The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CASE I – 475380 (AFIP 2992505)

Signalment: Two-month-old, male, Asaf lamb.

History: The lamb was submitted for postmortem examination with a history of mild fever (40.8°C), mild dyspnea, and coughing. Several other lambs in the herd showed similar clinical signs and were treated with antibiotics.

Gross Pathology: On postmortem examination mucopurulent discharge around the nostrils with multifocal erosions on the oral mucosa, lips, tongue, and hard and soft palate were seen. Small amounts of blood-tinged contents were found in the gastrointestinal tract, especially in the small intestine. In the respiratory tract, multifocal hemorrhages and ulcerations on the laryngeal mucosa with flakes of mucopurulent discharge were present. The lungs were partially consolidated, especially the anteroventral lung lobes.

Laboratory Results: Peste des petits ruminants was diagnosed by immunofluorescence, PCR, Agar Gel Precipitation Test (AGPT) and IHC.

Histopathologic Description: Small intestine, the mucosa is heavily infiltrated by neutrophils, histiocytes and lymphocytes. Many crypts are filled with cell debris and neutrophils. The villi are shortened and blunted and Peyer's patches consistently show areas of lymphoid depletion. Typical syncytial cells and occasionally intranuclear and intracytoplasmic eosinophilic inclusions in the epithelial cells or in syncytial cells are seen. Multifocal coccidian oocysts in the epithelial cells with no significant infiltrate are also present.

Contributor's Morphologic Diagnosis: Small intestine: necrotizing enteritis, acute, diffuse, severe, with crypt abscesses and loss, villous atrophy and fusion, lymphoid depletion, syncytial cells and intranuclear & intracytoplasmic eosinophilic inclusions, "Asaf" lamb, etiology consistent with peste des petits ruminants virus (PPR). Coccidian oocysts were also seen, consistent with concomitant ovine coccidiosis

Contributor's Comment: The histological changes in the lungs (not submitted) were broncho-interstitial, necrotizing, subacute, diffuse, severe, pneumonia with type II pneumocyte and bronchiolar epithelial hyperplasia, syncytial cells, and eosinophilic intranuclear and intracytoplasmic inclusion bodies.

Peste des petits ruminants (PPR) is a contagious viral disease of sheep and goats. PPR is similar clinically and pathologically to rinderpest in cattle and frequently manifests as diarrhea, stomatitis, oculonasal discharge, and pneumonia. The causative pathogen is a morbillivirus of the family Paramyxoviridae. In natural infections, the virus causes disease in goats and sheep, but not cattle or swine. Goats are considered to be more susceptible than sheep. 1,2,3 Morbilliviruses are important pathogens of humans and animals in addition to PPR, classic members of this genus cause rinderpest in cattle, distemper in dogs, and measles in humans. Phocine and cetacean morbilliviruses have been described recently. 2,3,4

PPR is an economically important disease that was first reported in the Ivory Coast of Africa in 1942, and has since spread east to parts of Asia, including India and Pakistan. The African and Asian strains seem to be antigenically distinct. There is current concern that the virus may pose a serious threat to endangered wild goats and sheep in the Himalayas through contact with infected domestic sheep and goats. Transmission occurs by inhalation of aerosols from closely associated animals, by direct contact through licking and nuzzling, and occasionally through fomites such as water troughs and feed bunks recently used by infected animals. 5

Pathological changes associated with PPR include erosive stomatitis and enterocolitis, similar to that of rinderpest in cattle, and proliferative and necrotizing broncho-interstitial pneumonia. Lymphoid depletion or necrosis occurs in the spleen, Peyer's patches, and lymph nodes. In fatal cases, pneumonia may not be as severe in younger goats (less than 4 months) than in older animals (over 6-7 months), probably because young lambs succumb due to dehydration caused by diarrhea before pulmonary lesions can fully develop. 3,5

Coccidiosis is a contagious disease, especially in young kids and lambs, with a world wide distribution. The disease is caused by one or more of approximately 12 different species of protozoa, *Eimeria*, which parasitize cells lining the intestinal tract. An infected animal sheds thousands of microscopic coccidial oocysts in its

feces daily. Transmission of coccidiosis to other kids or lambs occurs when infected animals shed the organisms in feces, resulting in contaminated feed or water. When first passed, the oocysts are harmless to other goats. However, under favorable conditions of warmth and moisture, each oocyst matures (sporulates) in 1 to 3 days to form 8 infective sporozoites. If a young kid swallows the sporulated oocyst, the sporozoites are released and rapidly penetrate the intestinal cells. From here, the coccidian passes through several periods of multiplication during which large schizonts are formed. The intestinal cell of the goat is destroyed and thousands of small forms called merozoites break out and invade other intestinal cells. Eventually, sexual stages are reached and new oocysts are produced. The entire life cycle from oocyst to new oocyst takes 2-3 weeks.

In the case of a young kid suddenly exposed to many sporulated oocysts, it may become severely ill in1-2 weeks. Young kids may die rapidly due to a severe attack of coccidiosis. While others, those stronger or less heavily infected, will develop a chronic disease characterized by intermittent diarrhea and poor growth.

AFIP Diagnoses: 1. Small intestine: Enteritis, necrotizing, acute, diffuse, moderate, with crypt abscesses, villous blunting and fusion, syncytia, and intracytoplasmic and intranuclear eosinophilic inclusion bodies, etiology consistent with peste des petits ruminants virus, Asaf, ovine.
2. Small intestine: Intraepithelial coccidia, multifocal, numerous, etiology consistent with *Eimeria* sp.

Conference Comment: The contributor provides an excellent review of Peste des petits ruminants (PPR) and *Eimeria* sp. infection in sheep. Attendees were able to diagnose the disease based on the presence of enteric syncytial cells which occasionally contain characteristic eosinophilic intracytoplasmic and intranuclear inclusion bodies. While several viruses cause the formation of syncytial cells and either intracytoplasmic or intranuclear inclusion bodies, only morbilliviruses cause the formation of syncytial cells and both eosinophilic intracytoplasmic and intranuclear inclusion bodies. The paucity of lymphoid cells and follicles supports the diagnosis as morbillivirus infection commonly results in profound lymphoid depletion. All agreed that the *Eimeria* sp. probably contributed very little, if any, to the necrosis within this section of small intestine.

As mentioned in the contributor's comments, PPR is caused by a morbillivirus, family Paramyxoviridae. Paramyxoviruses are single-stranded RNA viruses which are helical in shape and range from 100-300 nm in diameter. Below is a simplified taxonomic chart adapted from the International Committee on Taxonomy of Viruses

(ICTV) outlining the most important Paramyxoviridae and the diseases they cause in animals:

Family: Paramyxoviridae
- Subfamily: Paramyxoviridae
  - Genus: Respirovirus
    - Species: Bovine Parainfluenza Type 3 virus (PI3)
      - Sendai virus (mice)
      - Simian virus 10
  - Genus: Rubulavirus
    - Species: Porcine rubulavirus
      - Simian parainfluenza type 5 & 41
      - Mumps (human)
  - Genus: Morbillivirus
    - Species: Canine distemper virus
      - Rinderpest virus (mostly cattle)
      - Peste-des-petits-ruminants virus (mostly sheep)
      - Cetacean Morbillivirus (dolphins, porpoises, whales)
      - Phocine distemper virus (seals, sea lions)
      - Measles virus (primates)
  - Genus: Henipavirus (Note: There is a lot of new information concerning these two diseases as both have been transmitted to humans.)
    - Species: Hendra virus (affects mostly horses)
      - Nipah virus (affects mostly pigs)
  - Genus: Avulavirus
    - Species: Newcastle disease virus (END, poultry)
- Subfamily: Pneumovirinae
  - Genus: Pneumovirus
    - Species: Bovine respiratory syncytial virus (BRSV)
      - Murine pneumonia virus
  - Genus: Metapneumovirus
    - Species: Turkey rhinotracheitis virus

For additional information concerning morbilliviruses please see Wednesday Slide Conference 25, Case 4, 2004-2005, Canine Distemper Virus in a ferret.

The life cycle of *Eimeria* and *Isospora* sp. is both host-specific and direct. Unsporulated oocysts are shed in the feces and sporulate in the environment to become infectious. Following ingestion, sporozoites excyst, invade intestinal epithelial cells, form trophozoites and undergo asexual multiplication (schizogony, merogony) within a schizont or meront. Merozoites are released and eventually form sexual stages (micro- and macrogametes), which unite to form oocysts (9).

Some common coccidia species of domestic and wild mammals and birds include the following (8,9,10):

Animal	Coccidia	Organ affected
Cattle	*E. bovis*	1st gen schizont – Jejunum 2nd gen schizont – Cecum and colon
Sheep	*E. ahsata* *E. bakuensis* *E. ovinoidalis*	Small intestine Small intestine Ileum/Large intestine
Goats	*E. christenseni* *E. arloingi* *E. ninakohlyakimovae*	Small intestine Small intestine Large intestine
Equine	*E. leuckarti*	Small intestine
Swine	*I. suis*	Small intestine
Canine	*I. canis*	Ileum, colon occasionally
Feline	*I. felis*	Small intestine, colon occasionally
Mice	*E. falciformis*	Colon
Rabbit	*E. stiedae* *E. intestinalis* *E. flavescens*	Bile ducts Ileum & cecum Ileum & cecum
Birds Chickens Turkey Geese & ducks 	 *E. acervulina* *E. necatrix* *E. maxima* *E. tenella* *E. adenoeides* *E. meleagrimitis* *E. gallopavonis* *E. truncata* *E. anseris*	 Duodenum Mid-intestine Mid-intestine Ceca Ceca Mid-intestine Colon, rectum Kidney Mid-intestine

For additional information concerning *Eimeria* sp. parasites please reference Wednesday Slide Conference 19, Case 3, 2004-2005, *Eimeria* sp. in a calf.

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CASE II – 050309 (AFIP 2983861)

Signalment: 3-year-old, female, Boer cross, goat, caprine.

History: This 3-year-old goat had been at USAMRIID for 16 months. On February 23, 2005, the animal was observed with labored breathing, discharge from the nose, panting, encrusted nares, increased breath sounds bilaterally, and muffled heart sounds; the goat ate readily when provided grain. During physical examination the following clinical data were measured: body temperature - 102.4; pulse – 132; respiratory rate - 60. Broad-spectrum antibiotic therapy was initiated with 2 ml of Naxcel® (ceftiofur) once daily for 5 days; and 1.5 ml of LA-200® every other day for three treatments. After treatment, the goat was returned to the herd with noticeable clinical improvement. On March 3, 2005, the goat was removed from the herd and returned to treatment barn with increased breath sounds, the right side worse than the left, and moist lung sounds, especially in the ventral lung

fields. The goat was restarted on Naxcel antibiotic therapy per protocol above. The animal again seemed to respond clinically to antibiotic therapy, was observed eating in the treatment stall, and returned to the herd after the second course of treatment. On March 10, 2005, the animal was found dead in the pasture during morning rounds.

Gross Pathology: The carcass of the goat was in good nutritional condition with abundant body fat stores. The subcutaneous tissues over the ventral abdomen were moderately edematous. The abdominal cavity contained approximately 100 ml of clear yellow fluid. Several strands of fibrin extended between the liver lobes and adjacent omentum, intestines, and rumen. Fibrinous adhesions were present between the liver and diaphragm. The right side of the thoracic cavity contained approximately 50 ml of clear yellow fluid that congealed after exposure to air. The left side of the thoracic cavity contained approximately 100 ml of serosanguineous fluid. The pericardial sac was markedly distended with approximately 200 ml of cloudy, orange fluid. The pericardial wall was edematous and fibrous, measuring up to 5 mm in thickness. The internal surface of the pericardium and the surface of the epicardium were diffusely covered by a dense mat of villous, yellow to pink, fibrinous material, measuring up to 1.5 cm in thickness. Numerous fibrinous adhesions were present between the pericardium and the epicardium. The epicardium was diffusely fibrous and edematous, measuring up to 1 cm in thickness. The ventral and cranial aspects of all lung lobes were red, consolidated, and had numerous fibrous adhesions to the pericardium. Fibrous adhesions were also present between the right cranial lung lobe and the body wall. Multiple yellow-green, caseous nodules measuring up to 2 cm in diameter were scattered throughout the lungs and were most numerous in the areas of consolidation. Mediastinal lymph nodes were enlarged and edematous, measuring up to three times normal. The mesenteric, gastrosplenic, and retropharyngeal lymph nodes were congested, edematous, and similarly enlarged. The right inguinal and caudal cervical lymph nodes were enlarged approximately twice normal size. Reduced amounts of ingesta were present in the forestomachs. All other organs were unremarkable.

Primary Gross Diagnoses
1. Heart and pericardium: chronic diffuse fibrinosuppurative epicarditis and pericarditis (“bread and butter pericardium”; “shaggy heart”)
2. Lung: multiple pulmonary abscesses, with fibrinosuppurative pleuropneumonia and multiple fibrous adhesions
3. Abdominal cavity: fibrinous serositis, with adhesions
4. Haired skin, subcutis, ventral abdomen: interstitial edema, diffuse, mild

Laboratory Results: *Corynebacterium pseudotuberculosis* was cultured from the pericardial exudate and one of the pulmonary abscesses. Bacterial cultures of the

thoracic fluid, abdominal fluid, and cerebrospinal fluid were negative for aerobic bacterial growth.

Histopathologic Description: The tissue Gram stain demonstrated many gram-positive bacteria morphologically consistent with *C. pseudotuberculosis* within inflammatory lesions in the lungs, epicardium, and pericardium. The Congo red stain demonstrated varying amounts of amyloid deposition in the liver, spleen, kidneys, rumen, reticulum, and multiple lymph nodes; the amyloidosis was attributed to severe and prolonged inflammation within the thoracic organs.

Contributor's Morphologic Diagnoses: 1. Heart: diffuse chronic (organizing) fibrinosuppurative epicarditis, severe, with gram-positive bacillary bacteria, granulation tissue, multiple chronic abscesses, and multifocal myocardial edema with acute to subacute inflammation
2. Pericardium: diffuse chronic (organizing) fibrinosuppurative pericarditis, severe, with Gram positive bacillary bacteria, granulation tissue, and multiple chronic abscesses (histoslides not submitted)
3. Lung: multiple chronic abscesses, with Gram positive bacillary bacteria, diffuse chronic pleuropneumonia, congestion, and multiple fibrous adhesions (histoslides not submitted)
4. Liver: diffuse centrilobular fibrosis, mild to moderate, with congestion and multifocal proliferative endophlebitis (histoslides not submitted)
5. Spleen; kidney; liver; rumen; reticulum; and inguinal, cervical, and mesenteric lymph nodes: amyloidosis, diffuse, mild to moderate (histoslides not submitted)

Contributor's Comment: The pericarditis and epicarditis in this goat were sufficiently severe to have caused death of the animal; the inflammatory lesion in the heart and pericardial sac most likely developed secondarily from pulmonary infection with *C. pseudotuberculosis*. Centrilobular hepatic fibrosis, pulmonary and hepatic congestion, and the proliferative vascular changes in the hepatic veins (histoslides not submitted) reflect the effects of increased venous pressure secondary to congestive heart failure. Right-sided heart failure developed in this animal as a result of the restrictive pericarditis and epicarditis secondary to chronic fibrinosuppurative inflammation.

Corynebacterium pseudotuberculosis, the cause of caseous lymphadenitis in sheep and goats, was formerly known as *C. ovis*. Infection with *C. pseudotuberculosis* may be more severe in goats than sheep, and usually results in the development of lymph node abscesses in the head and neck regions that may resemble melioidosis or pseudoglanders[1]; we have observed lymph node abscesses in these anatomic areas as incidental findings during necropsy of other animals in this goat herd. Bacteria may occasionally spread from infected lymph nodes to the lungs, and pulmonary lesions are most common in older animals[1]. Chronic subpleural

pulmonary abscesses and/or bronchopneumonia with secondary pleuropneumonia may develop in animals that have had previous episodes of "caseous lymphadenitis" due to *C. pseudotuberculosis*. Bacterial spread to other organs is uncommon, but has reported to occur in the kidney, spleen and liver[1].

Infection with *C. pseudotuberculosis* in sheep typically results in enlargement and abscessation of the superficial and visceral lymph nodes. Infection is spread to other animals directly through oral and nasal secretions, and ruptured peripheral lymph node abscesses[2]. Affected lymph nodes become enlarged and filled with caseous, inspissated, green to chalky-colored material; on cross-section, the encapsulated abscesses have a characteristic “onion ring” appearance due to the concentric lamellations of the material. Hematogenous spread may lead to internal lymph node abscessation and pneumonia[2]. Other pathogenic corynebacteria and their associated disease conditions in animals include: 1) *C. renale*, *C. pilosum*, and *C. cystitidis* as causes of cystitis and pyelonephritis in cattle; 2) *C. bovis* and *C. ulcerans* as causes of mastitis in cows; and 3) *C. kutscheri* as the cause of caseopurulent lesions in the lungs, liver, and lymph nodes in rats[3].

Corynebacteria are small, pleomorphic gram-positive bacteria with varied morphology, including coccoid, club, and rod forms; in stained smears they may be found singly, arranged in palisades of parallel cells, or angular clusters (Chinese letters)[2]. Many members of the group are found as commensals on the mucous membranes, and some species, such as *C. pseudotuberculosis*, can survive for several months in the environment[2]. The pathogenicity of some corynebacteria is known to be related to the elaboration of certain exotoxins. For example, the virulence of *C. pseudotuberculosis*, a facultative intracellular bacterium capable of surviving and replicating in phagocytes, is related to its cell wall lipid and to the production of the exotoxin phospholipase D[2]. Other virulence factors in the corynebacteria group are related to the use of bacterial attachments and production of proteases. *Corynebacterium renale*, the urinary tract pathogen causing cystitis and pyelonephritis in cattle, possesses fimbriae which facilitate attachment to the urothelium and produces urease to hydrolyze urea[2].

Fibrinous pericarditis (and by extension epicarditis) usually occurs by hematogenous routes, but lymphatic spread or direct extension of the inflammatory or infectious process to the adjacent tissue is also possible[4]. There are a multitude of infectious causes of fibrinous pericarditis in domestic farm animals, including: 1) in cattle, contagious bovine pleuropneumonia, clostridial infections, pasteurellosis, sporadic bovine encephalomyelitis, and neonatal coliform infections; 2) in swine, Glasser’s disease, pasteurellosis, porcine enzootic pneumonia, and salmonellosis and streptococcosis in piglets; 3) in sheep, pasteurellosis and streptococcosis (lambs); and 4) in horses, streptococcal infections[4].

Ventral subcutaneous edema and effusions in the abdominal, thoracic, and pericardial spaces were prominent gross findings in this animal. While the inflammatory process in the pleural and pericardial spaces was likely the major contributing factor to fluid accumulation in these areas, the ventral subcutaneous edema and abdominal effusion suggest that other mechanisms may have resulted in extravascular fluid sequestration at these anatomic sites. The primary factors that generally govern fluid balance between the vascular and interstitial spaces are vascular hydrostatic pressure and plasma colloid osmotic pressure[5]. Given the gross and histologic findings, this goat may have had derangements in both mechanisms: 1) right-sided congestive heart failure likely resulted in generalized increase in venous pressure and subsequent systemic edema via activation of the renin-angiotensin-aldosterone axis; and 2) reduced plasma osmotic pressure may have been present due to decreased production of albumin in the liver due to hepatic amyloidosis and increased loss of albumin into extensive areas of chronic suppurative inflammation in the pleural and pericardial areas. Unfortunately, albumin levels were not measured in this animal before death, and decreased plasma osmotic pressure cannot be confirmed.

AFIP Diagnosis: Heart: Epicarditis, pyogranulomatous and fibrinous, chronic, diffuse, severe, Boer cross, ovine.

Conference Comment: The contributor provides a thorough review of *C. pseudotuberculosis* infection in sheep and goats as well as differential diagnoses and diseases in other species caused by *Corynebacteria* sp.

Conference attendees did not have the benefit of the animal's history of antibiotic administration or Gram stains. Expecting to see large colonies of "Chinese characters" coccobacilli associated with *C. pseudotuberculosis* infection, attendees initially placed it lower on the differential diagnosis list. All agreed that the repeat administration of antibiotics is probably responsible for the absence of large bacterial colonies from these lesions and may have contributed to the protracted development of the "bread and butter" lesions of the pericardium and epicardium.

A Brown-Brenn stain revealed the scattered clusters and individual Gram-positive coccobacilli within the epicardium.

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* Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

**The opinions, interpretations, conclusions, and views expressed herein are those of the author(s) and do not reflect the official policy of the Department of the Army, the Department of Defense, or the U.S. Government.

CASE III – G 6288 (AFIP 2991429)

Signalment: 6-year-old, intact female, cynomolgus monkey (*Macaca fascicularis*), non-human primate.

History: Six animals of different age and sex died in a group of 35 cynomolgus monkeys housed indoor-outdoor within a short time period from late summer to late autumn. The animals became lethargic and febrile and died with unspecific clinical symptoms after one or two days of illness. The monkeys were considered to be serological negative for Herpes B, Herpes simplex I and II, Varicella Zoster virus,

Cytomegalovirus, Epstein-Barr virus, SIV, and HTLV. All six deceased monkeys exhibited similar clinical and pathomorphological findings.

Gross Pathology: Main gross necropsy findings occurred in the respiratory tract. The pharyngeal lymphoid tissue was enlarged, heavily inflamed and showed multifocal caseous abscess formations. The lung showed multinodular to diffuse red to grey areas of consolidation in affected lobes and a severe multifocal granulomatous pneumonia. Necropsy findings included marked splenomegaly and mild hepatomegaly with numerous white foci up to 1 mm in diameter distributed throughout the parenchyma. The liver was friable with blunted margins.

Laboratory Results: Routine bacteriology was negative.

Histopathologic Description: The main histologic finding was multifocal granulomatous pneumonia accompanied by a severe purulent bronchitis. Granulomas were diffusely distributed within the lung tissue but were always found in close association to larger lung vessels. The granulomas were sharply demarcated and consisted of central necrotic areas, hemorrhage and mild infiltration of inflammatory cells. They were surrounded and infiltrated by activated alveolar macrophages. The spleen contained coalescing foci of necrosis of both white and red pulp. The lesions consisted of amorphous cellular debris and were infiltrated by few lymphocytes and neutrophils. In general, they resembled the inflammatory reaction occurring in the lung. The submandibular lymph nodes were grossly enlarged and necrotic. Caseous granulomatous inflammation occurred within the pharyngeal tissue. Furthermore, a suppurative leptomeningitis was found.

Immunohistochemistry was performed on paraffin embedded sections of altered tissue using a specific monoclonal anti *Francisella tularensis* antibody. A widespread distribution of *Francisella* antigen within the pharyngeal lesion, the inflamed lymph nodes and the spleen could be demonstrated. Identification of the causative agent was achieved by culture, enzyme-linked immunosorbent assay (ELISA) and molecular techniques.

Special stains like Gram, Giemsa, PAS-reaction, Grocott and Ziehl-Neelsen staining failed to demonstrate intralesional microorganisms in any of the affected organs.

Contributor's Morphologic Diagnoses:

1. Lung: Pneumonia, granulomatous, multifocal, severe, Cynomolgus monkey
2. Spleen: Splenitis, granulomatous, multifocal to coalescing, severe
3. Liver: Hepatitis, necrotizing, focal, mild
4. Pharynx: Pharyngitis, pyogranulomatous to caseous, severe
5. Lymph node: Lymphadenitis, necrotizing, focal, mild

Etiologic agent: *Francisella tularensis* subsp. *holarctica*

Contributor's Comment: The pathologic findings in our cases correspond to the histologic features in naturally acquired and experimentally induced tularemia in nonhuman primates (Baskerville et al. 1978, Calle et al. 1993, Emmons et al. 1970, Hoelzle et al. 2004, Nayar et al. 1979, Posthaus et al. 1998). *Francisella tularensis* is a zoonotic gram-negative bacterium which causes the disease tularemia. It is widespread in North America as well as in parts of Europe and Asia. The organism is able to infect different species of mammals, and even some species of birds and reptiles. Rodents and lagomorphs are highly susceptible and are considered to be the main reservoir hosts in many areas of the world. Hematophagous arthropods like mosquitoes and ticks have been suggested as main vectors (Ellis et al. 2002). Transmission is often associated with an arthropod vector, but the infection can also be acquired orally, via the respiratory route, by bites of infected vertebrates or from direct contact with infected tissue. Six classic forms of tularemia are described in human medicine. The predominant manifestations of human disease are the ulceroglandular, glandular, occuloglandular, pharyngeal, typhoidal and the pneumonic form; however, overlapping of the different symptoms is observed frequently (Lamps et al. 2004). The presented case mostly resembles the pharyngeal form with pneumonic manifestation. The case illustrates that in addition to classical nodal and pulmonary involvement tularemia usually affects the liver and spleen with hepatosplenomegaly and necrotic lesions within the altered organs.

The source of infection of this epizootic is still under investigation. The indigenous rodent population appeared to be unusually high. Starting several weeks prior to the epizootic, many dead mice had been found in the facility. A murine tularemia epizootic was the most likely source of infection for the nonhuman primates. The most probable route of infection is assumed via ingestion of infected mice or food contaminated by infected mice, feces or urine.

AFIP Diagnoses: 1. Lung: Pneumonia, granulomatous, multifocal, moderate, cynomolgus monkey (*Macaca fascicularis*), primate.
2. Adipose tissue and attached skeletal muscle (pharyngeal region): Pyogranuloma, with muscular atrophy and replacement fibrosis.

Conference Comment: Tularemia is endemic worldwide and primarily causes disease in wild rabbits and rodents. In the United States, the main reservoir is wild rabbits and contact with infected wild rabbits can result in fatal infection to humans.

F. tularensis is a small, pleomorphic, gram-negative, intracellular coccobacillus that is surrounded by a thick, lipid-rich capsule. Isolates are antigenically similar, but subdivided according to virulence, and epidemiologic and biochemical characteristics into three subspecies or biovars:

1. *F. tularensis* subsp. tularensis (Type A): most virulent; found in North America; associated with tick-borne tularemia in rabbits and zoonotic disease.
2. *F. tularensis* subsp. palaearctica (Type B): less virulent; found worldwide except for Australia and Antarctica; associated with waterborne disease of rodents.
3. *F. tularensis* subsp. mediasiatica: found in central Russia.

Disease susceptibility varies with species infected. Rodents and lagomorphs are most susceptible, and usually suffer fatal septicemia. Other herbivores, ruminants and birds are susceptible, but mortality is unusual. Carnivores are least susceptible, require a large infective dose, rarely develop bacteremia, and only occasionally manifest overt disease.

F. tularensis is transmitted through arthropod bites, penetration of the skin, cuts, abrasions, exposure of mucous membranes, ingestion or inhalation. *Dermacentor* spp. and *Amblyomma* spp. ticks pass the organism transstadially and transovarially, functioning as both vectors and reservoirs. Mosquitoes, fleas, horseflies, deer flies (especially important in N. America) and lice can also transmit the disease. Carnivores may be infected by ingesting infected carcasses and there are numerous reports of zoonotic infections resulting from dog and cat bites.

Following infection, bacteria are phagocytosed by local macrophages. Intrahistiocytic replication occurs in local lymph nodes. After 3-14 days, bacteria disseminate to the spleen, liver, lymph nodes and bone marrow resulting in septicemia. Consistent gross necropsy findings include numerous small, white foci on the surface of an enlarged liver, spleen, lymph nodes, and, less often, kidneys. Microscopically, these small white foci are identified as coalescing areas of caseous to lytic necrosis, surrounded by lymphocytes, neutrophils and macrophages. Additional microscopic findings include vasculitis and thrombosis resulting from bacterial invasion and damage to the vascular endothelium. In acute septicemia, an initial neutrophilia is often followed by neutropenia.

Francisella tularensis can be grown in culture, but the potential risk of human infection requires extra caution. The preferred diagnostic test is serology. A four-fold rise in antibody titer between acute and convalescent serum is considered diagnostic; however, cross-reaction with *Brucella* antigen can occur. IFA is available. *F. tularensis* bacteria are visible on smears stained with new methylene blue.

Participants briefly discussed *F. tularensis* as a potential biological weapon. The Centers for Disease Control (CDC) lists *F. tularensis* as a Category A agent. Category A agents have the greatest potential for inflicting high numbers of human casualties, can be manufactured and disseminated on a large scale, require significant efforts in public health preparedness, and are most familiar to the public.

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CASE IV – 13125-05 (AFIP 2988628)

Signalment: Two-weeks-old Angus (*Bos taurus)* bovine

History: Owner's fall calving cows were obese and were experiencing dystocia. Some calves were born weak, failing to thrive probably due to dystocia (as per submitting veterinarian). Owner had treated calves without examination by a

veterinarian. The following antibiotics were administered to this calf; ceftiofur, penicillin, spectinomycin sulfate, danofloxacin, tilmicosin, and florfenicol.

Gross Pathology: Discrete areas of dark discoloration 2.5 cm or larger were observed in the forestomachs. Small areas of cranioventral consolidation were noted in the lung.

Contributor's Morphologic Diagnoses: 1. Severe subacute diffuse necrosuppurative, erosive, ulcerative and transmural rumenitis with myriad intralesional fungi
2. Severe subacute necrotic vasculitis with thrombosis and intralesional fungal hyphae

Contributor's Comment: Two predominant types of fungi are seen in these sections. Those which are approximately two to three microns wide and septate with parallel sides are limited to the keratinized layer. These hyphae are branching with buds and free chlamydospores and were presumed to be *Candida sp.* The fungi, which are non-branching with non-parallel sides and approximately eight microns wide, are observed in all levels of the sections as well as in thrombosed blood vessels and muscular layers. These fungi are considered to belong to the class of fungi, *Zygomycetes*. Included in this class are *Absidia*, *Mucor*, and *Rhizopus*.[1] Spread to other organs occurs via a hematogenous route from the initial site of infection.[1]

Mycotic infection of the forestomachs of ruminants is generally the result of opportunistic infection secondary to other predisposing factors.[1,2,3,4] This case is somewhat unusual in that at least two species of fungi were identified in the sections. The predisposing factor is this case was believed to be the wide spectrum of antibiotics that were administered over the course of nine days.

In general, other predisposing factors include ruminal acidosis secondary to carbohydrate engorgement, rumen stasis, and reflux of abomasal contents into the omasum.[1,2] Rumen stasis and abomasal reflux may occur secondary to acid base disequilibrium, toxemia, fever, parturient hypocalcemia, and peritonitis.[2]

AFIP Diagnosis: Rumen: Rumenitis, necrosuppurative, erosive, subacute, transmural, diffuse, moderate, with vasculitis, thrombi, and fungal hyphae, Angus, bovine.

Conference Comment: There is some variation among slides. Participants may receive sections with an ulcerated rumen mucosa; whereas, other sections may

simply have erosions. Some sections do not contain *Candida*. The contributor provides an excellent case of mycotic rumenitis. Attendees identified the larger hyphae as Zygomycetes class fungi and debated whether or not the few *Candida* organisms are pathologic or simply a commensal organism identified within the superficial epithelium of the rumen.

Zygomycetes are ubiquitous saprophytic molds associated with water, soil, decaying matter, and substrates high in carbohydrates. Zygomycetes are opportunistic invaders; predisposing factors include antibiotic therapy, ruminal acidosis (grain overload), reflux of acidic abomasal contents and erosive viral disease such as infectious bovine rhinotracheitis (IBR) or bovine pestivirus (BVD-mucosal disease). Focal or disseminated infections can occur in cattle of all ages.

The pathogenesis for mycotic rumenitis is another "classic" which every pathology resident must understand and be able to rapidly regurgitate. Briefly, antibiotic therapy, grain overload (or another predisposing factor) leads to the disruption of the normal rumen flora and proliferation of *Streptococcus bovis*. *S. bovis* utilizes the carbohydrates to produce an abundance of lactic acid which decreases the rumen pH resulting in ruminal acidosis, ruminal atony, chemical rumenitis and a favorable environment for growth of Zygomycetes class fungi (*Mucor, Rhizopus*, *Absidia* spp.). The inflamed mucosa becomes ulcerated allowing the fungal hyphae the opportunity to penetrate and invade the vasculature resulting in thrombosis, infarction, acute necrosis, and direct or hematogenous spread (1).

Typical clinical findings include anorexia, depression, rumen atony, foul-smelling feces and increased cardiac and respiratory rates due to acidosis. Clinical pathology findings include hemoconcentration due to the movement of fluid from the circulation into the rumen.

Gross findings include multifocal, well-circumscribed necrotic foci surrounded by zones of hyperemia in the rumen, reticulum, and omasum. Necrotic areas are red to black, firm, leathery, and thickened up to 1 cm. Usually the ventral portion of the rumen is most severely affected; however, up to 70% of the rumen may be involved. Severe cases may develop fibrinohemorrhagic peritonitis and, within the liver, necrotizing thrombophlebitis of portal triads.

Microscopically there is transmural, coagulative necrosis with necrotizing vasculitis, a fibrinous exudate and variable amounts of inflammation. *Zygomycetes* are described as rarely septate, thin-walled, 3-25 um wide hyphae with non-parallel walls, non-dichotomous, irregular branching, and focal bulbous dilatations. Frequently, hyphae are collapsed, folded and twisted and resemble a ribbon. Hyphae may be angiotropic, and perineural invasion is common.

Candida albicans (Class Blastomycetes, Family Cryptococcaceae) typically form round to oval budding yeasts (blastospores), masses of branching, septate hyphae (3-6 microns in diameter), and pseudohyphae (budding yeast that remain attached to each rather than breaking free) (5). Infection of the squamous epithelium in young people and animals is called "thrush". *C. albicans* is also an opportunistic invader of aged and immunosuppressed people and animals.

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