from the remaining members of the flock found 87% of adult sheep were positive for SASd, while all lambs were negative. Possible explanations for this striking difference include: 1) maternal antibody protection; 2) a prerequisite for immunosuppression (e.g. pregnancy); and/or 3) prior or concurrent infection with a yet undetermined organism. This discovery indicates it may be possible to suppress or eliminate SASd from flocks by separating lambs from their dams and other remaining adults prior to colonization and infection during their first year of life. However, the feasibility of eradication utilizing this method is purely theoretical and additional investigation is needed.[6]

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