The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2002-2003

CONFERENCE 3
25 September 2002

Conference Moderator: LTC Peter Vogel, DVM, PhD, Diplomate, ACVP
Director, Division of Pathology
Walter Reed Army Institute of Research
Silver Spring, MD 20910-7500

CASE I – 01-7164 (AFIP 2839250)

Signalment: Two-year-old, doe, Nubian goat

History: Acute onset of mastitis followed by death within 24 hours.

Gross Pathology: The right half of the udder was dark red/black and exuded watery red fluid from the cut surface.

Laboratory Results: Culture of the mammary gland yielded *Bacillus cereus*.

Contributor's Morphologic Diagnosis: Necrotizing mastitis, peracute, diffuse, severe, with bacilli. Etiology: *Bacillus cereus*

Contributor's Comment: There is diffuse necrosis of mammary gland acini and septal tissue with colonization by Gram-positive, spore-forming bacilli. There is widespread lobular hemorrhage. Inflammatory infiltrates consist of a few degenerating neutrophils within lobules and interstitium.

Mastitis in small ruminants has been divided into four categories: 1) small fibrotic lesions in the udder with normal secretions (a chronic lesion), 2) extensive fibrosis of the udder with normal to purulent milk or no milk due to teat obstruction (also a chronic lesion), 3) extensive swelling of the udder with white to serum-like or purulent milk (an acute lesion), and 4) peracute mastitis characterized by complete udder involvement with severe inflammation and serum-like secretions that contain variable amounts of fibrin and purulent material. This case fits the peracute category. Peracute mastitis occurs most often in the period from lambing/kidding until 2-4 weeks post-partum. There is another spike in incidence just after weaning. Clinical signs include fever and depression, followed by hypothermia, dehydration, anorexia and a swollen, discolored (often purple) gland. Case fatality rates are high, often reaching 30-40%. In sheep and goats, *Staphylococcus aureus* is the most common isolate, with occasional isolates of *Mannheimia sp., Pseudomonas aeruginosa* and coliforms.

Bacillus cereus is a motile, aerobic or facultative anaerobic, spore-forming, Gram-positive or Gram-variable bacillus. It is a widespread, ubiquitous soil organism. The organism is an opportunistic pathogen well recognized as a cause of human food-borne illness, but also causes a wide range of other illnesses in man. These include skin and joint infections, meningitis, septicemia, pneumonia, endocarditis and intraocular infections. It produces a variety of toxins including enterotoxin, hemolysin, phospholipase C, and the emetic toxin. The necrotizing enterotoxin has been proposed as the major virulence factor in non-gastrointestinal lesions. The hemolysins and phospholipases are thought to contribute to tissue injury. Various host factors are thought to play a role in infections by the organism. These include immunosuppression, indwelling catheters or implants and intravenous drug use.

Bacillus cereus is a sporadic cause of bovine mastitis. In the cow, the organism causes an acute gangrenous mastitis. The gross and microscopic lesions resemble those illustrated in the present case. The cause of the infection was associated with teat injury, teat surgery, teat dilation or intra-mammary infusion. In one case, the infusion was contaminated with *Bacillus cereus*. This goat had no history of mammary gland treatment prior to the onset of the infection.

AFIP Diagnosis: Mammary gland: Mastitis, necrotizing, peracute, diffuse, severe, with hemorrhage, edema, and bacilli, Nubian goat, caprine.

Conference Comment: The contributor has provided a concise summary of caprine mastitis caused by *Bacillus cereus*. Conference participants considered other causes of caprine mastitis, including avocado leaf toxicity (*Persea americana*), *Escherichia coli*, and *Staphylococcus aureus*.

Contributor: Arizona Veterinary Diagnostic Laboratory, 2831 N. Freeway, Tucson, AZ 85705

References:

1. Gaur AH and Shenep JL: The expanding spectrum of diseases caused by *Bacillus cereus*. Pediatr Infect Dis J **20**:533-534, 2001
2. Menzies PI and Ramanoon SZ: Mastitis of sheep and Goats. *In*: Veterinary Clinics of North America, Food Animal Practice, ed. Van Metre DC, vol 17, pp. 333-358. WB Saunders Co., Philadelphia, PA, 2001
3. Schiefer B, Macdonald KR, Klavano GG and van Dreumel AA: Pathology of *Bacillus cereus* mastitis in dairy cows. Can Vet J **17**:239-243, 1976
4. Turnbull PCB, Jorgensen K, Kramer JM, Gilbert RJ, Parry JM: Severe clinical conditions associated with Bacillus cereus and the apparent involvement of exotoxins. J Clin Path **32**:289-293, 1979

CASE II - 02-2965 (AFIP 2839957)

Signalment: 12-year-old, spayed female, Rottweiler-Shepherd dog.

History: Intermittent diarrhea and vomiting.

Gross Pathology: Numerous (over 40), firm, off-white nodules, varying in size from 0.5 to 1 cm in diameter were located in the serosa of the small intestine, at the site of the mesenteric attachment. These nodules were more prominent and numerous in the upper small intestine compared to the lower small intestine. In the affected areas, 2-3 mm in diameter nodules followed the tracts of the mesenteric lymphatic vessels, which were markedly thickened. A few nodules measuring up to 2 mm in diameter were located in the serosal surface of the duodenum. The wall of the small intestine was thickened (1.5 to 2 times normal) and cut surfaces revealed a thickened, off-white mucosa. The cortical area of both kidneys had irregularly-shaped, off-white to pale yellow areas of depression measuring up to 1cm in greatest dimension.

Laboratory Results: None

Contributor's Morphologic Diagnoses:
1. Intestinal lymphangiectasia and severe multifocal granulomatous lymphangitis.
2. Membranous glomerulopathy, renal infarcts and chronic interstitial nephritis.

Contributor's Comment: This section of duodenum is characterized by marked dilation of lacteals at the tip of the villi. Villi are hypercellular, often fused and eroded. Hypercellularity is due to infiltration by lymphocytes, plasma cells and fewer numbers of neutrophils. A few eosinophils are seen in the deep mucosa. Multifocal to coalescent areas of inflammatory cell infiltration (macrophages with foamy cytoplasm, lymphocytes and plasma cells) are located in the muscularis externa and serosa layers, and extend into the mesentery. In the center of some of these areas, the lumen of lymphatic vessels is identified. Mesenteric lymphatic vessels are markedly dilated and surrounded by a prominent inflammatory infiltrate composed of macrophages, lymphocytes, plasma cells and neutrophils. Macrophages often contain foamy, pale basophilic material within the cytoplasm. Amorphous eosinophilic and pale amphophilic material is seen within the lumen of these lymphatics.

In this section of kidney, in addition to focal areas of cortical fibrosis, (interpreted as healed infarcts) and interstitial inflammation, glomerular changes are detected. These changes are prominent near the infarct and consist of hyaline thickening of Bowman's capsule and occasional periglomerular fibrosis, thickening of glomerular mesangia, reduction in size and sclerosis of glomerular tufts.

Intestinal granulomatous lymphangitis and lymphangiectasia have been described in dogs. The pathogenesis of these lesions is unclear. It has been suggested that primary lymphangiectasia can lead to leakage of lipid-rich lymph resulting in inflammatory response. A relationship between protein-losing enteropathy and protein losing nephropathy has been described in Soft Coated Wheaten Terriers and Basenjis. Proposed mechanisms linking the intestine and kidney lesions include: 1) immunoregulatory dysfunction, 2) altered response to an environmental trigger due to intolerance or cross- reaction with endogenous epitopes, 3) ultrastructural or developmental defect of lymphatics, vasculature, epithelia, and/or basement membrane.

AFIP Diagnoses:
1. Small intestine and mesentery: Lymphangitis, lipogranulomatous, multifocal, moderate, with lymphangiectasia, Rottweiler-Shepherd cross, canine.
2. Kidney: Fibrosis, focally extensive, moderate, with tubular loss, stromal collapse, glomerulosclerosis, and multifocal lymphoplasmacytic interstitial nephritis.

Conference Comment: Intestinal lymphangiectasia is the most common cause of protein-losing enteropathy in dogs. Dilated lacteals, lymphopenia, hypoproteinemia and hypocalcemia are characteristic findings. Clinical signs of lymphangiectasia include diarrhea, steatorrhea, flatulence, ascites, and vomiting. Congenital or primary lymphangiectasia results from a developmental disorder of lymph vessels. Acquired or secondary lymphangiectasia is most commonly idiopathic, or is secondary to functional obstruction of the lymph vessels, heart disease, intestinal neoplasia, or mesenteric inflammation. Lymphopenia results from lymphatic duct blockage and lymphoid infiltration of the intestine, preventing recirculation of lymphoid cells to peripheral blood. Hypoproteinemia, hypoalbuminemia, and hypocalcemia are clinicopathologic abnormalities associated with a generalized protein losing enteropathy.

Lipogranulomatous lymphangitis is uncommonly associated with lymphangiectasia, and characterized by transmural enteric lipogranulomas. The granulomatous inflammation often surrounds a central remnant of a dilated lymph vessel. Suggested dietary control measures include changing the diet from long chain triglycerides to medium chain triglycerides.

Contributor: San Diego Animal Disease Diagnostic Laboratory, Office of the County Veterinarian, 5555 Overland Ave Bldg 4, San Diego, CA 92123-4268

References:
1. Gelberg HB: Alimentary System. *In*: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 39-41. Mosby Inc., St. Louis, MO, 2001
2. Littman MP, Dambach DM, Vaden SL, Giger U: Familial protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers: 222 Cases (1983-1997). J Vet Intern Med **14**:68-80, 2000
3. Meschter CL, Rakich PM, Tyler DE: Intestinal lymphangiectasia with lipogranulomatous lymphangitis in a dog. J Am Vet Med Assoc **190**:427-430, 1987
4. Van Kruiningen HJ, Lees GE, Hayden DW, Meuten DJ, Rogers WA: Lipogranulomatous lymphangitis in canine intestinal lymphangiectasia. Vet Pathol **21**:377-383, 1984

CASE III –TAMU-1 (AFIP 2840168)

Signalment: Two year-old Indian Black Buck Antelope (*Antilope cervicapra*)

History: This antelope had a history of depression and moving away from the herd 5 days prior to when it was presented to the hospital with a mild fever, 104.4 degrees

Fahrenheit. The animal died, soon after being given fluid subcutaneously. The owner reported previous loss of two animals with similar clinical signs. No further history on these other animals is available.

Gross Pathology: Besides fecal staining of the perineum, no macroscopic lesions were noted at necropsy.

Laboratory Results:
WBC = 2900/mm^3; Neutropenia 1798/mm^3; Lymphocytes 1044/mm^3
Clinical chemistry values of note included:
Total protein 5.4 g/dl; BUN 216 mg/dl; Creatinine 7.6 mg/dl; Phosphorous 11.7 mg/dl; Calcium 6.7 mg/dl
The GGT, AlkP, SGPT, and CK were within our laboratory normal values.

Contributor's Morphologic Diagnosis: Non-suppurative interstitial, especially perivascular and periglomerular, nephritis with necrotizing vasculitis.

Contributor's Comment: The histologic lesions of the kidney are characteristic of Malignant Catarrhal Fever (MCF). The pleocellular infiltrate concentrates in the interstitium around blood vessels and glomeruli. Many vessels are necrotic and/or infiltrated by the inflammatory cells. The interesting feature of this case was the presentation as a renal failure. Others have noted the severe nature of the renal lesions (both bladder and kidney lesions), and in this case, the nephritis and the prerenal (dehydration) complications resulted in a significant elevation of BUN and creatinine, with changes in calcium and phosphorous concentrations as well. Leptosporosis serology was negative and silver stains for spirochetes were negative.

The animal had vasculitis in all organs as well as acute, superficial, necrotizing lesions of many mucosal surfaces. A meningoencephalitis and intense periportal infiltration of the liver were present. A lymph node submitted for ovine herpesvirus 2 (OHV-2) PCR testing was positive.

Sheep-associated MCF in wild ungulates often has unimpressive or subtle lesions. Superficial erosive or ulcerative mucosal lesions are often missed or ignored in deer. Diarrhea or perineal fecal staining in an adult wild ungulate may be a clue to the diagnosis, and in deer, gastrointestinal (abomasum, intestine, colon) lesions are often severe. "Milky eye" is a lay term for anterior uveitis seen with MCF in farmed wild ungulates. In this animal, the uveitis was mild and only seen histologically. Most cases can be associated with sheep. The OHV-2 has not been isolated, but has been "established" in lymphoblastoid cell lines by a number of labs. This animal's lymph node was tested using specific primers for the non-glycosylated, tegument structural protein that is specific and can distinguish OHV-2 from the exotic gammaherpes found in African ungulates and imported zoo ruminants in the world. On this farm, Dall sheep have contact with antelope. OHV-2 is widespread in the United States. Lambs begin shedding it after a couple of months of birth, but their peak excretion is between 8 and 10 months of age. The old wisdom was that the cortisol associated with parturition induces increased shedding of MCF virus. That has been shown to be untrue in both wildebeests with alcelaphine herpesvirus 1 (AHV-1) and sheep with OHV-2. OHV-2

lethally infects a variety of domestic and wild ungulates; however, the fallow deer seems resistant. Some animals have been shown to recover from clinical infection (though rarely). Recently, OHV-2 has been associated with a vasculitis and encephalitis in pigs in Switzerland and Norway. The owner is trying to decide if he wants to keep his antelope or his sheep. Should we start testing feral hogs for OHV-2?

AFIP Diagnosis: Kidney: Arteritis, fibrinonecrotic, lymphocytic, multifocal, moderate, with perivascular and periglomerular interstitial nephritis, black buck antelope (*Antilope cervicapra*), cervid.

Conference Comment: Malignant catarrhal fever is caused by a gammaherpesvirus, which is an enveloped, linear, doubled-stranded DNA virus that measures 150 nm in diameter. Gammaherpesviruses replicate within lymphoblastoid cells, and in the case of malignant catarrhal fever, specifically targets T cell lymphocytes. There is a lymphoproliferative response of T helper and T cytotoxic cells, resulting in a generalized lymphadenopathy. There are two gammaherpesviruses, both of which cause similar lesions and clinical signs of MCF: alcelaphine herpesvirus 1 - natural host is the wildebeest; and ovine herpesvirus 2 - natural host is the domestic sheep. In both types, the natural host is subclinically infected. Aberrant hosts become severely infected, and most animals die suddenly, with few gross lesions other than hemorrhagic enterocolitis. In chronically infected animals, there is necrotizing lymphocytic vasculitis, obliterative arteriopathy, gastrointestinal erosion, edema, and generalized lymphadenopathy.

The differential diagnosis for malignant catarrhal fever includes bovine viral diarrhea-mucosal disease (*Pestivirus*), bluetongue and epizootic hemorrhagic disease (*Orbivirus*), rinderpest (*Morbillivirus*), foot and mouth disease (*Aphthovirus*), vesicular stomatitis (*Vesiculovirus*) and alimentary toxicosis.

Contributor: Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467

References:

1. Barker IK, Van Dreumel AA, Palmer N: The Alimentary System. *In*: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N., 4th ed., vol. 2, pp. 163-172. Academic Press, San Diego, CA, 1993
2. Heuschele WP, Reid HW: Malignant Catarrhal Fever. *In*: Infectious Diseases of Wild Mammals, eds. Williams ES, Barker IK, 3rd ed., pp. 157-163. Iowa State University Press, Ames, IA, 2001
3. Li H, Snowder G, O'Toole D, Crawford TB: Transmission of ovine herpesvirus 2 in lambs. J Clin Microbiol **36**:223-226, 1998
4. Li H, Dyer N, Keller J, Crawford TB: Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). J Clin Microbiol **38**:131-1318, 2000
5. Li H, Westover WC, Crawford TB: Sheep-associated malignant catarrhal fever in a petting zoo. J Zoo Wildlife Med **30**:408-412, 1999
6. Loken T, Aleksandersen M, Reid H, Pow I: Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. Vet Rec 143:464-467, 1998

7. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Veterinary Virology, 3rd ed., p. 309. Academic Press, San Diego, CA, 1999
8. O'Toole D, Li H, Miller D, Williams WR, Crawford TB: Chronic and recovered cases of sheep-associated malignant catarrhal fever in cattle. Vet Rec **140**:519-524, 1997
9. O'Toole D, Li H, Roberts S, Rovnak J, DeMartini J, Cavender J, Williams B, Crawford T: Chronic generalized obliterative arteriopathy in cattle: a sequel to sheep-associated malignant catarrhal fever. J Vet Diag Invest **7**:108-121, 1995
10. Wobeser G, Majka JA, Mills JHL: A disease resembling malignant catarrhal fever in captive white-tailed deer in Saskatchewan. Can Vet J **14**:106-109, 1973

CASE IV - 21446-02 (AFIP 2840461)

Signalment: Adult male fox squirrel (*Scirurus niger*)

History: Numerous squirrels had been identified in the area with generalized alopecia and emaciation. One squirrel was submitted for laboratory examination.

Gross Pathology: The carcass displayed marked generalized alopecia with broad areas of increased thickness of the skin. In many areas, crusts were present on the surface of the skin. The carcass was emaciated with boney prominences easily palpable.

Laboratory Results: None

Contributor's Morphologic Diagnoses: Haired skin: 1. Severe diffuse orthokeratotic hyperkeratosis, with ballooning degeneration, epidermal hyperplasia, and intracytoplasmic eosinophilic inclusion bodies.
2. Moderate diffuse superficial dermal fibrosis.

Contributor's Comment: Poxviruses are known to cause tumors or tumor-like lesions in man and in a variety of animals. These include Yaba disease in African monkeys, *Molluscum contagiosum* virus in man, lumpy skin disease in African cattle, and fibromatosis in cottontail rabbits. As in squirrel fibroma, the hallmark lesions are ballooning degeneration with large intracytoplasmic eosinophilic inclusion bodies. This particular case represents one of an outbreak of poxvirus infection in the local squirrel population.

These sections of haired skin display modest to marked diffuse orthokeratotic hyperkeratosis and diffuse acanthosis. Numerous intracytoplasmic eosinophilic inclusion bodies can be seen in the epidermis in the stratum spinosum and stratum granulosum. The dermis is diffusely thickened due to fibroplasia.

AFIP Diagnosis: Haired skin: Epidermal hyperplasia and ballooning degeneration, diffuse, moderate, with hyperkeratosis, dermal mesenchymal cell proliferation, and eosinophilic intracytoplasmic inclusion bodies, fox squirrel (*Scirurus niger*), rodent.

Conference Comment: Leporipoxvirus is a member of the family *Poxvirus,* which is a linear, double-stranded DNA virus that has a brick shaped virion measuring 200-400 nm in length. Leporipoxviruses include rabbit (Shope) fibroma virus, squirrel fibroma virus, hare fibroma virus and myxoma virus. Transmission of leporipoxvirus is via arthropod percutaneous inoculation, close contact with discharges, or mechanical trauma. Squirrel fibroma virus may cause solitary or numerous cutaneous nodules. The virus replicates in the dermis and epidermis, and there may be viremia to internal organs, such as the lungs and liver. In this case, eosinophilic intracytoplasmic inclusion bodies were also noted within the mesenchymal cell population of the dermis.

Contributor: Veterinary Diagnostic Center, Fair Street and East Campus Loop, Lincoln, NE, 68583-0907

References:

1. Hirth RS, Wyand DDS, Osborne AD, Burke, CN: Epidermal changes caused by squirrel poxvirus. J Am Vet Med Assoc **155**:1120-1125, 1969
2. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Veterinary Virology, 3rd ed., p. 278, 287-288. Academic Press, San Diego, CA, 1999
3. O'Connor DJ, Diters RW, Nielsen SW: Poxvirus and multiple tumors in an eastern grey squirrel. J Am Vet Med Assoc **177**:792-795, 1980
4. Robinson AJ, Kerr PJ: Poxvirus Infections. *In*: Infectious Diseases of Wild Mammals, eds. Williams ES, Barker IK, 3rd ed., pp. 179-195. Iowa State University Press, Ames, IA, 2001

Kathleen A. Ryan, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.