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Conference Moderator:
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CASE I: A07/10654 (AFIP 3065885).

Signalment: 8-week-old, male, Holstein calf (*Bos taurus*).

History: Fifty dairy calves were purchased as neonates at a sale barn in Ohio, weaned at 5 weeks of age and in the feedlot for 3 weeks. All calves in the group reportedly developed diarrhea at 4 weeks of age with wasting, weakness and 40% mortality. One live calf was submitted for diagnostic studies.

Gross Pathology: The calf was thin (48 kg body weight) and dehydrated; the perineum was stained with liquid brown feces. The wall of the small intestine, especially the ileum, was thickened with a dark pink serosal surface. The ileal mucosa was coated by a yellow fibrinous membrane. The cecum and colon contained abundant yellow-green malodorous fluid. The wall of the gallbladder was thickened by edema; the lumen was distended by a fibrin clot. The mesenteric lymph nodes were enlarged, pale pink, and wet.

Laboratory Results: *Salmonella enteritidis* serotype Dublin was isolated from gallbladder, liver, spleen, small intestine and mesenteric lymph node. Fluorescent antibody tests were negative for bovine viral diarrhea virus, rotavirus, and coronavirus. Virus isolation was also negative.

Histopathologic Description: Gallbladder: The mucosa is expanded by infiltration of lymphocytes, macrophages, plasma cells and fewer neutrophils mixed with fibrin. Some capillaries and venules contain fibrin thrombi or are dilated and filled with neutrophils. Leukocytes also infiltrate the wall of some venules. The mucosal epithelium is multifocally attenuated or lost and has a few clusters of coccobacilli. A pleocellular (mostly lymphohistiocytic) inflammatory infiltrate dissects the tunica muscularis and infiltrates the edematous adventitia.

Liver: Periportal inflammation consists of lymphocytes and macrophages with rare neutrophils. This infiltrate is particularly intense around bile ductules, some of which have attenuated epithelium and lumina distended by bile. Random foci of lytic hepatocellular necrosis are infiltrated by histiocytes, lymphocytes, and few neutrophils. Vascular lesions are similar to those in the gallbladder. There are increased numbers of sinusoidal leukocytes and hypertrophy of Kupffer cells.

Small intestine: A thick layer of fibrin, neutrophils, degenerated leukocytes, and red blood cells with abundant Gram-negative bacterial coccobacilli and Gram-positive robust bacilli covers the focally denuded mucosa. Villi are blunted; goblet cells are scarce. The lamina propria is congested with numerous lymphocytes and histiocytes. Dilated crypts contain neutrophils and cellular debris. Lymphoid follicles of Peyer's patches are depleted of lymphocytes with replacement by reticulohistiocytic cells. Vascular lesions are similar to those in the gallbladder.

Contributor's Morphologic Diagnosis: 1. Small intestine: Fibrinosuppurative and necrotizing enteritis.
2. Gallbladder: Ulcerative and fibrinous cholecystitis.
3. Liver: Lymphohistiocytic cholangiohepatitis and necrotizing hepatitis.

Contributor's Comment: Gross and histologic lesions in the small intestine, liver, and gallbladder are typical of those of enteric and septicemic salmonellosis. *Salmonella* Dublin and *S.* Typhimurium are the most common serovars associated with salmonellosis in calves.(1,2) In this case, *S.* Dublin was isolated from the small intestine,

mesenteric lymph node, gallbladder, liver, and spleen. *S*. Dublin is considered a bovine-specific serovar and seldom infects other species of animals.(2) The Dublin serovar is also more commonly associated with septicemia than is *S*. Typhimurium due to the more common presence of plasmid-encoded *spv* genes that enhance intracellular proliferation in the former.(2) Disease usually develops in calves between 6 and 12 weeks of age and affects dairy calves much more frequently than beef calves, especially those that are purchased from sale barns.(2)

Calves with diarrhea and carrier cattle, including adult cattle, are the source for the fecal-oral route of infection.(2) *Salmonella* bacteria first colonize the distal small intestine, where pili facilitate attachment to the surface of enterocytes. The bacteria are taken up by enterocytes, macrophages and neutrophils. Production of enterotoxin results in the secretory diarrhea of enteric salmonellosis with loss of fluid and electrolytes; endotoxin production is important in septicemic salmonellosis. Some calves develop both syndromes (diarrhea and septicemia).(2)

The presence of gross lesions at necropsy examination helps to distinguish salmonellosis from enteric colibacillosis. Enteritis with yellow fibrinous exudate covering the mucosa is usually most severe in the ileum.(1) Mesenteric lymph nodes are enlarged and edematous.(1,2) The gallbladder is distended with an edematous wall and fibrinous mucosal inflammation.(2) Gray-white foci of hepatic necrosis may be observed macroscopically,(2) but were not obvious in this calf. These 'paratyphoid nodules' are easier to detect histologically as foci of lytic necrosis with infiltration by a few macrophages.(1,2) Lymphoid depletion is obvious in the mesenteric nodes and intestine; fibrin thrombi in capillaries and venules with phlebitis may be observed in the intestine, liver, gallbladder or other tissues. (1,2)

AFIP Diagnosis: 1. Small intestine: Enteritis, fibrinonecrotic, subacute, diffuse, severe, with erosions, diphtheritic membrane, and edema.
2. Gallbladder: Cholecystitis, transmural, fibrinonecrotic, diffuse, severe, with edema.
3. Liver: Cholangiohepatitis, lymphohistiocytic and neutrophilic, diffuse, mild to moderate, with multifocal, random lytic hepatic necrosis (paratyphoid nodules).

Conference Comment: The conference moderator and participants were impressed by the strikingly similar transmural necrosis and inflammation in both the small intestine and gallbladder. Most slides lacked the fibrin thrombi in the intestinal submucosa that are typically found in cases of salmonellosis. Characteristic gross and histologic lesions of salmonellosis include a fibrinonecrotic (diphtheritic) membrane in the small intestine; the combination of hepatic and intestinal lesions; a characteristic odor of blood within intestinal contents; and paratyphoid nodules composed of mononuclear cells surrounding and infiltrating areas of coagulative necrosis with variable numbers of neutrophils.(1) Conference participants discussed the differential diagnosis for diphtheritic membranes in different species, which in the horse includes salmonellosis, colitis X (clostridial infection) and right dorsal colitis. The moderator noted that in the ox, diphtheritic membranes are highly suggestive of salmonellosis.

There are now two recognized species of *Salmonella*: *S. enterica* and *S. bongori*. *S. enterica* has six subspecies: *enterica*, *salame*, *arizonae*, *diarizonae*, *indica* and *houtenae*. *S. enterica enterica* causes a majority of the cases of salmonellosis in humans and domestic animals, and contains approximately 60% of the more than 2,200 distinct serovars and serotypes of *Salmonella* that have been identified. Besides *S.* Dublin, other important serovars include *S*. Enteritidis, *S*. Pullorum-Gallinarum, *S*. Arizonae., *S*. Choleraesuis, *S*. Typhisuis, *S*. Typhi, and *S*. Typhimurium (especially the multi-drug resistant definitive phage type 104 (DT104)). *S. bongori* are primarily recognized as tropical strains.(1)

An informative review by Santos et al(3) details the fascinating mechanisms by which the non-typhoidal *Salmonella* (NTS) serotypes adapt and thrive in the milieu of the inflamed intestine. Briefly, two key abilities enable *S.* Typhimurium to incite inflammation: 1) penetration of intestinal epithelium, and 2) survival within macrophages. These traits are conferred by two type III secretion systems (T3SS): T3SS-1, the invasion-associated T3SS encoded by *Salmonella* pathogenicity island (SPI) 1; and T3SS-2, responsible for survival in macrophages, encoded by SPI2. Mucosal barrier functions against NTS dissemination are activated via the interleukin (IL)-18/interferon-γ and IL-23/IL-17 axes, but the pathogen is adapted to survive the antimicrobial defenses of the inflamed intestininal lumen, allowing perpetuation of fecal/oral transmission.(3)

In general, three mechanisms account for the diarrhea. The first is malabsorption due to villous atrophy; there is reduced surface area for digestion and uptake of nutrients which then remain in the intestinal lumen exerting an osmotic effect which draws water. Second is secretory diarrhea due to an imbalance that results in a net excess of secretion over absorption; this is most often due to diarrheagenic enterotoxins of pathogenic bacteria, two classic

examples of which are *Vibrio cholera* and *Escherichia coli*. The enterotoxins typically alter normal cellular secretory and absorptive functions of enterocytes. The final mechanism is effusive diarrhea, wherein there are changes in Starling forces or increased vascular permeability which allow fluid and solutes to move in a retrograde manner from the lateral intercellular space to the lumen. Interestingly, salmonellosis causes diarrhea via all three of these mechanisms. The secretory component is due to effector proteins, such as those encoded by SPI1 and SPI5, which block chloride channel closure or promote hypersecretion of chloride by enterocytes, respectively. Malabsorption is due to reduction of mucosal surface area and enterocyte function. Effusion of inflammatory exudates is the final component of this process.(1,2)

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CASE II: 08-91 (AFIP 3133650).

Signalment: 148-day-old, female, Finn x Dorset lamb (*Ovis aries*).

History: This lamb was used in a medical device study.

Gross Pathology: The left lung (135 g) was pink and spongy with mild patchy atelectasis. The right lung (180 g) was pink and spongy, except for the right cranial lobe. Greater than 80% of the cranioventral right cranial lobe was pale tan-grey and firm (consolidation). On cut surface there were prominent lymphoid nodules with several mucus casts present in bronchioles.

Laboratory Results: Routine aerobic cultures from the right cranial lung lobe produced no growth. Serum obtained at necropsy was sent to the Washington Animal Disease Diagnostic Laboratory (WADDL). This animal had a serologic titer of >1:2560 to *Mycoplasma ovipneumoniae*. Immunostaining for *Mycoplasma* performed at the Michigan State University Diagnostic Center for Population and Animal Health was positive.

Histopathologic Description: In submitted sections of lung there is extensive consolidation of greater than 65% of the pulmonary parenchyma with prominent chronic "cuffing" bronchial/bronchiolar inflammation. Many bronchi/bronchioles are ectatically dilated and filled with a mixture of eosinophilic proteinaceous material, wispy basophilic mucus, and variable numbers of viable and degenerate neutrophils and sloughed epithelial cells (casts). Discrete foci of mineralization are present in the exudate in some sections. The respiratory epithelium is crowded and hyperplastic. The bronchial/bronchiolar submucosa is infiltrated and expanded by dense cuffs of moderate to large numbers of lymphocytes with frequent macrophages and fewer plasma cells, eosinophils, and neutrophils. Inflammatory cells are present within the respiratory epithelium and extend into the adjacent pulmonary interstitium and parenchyma. Lymphocytes frequently form discrete nodular aggregates, often with pale germinal centers containing large lymphoblasts and rare tingible body macrophages. Alveolar spaces are diffusely filled with variable but often large numbers of neutrophils and macrophages, the latter often containing abundant foamy cytoplasm. There are rare foreign body type multinucleate cells, with occasional lymphocytes and plasma cells. Scattered alveoli are lined by plump cuboidal epithelium (type II pneumocyte hyperplasia). There are occasional foci of squamous metaplasia.

Contributor's Morphologic Diagnosis: Lung, bronchitis and bronchiolitis, lymphocytic (cuffing) and proliferative, multifocal, chronic, severe, with bronchiectasis and chronic suppurative bronchopneumonia.

Contributor's Comment: Observed gross and histologic lesions are consistent with those previously reported for *Mycoplasma ovipneumoniae*.(1,11) One commonly reported histologic feature of this disease, the nodular "hyaline scar," was not observed in this case. These hyaline scars consist of polypoid masses of fibrous connective tissue protruding into the airway lumen, similar to bronchiolitis obliterans.(5)

Mycoplasma ovipneumoniae is considered the causative agent of "atypical" or "chronic, non-progressive pneumonia," a mild or subclinical respiratory disease of lambs in the first year of life.(6) Mixing of animals, high stocking density, and poor air quality are considered to be important risk factors. Concurrent or secondary infection with *Mannheimia haemolytica* is frequently reported and contributes to suppurative inflammation.(6,11)

Experimentally, *M. ovipneumoniae* adheres to luminal ciliated epithelial cells and induces ciliostasis.(4,8) In one study, infection with *M. ovipneumoniae* was correlated with the development of autoantibodies to the cilia.(7) Additionally, *M. ovipneumoniae* has been shown to inhibit alveolar macrophage phagocytosis *in vitro*.(9) In this case, the presence of inspissated mucus casts, bronchiectasis, and mixed suppurative and histiocytic inflammation in the alveoli are strongly suggestive of impaired mucociliary clearance. Colonization of the ciliated epithelium with ciliostasis and deciliation are features of other *Mycoplasma* pneumonias.(2,5) Other virulence factors include alteration of host prostaglandin synthesis, membrane alterations, induction of apoptosis in lymphocytes and other cells, as well as the presence of superantigens in the mycoplasmal plasma membrane. This latter feature is thought to give rise to the typical "lymphocytic cuffing" that is a feature of many pulmonary mycoplasmoses.(2)

Mycoplasma ovipneumoniae has been implicated as a causative agent of enzootic and epizootic pneumonia in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*).(1) The gross and histologic lung lesions induced by *M. ovipneumoniae* are similar to that of *M. pulmonis* (and cilia-associated respiratory bacillus) in laboratory rats (and mice)(10) and *M. hyopneumoniae* in pigs,(2,5) but differ markedly from the typical appearance of *Mycoplasma bovis* pneumonia in cattle.(2,3)

AFIP Diagnosis: Lung: Bronchopneumonia, suppurative, chronic-active, diffuse, severe, with peribronchiolar nodular lymphofollicular hyperplasia, bronchiolar epithelial and type II pneumocyte hyperplasia and hypertrophy, bronchiectasis, and interlobular edema.

Conference Comment: The most striking microscopic feature of the submitted section of lung tissue is the formation of lymphoid nodules with germinal centers, which in combination with the cuffing lymphoid infiltrates and bronchiolar epithelial hyperplasia and hypertrophy, is characteristic of mycoplasmal pneumonia. Suppurative bronchopneumonia, prominent in several examined slides, is not, however, typical of mycoplasmal pneumonia, suggesting a secondary infection. In addition to *Mannheimia haemolytica* (formerly *Pasteurella haemolytica* biotype A, multiple serotypes excluding 11), common secondary infections in ovine pulmonary mycoplasmosis include *Bibersteinia trehalosi* (*Pasteurella trehalosi*, formerly *P. haemolytica* biotype T) and *Mannheimia glucosida* (formerly *P. haemolytica* biotype A, serotype 11).(5) Tissue Gram stains did not demonstrate bacteria in submitted serial unstained slides.

Conference participants reviewed and discussed the three primary respiratory tract defense mechanisms: 1) mucus and the mucociliary escalator system; 2) antibody and innate defense proteins; and 3) alveolar macrophages and recruited neutrophils. Hairs and air turbulence in the nasal passages and sinuses prevent particles larger than 10 μm in size from advancing further in the respiratory tract. Particles entrapped in mucus are cleared via the mucociliary escalator and/or coughing. The mucociliary escalator ends at the level of the terminal bronchioles, making this anatomic area in the respiratory tree the most susceptible to establishment of infection. The airway mucosa contains many non-specific antimicrobial factors, including lactoferrin, B-defensins, collectins, lysozyme, the complement membrane attack complex and lactoperoxidase, among others. Opsonizing agents in the respiratory tract include IgG, C3b from the complement system and surfactant proteins A and D. Primary defense and clearing of debris in the alveolus is accomplished by alveolar macrophages; T lymphocytes and natural killer cells assist in sampling alveolar material.(2) Conference participants discussed the importance of impaired mucociliary clearance and ciliostasis in the pathogenesis of myocoplasmal pneumonia, and noted additional causes of ciliostasis, including cilia-associated respiratory bacillus (CAR bacillus) of rodents, *Bordetella bronchiseptica*, alcohol, smoking, and primary ciliary dyskinesia.

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CASE III: NCAH 2009-2 (AFIP 3134595).

Signalment: 7-week-old, specific pathogen free, White Leghorn chickens, (*Gallus gallus domesticus*).

History: The chickens were one component of an infectious bursal disease research project that included one group which had received topical ocular inoculations of infectious bursal disease virus (variant strain E) and another group of non-challenged control chickens. Four days post inoculation both groups of birds were euthanized. The challenged and control chickens were clinically normal throughout the duration of the study. Each slide contains tissues from one challenged animal and one age-matched control.

Gross Pathology: Subjectively, the bursa appeared approximately 10% smaller in the challenged group than the control group**.**

Histopathologic Description: Bursa of Fabricius (cloacal bursa): In the affected tissue there is marked atrophy of bursal follicles due to loss of lymphocytes in the medulla and cortex. Numerous large, foamy, macrophages containing phagocytosed cellular debris are within the collapsed follicles. The endodermal epithelium separating the medulla from the cortex is prominent due to collapse of the follicles and mild epithelial hypertrophy and hyperplasia. The interfollicular epithelium covering the surface of the bursa is also mildly hyperplastic. Diffusely, the follicular cortex and interfollicular interstitium contain increased clear space (edema), congested vessels, and multifocal areas of mild hemorrhage. Multifocally, the lamina propria, muscularis, and serosa are expanded by edema with small numbers of lymphocytes, plasma cells, and macrophages.

Contributor's Morphologic Diagnosis: Bursa of Fabricius (cloacal bursa): Lymphoid depletion, diffuse, severe, subacute, White Leghorn *(Gallus gallus domesticus),* avian.

Contributor's Comment: Infectious bursal disease, or Gumboro disease, is an acute, highly contagious, immunosuppressive disease caused by serotype 1 infectious bursal disease virus (IBDV), a double-stranded RNA virus in the family *Birnaviridae* and genus *Avibirnavirus*.(2) Chickens are the only animals known to develop

clinical disease and distinct lesions when exposed to IBDV, although other species may replicate the virus and develop neutralizing antibodies. Virtually all flocks in major poultry producing areas worldwide are exposed to the virus through natural infection or vaccination. Eliminating the virus from infected farms can be difficult, despite routine cleaning and disinfection, because the virus has been shown to survive in the environment for several months and it is relatively resistant to heat and certain disinfectants. Clinical signs of disease appear within 2-3 days after exposure and include soiled vent feathers, diarrhea, dehydration, anorexia, depression, ruffled feathers, trembling, prostration, and death. Clinical disease, although uncommon in the United States, is primarily seen in chickens 3-6 weeks old. Infection in chickens younger than 3 weeks of age is typically subclinical, but results in prolonged immunosuppression that can cause adverse economic impact to the producer due to secondary infections and poor response to routine flock vaccinations. Infected birds older than 6 weeks of age seroconvert but usually do not develop clinical signs.(2)

There are two serotypes: type 1 and type 2. The type 1 serotype is further divided into the standard (or classic) strain and variant strains. In Europe, Asia, Africa, and South America highly pathogenic serotype 1 strains have been identified and are termed very virulent infectious bursal disease (vvIBD).(2) Experimental inoculation elicits different responses depending on the strain of virus. Both the very virulent and classic strains induce typical clinical signs and gross lesions with mortality rates of 10-50% for classical strains and up to 50-100% during infections with vvIBDV.(2) Variant strains such as the type E strain used to infect this chicken typically cause bursal lesions to develop but rarely induce acute clinical signs or mortality.(5) In general, serotype 2 virus is not pathogenic and does not protect against challenge with serotype 1 viruses.(2)

Grossly the bursa initially increases in size due to edema and hyperemia, but then begins to atrophy due to lymphoid depletion and eventually decreases in size to one-third of its original weight.(1) The bursa may have necrotic foci with mild to extensive hemorrhage and the spleen may be mildly enlarged with uniform small gray foci on the surface.(2) Hemorrhages of the thigh and pectoral muscles are frequently present and severely dehydrated chickens commonly develop non-specific renal lesions.(2)

Microscopically the cloacal bursa is the most severely affected organ, but other lymphatic tissues, such as the spleen, thymus, and Harderian gland, have similar but milder lesions than the bursa.(3) Initially there is degeneration and necrosis of lymphocytes in the bursal medulla as early as 36 hours after infection.(1) As the disease progresses, heterophils, macrophages, and eventually fibroblasts accumulate in the follicular and interfollicular areas. In the spleen there may be hyperplasia of splenic reticuloendothelial cells along with lymphoid necrosis and the Harderian gland can have areas of necrosis.(1,3)

Multiple virus and host variables, including strain of virus, age and breed of chicken, and maternal immunity and vaccine status affect the development of infection/disease.(4) The pathogenesis of oral infection results in an initial viremia within five hours and a second massive viremia may occur following infection of the bursa.(2) The virus targets immature dividing B lymphocytes resulting in necrosis and apoptosis of B cells with activation of macrophages, T cells, and NK cells.(2,4) Recovery from complete immunosuppression can occur if large follicles capable of replenishing the B cell population are reconstituted from endogenous stem cells that survived the initial infection.(2,9) The long-term effect of IBD is frequently immunosuppression due to decreased humoral immunity, and to a much lesser degree, cellular immunity resulting in increased susceptibility to disease and poor immune response to vaccination.(7)

AFIP Diagnosis: Bursa of Fabricius (cloacal bursa): Lymphoid necrosis and depletion, diffuse, marked, with stromal collapse and interstitial edema.

Conference Comment: Compared to tissue from the aged-matched control, there is increased prominence of reticular cells and macrophages in the section of bursa from the infected animal. One of the focal points of discussion among conference participants was the distinction between true inflammation versus increased prominence of resident reticular cells and monocytes due to follicular collapse. Additionally, some participants' slides contained few discrete foci of heterophilic inflammation.

Based on the histomorphologic findings of lymphoid necrosis and depletion, conference participants were evenly divided between infectious bursal disease virus (IBDV) and chicken anemia virus (CAV) (*Circoviridae, Gyrovirus*) as the underlying etiology. Chicken anemia virus infects hemocytoblasts in the bone marrow and lymphoblasts in the cortex of the thymus, resulting in anemia with a hematocrit of 6-27% and pancytopenia. Gross lesions of CAV include thymic atrophy; bone marrow atrophy; hemorrhage in the proventricular mucosa, subcutaneous tissue, and

skeletal muscle; and, less commonly, bursal atrophy. Panmyelophthisis and generalized lymphoid atrophy are commonly observed in chickens infected with CAV, with the thymus exhibiting the most severe lesions. When present, bursal lesions caused by CAV are less severe than those induced by IBDV, and include lymphofollicular atropy, few areas of necrosis, and infolding of the epithelium with hydropic degeneration and proliferation of reticular cells. In contrast, infection with IBDV frequently results in marked to severe lymphofollicular bursal atrophy, hemorrhage, edema, heterophilic inflammation, and hyperplasia of the bursal epithelium, sometimes resulting in a pseudoglandular appearance.(2,6)

The contributor discusses immunosuppression and decreased humoral immunity caused by IBDV infection, which renders infected chicks more susceptible to disease. Immunosuppression is most severe in chicks exposed to the virus within 2-3 weeks of hatch, and manifests clinically as impaired protective response to vaccination, increased incidence of secondary infections, poor feed conversion, and increased rates of carcass condemnation at processing. Interestingly, although T lymphocytes are resistant to infection with IBDV, the virus does cause thymic atrophy, thymocyte apoptosis, and impaired cell-mediated immunity, in addition to the expected impairment of humoral immunity that results from depletion of IgM^+ B lymphocytes in the cloacal bursa, spleen, and cecal tonsils. Infection with IBDV is also thought to impair innate immunity, compromising the phagocytic activity of macrophages.(7) The Harderian gland is a component of the local immune system of the upper respiratory tract, and its function is also impaired by IBDV infection.(2)

The United States was considered free of the very velogenic (vvIBD) form of the disease; however, an outbreak of vvIBD was recently reported in 2009.(8) The source of the most recent disease outbreak has not been determined. Field veterinarians and diagnosticians should be aware of this report and consider vvIBD in the differential diagnosis when clinical signs, epidemiologic evidence, and diagnostic data indicate it as a potential etiology.

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CASE IV: 09-07233 (AFIP 3136516).

Signalment: 5-month-old, female Boer goat (*Capra hircus*).

History: This goat has a 2 week history of lethargy, anorexia, and loss of body condition.

Gross Pathology: The dependent subcutaneous tissues were wet and gelatinous. The thoracic cavity contained 600 mL of dark yellow, translucent fluid and multiple aggregates of translucent, straw colored gel. The pericardial sac was thickened and filled with multiple pockets up to 2 cm in diameter containing thick, yellow, creamy material and surrounded by a thick white capsule. Multifocally, the lungs were tightly adhered to the thoracic wall. The abdominal cavity was filled with approximately 500 mL of dark yellow translucent fluid and gel similar to that described in the thorax.

Laboratory Results: Bacterial culture yielded a heavy growth of *Corynebacterium pseudotuberculosis*.

Histopathologic Description: The pericardium is markedly thickened and contains multifocal to coalescing abscesses characterized by a central core of necrotic cellular debris, eosinophilic proteinaceous material, neutrophils, and karyolytic and karyorrhectic cells, and dark basophilic material (mineralization). The abscesses contain many randomly scattered macrophages filled with short rod shaped, Gram-positive bacteria both intracellularly within the cytoplasm and free in the surrounding debris. The foci are surrounded by lymphocytes, plasma cells, and macrophages and are walled off with a dense band of fibrous connective tissue. The fibrous tissue has fibroblasts with closely spaced, plump, vesiculated nuclei (active fibroplasia). The epicardium is expanded with moderate amounts of edema and fibrin admixed with lymphocytes and plasma cells. The underlying myocardium contains a few randomly scattered fibers that are pale, discontinuous, and have a loss of striation pattern.

Contributor's Morphologic Diagnosis: Pericarditis, fibrosing, with multifocal abscesses and intralesional bacteria.

Contributor's Comment: A heavy growth of *Corynebacterium pseudotuberculosis* was isolated from the pericardial abscesses. *C. pseudotuberculosis* serotype I is the etiologic agent of caseous lymphadenitis (CLA) in sheep and goats. Serotype II is often isolated from buffalo and cattle. A phospholipase D exotoxin is considered to be the primary virulence factor and facilitates spread of the infection from the primary site and results in intravascular hemolysis, necrosis, pulmonary edema, and shock. Inactivation of the *pld* gene encoding phospholipase D results in bacteria incapable of producing CLA.

C. pseudotuberculosis often enters the body through skin or oral wounds. The draining lymph node is then infected and subsequently the organism can spread to internal organs, typically the lungs. Treatment of animals with *C. pseudotuberculosis* often results in limited success due to the intracellular nature of the organism and the formation of abscesses leading to poor penetration of the drug. Typically, once the bacterium enters the lymph nodes, the infection is considered persistent except for the rare occasion when the node ruptures and drains through the skin to the outside of the body. A primary finding of pericarditis without lymphadenitis, as in this case, is unusual.

AFIP Diagnosis: Heart, pericardium: Pericarditis, caseonecrotic and fibrosing, diffuse, severe, chronic, with mineralization and many intrahistiocytic and extracellular small bacilli.

Conference Comment: As mentioned by the contributor, this case is unusual in that it features primary pericarditis in the absence of the lymphadenitis that typifies CLA; in fact, although participants readily identified the pathologic process in this case and considered *C. pseudotuberculosis* to be a likely etiology, many misidentified the affected tissue as lymph node.

Conference participants briefly reviewed bacteria able to survive in macrophages, including *Rhodococcus* spp., *Salmonella* spp., *Mycobacteria* spp., *Brucella* spp. and *Listeria* spp. Other less commonly encountered microbial agents in macrophages include rickettsial organisms, *Shigella* spp., *Trypanosoma* spp., *Coxiella* spp., chlamydial organisms, *Toxoplasma* spp., and *Legionella* spp.

The contributor provides a succinct synopsis of the pathogenesis of CLA in sheep; readers are referred to a comprehensive review by Baird and Fontaine(1) for more details. In addition to phospholipase D, *C. pseudotuberculosis* produces a waxy mycolic acid coat with well-described cytotoxic properties, which both contributes substantially to the organism's pathogenicity and enhances its ability to survive for extended periods in the environment. This latter attribute is common to the actinomycete family, of which the genera *Corynebacterium*, *Mycobacterium*, *Rhodococcus*, and *Nocardia* are members.(1)

In sheep, *C. pseudotuberculosis* has been hailed the "perfect parasite" because of its ability to evade the immune system, establish a chronic infection that often persists for the life of the host, and readily infect flock mates, all while only rarely producing fatal infections. Therefore, in regions where the disease is endemic, CLA may be of

considerable economic importance. Visceral lesions are far less common than superficial lymphadenitis in both sheep and goats, and they occur even less frequently in goats as compared to sheep. While abscesses in sheep due to CLA are most often found in the superficial lymph nodes of the torso, in goats the lesions manifest as lymphadenitis of the superficial nodes of the head and neck.(1)

In horses, *C. pseudotuberculosis* causes three distinct disease syndromes: 1) ulcerative lymphangitis; 2) contagious folliculitis and furunculosis, which is also known as equine contagious acne, Canadian horsepox, and equine contagious pustular dermatitis; and 3) deep-seated subcutaneous abscessation, also termed pigeon fever, Wyoming strangles, and false strangles. In farmed llamas and alpacas in South America, *C. pseudotuberculosis* is a commonly identified cause of purulent lymphadenopathy. In dairy cattle in Isreal, *C. pseudotuberculosis* has been implicated in several syndromes, including deep subcutaneous abscesses and mastitis. Sporadic and experimental infections have been reported in a variety of species.(1)

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