The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CONFERENCE 3
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CASE I – NADC MVP-1 (AFIP 3028509).

Signalment: 5-year-old, female, Suffolk ewe, (*Ovis aries*).

History: Ewe culled due to chronic weight loss and unthriftiness. Euthanized due to poor prognosis.

Gross Pathology: Little to no body fat; irregular thickening of the ileum and distal jejunum; mesenteric lymphadenomegaly; prominent lymphatics in region of distal jejunum and ileum.

Histopathologic Description: There is moderate to marked expansion of the lamina propria and submucosa due to infiltrates of large numbers of macrophages, lymphocytes and lesser numbers of neutrophils. In addition there is moderate ectasia of lymphatics within the lamina propria. Macrophages contain abundant eosinophilic cytoplasm and staining with the Ziehl-Neelsen technique reveals numerous intracellular acid fast bacteria. Granulomatous infiltrates extend deep through the muscularis mucosa, submucosa and are occasionally seen in the subserosa. In submucosal and subserosal regions small foci of granulomatous infiltrates are associated with lymphatic vessels. Associated lymph nodes are characterized by multifocal infiltrates of macrophages involving the cortex and to a lesser extent the medulla. These macrophages also contain variable numbers of acid fast bacilli.

Contributor's Morphologic Diagnosis: Small intestine: Enteritis, granulomatous, multifocal, moderate, chronic with lymphangiectasia and intralesional intracellular acid fast bacilli.

Lymph node: Lymphadenitis, granulomatous, multifocal, moderate with intralesional, intracellular acid fast bacilli.

Contributor's Comment: *Mycobacterium avium* subsp. *paratuberculosis* (Map) is the causative agent of paratuberculosis or Johne's disease. Infection usually occurs soon after birth and clinical disease in ruminants is characterized by chronic, progressive enteritis, often accompanied by protracted diarrhea and weight loss. Paratuberculosis is generally associated with domestic ruminants such as cattle, sheep and goats with sporadic occurrence in free ranging ruminants such as white-tailed deer, Key deer, bighorn sheep, Rocky Mountain goats, tule elk, and bison. Following oral exposure, the organism is generally believed to cross the small intestinal mucosa into the Peyer's patches via M-cells within the follicle associated epithelium.[1] Macrophages taking up Map accumulate in intestinal epithelium, associated lymphoid tissue as well as draining lymph nodes resulting in granulomatous lesions. Initial lesions generally contain few acid fast bacteria; however, in later stages of lesion development myriads of acid fast bacteria are common. Studies of naturally occurring cases of paratuberculosis in sheep and goats describe different types of lesions represented by two extremes, i.e., paucibacillary (tuberculoid) lesions and multibacillary (lepromatous) lesions. Three classifications of lesions have been suggested that span the spectrum of observed lesions.[2] It has been observed that paucibacillary forms of the disease are associated with a strong cell mediated immune response, while in multibacillary forms of the disease the humoral immune response dominates. The current case represents an example of the multibacillary form of paratuberculosis.

Map is known to exist as 2 phenotypically different strains designated the sheep (S) and cattle (C) strains. Sheep strains are generally more difficult to isolate in culture than cattle strains.[3] Recently, several large genomic deletions were identified in the S strain when compared to the C strain.[4] The cattle strain appears to have a broader host range than the sheep strain as the cattle strain can be frequently isolated from sheep; however, the sheep strain appears to be more host restrictive and uncommonly isolated from cattle. The age of onset of clinical disease tends to be younger in sheep than in cattle. Unlike cattle, chronic weight loss is the primary clinical sign rather than protracted diarrhea. Only 10-20% of clinical cases in sheep present with diarrhea.[4]

AFIP Diagnosis: 1. Small intestine: Enteritis, granulomatous, chronic, multifocal, moderate, with lymphangitis and edema, Suffolk sheep, ovine.
2. Lymph node: Lymphadenitis, granulomatous, chronic, multifocal, moderate.

Conference Comment: The contributor provides a concise summary of the pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* or Johne's disease in sheep and goats. *Mycobacterium avium* subsp. *paratuberculosis* are acid-fast, weakly Gram-positive, non-spore forming, non-motile, facultative intracellular bacilli. They are slow growing in culture and dependent on mycobactin as a source of iron.[8] The mycolic acid in the cell wall of Mycobacteria makes them acid-fast allowing them to retain carbolfuschin stains after treatment with an acid-alcohol wash. Once Mycobacteria enter macrophages they block phagosome-lysosome fusion by several mechanisms allowing replication within phagosomes.[5]

In cattle, paratuberculosis is characterized by profuse diarrhea, emaciation, and hypoproteinemia in animals over 19 months of age. In sheep and goats, the clinical signs are similar to those seen in cattle with the absence of diarrhea. However, the pygmy goat is unusual in that some develop an explosive diarrhea and die unexpectedly.[6] The moderator emphasized that *Mycobacterium avium* subsp. *paratuberculosis* is one of the top three differentials for chronic wasting in sheep (thin-ewe syndrome). The two other differentials are *Corynebacterium pseudotuberculosis* (caseous lymphadenitis) and malnutrition.

Gross lesions in cattle with Johne's disease include a diffusely thickened intestinal mucosa folded into transverse rugae; mesenteric lymphadenopathy with noncaseating granulomas that contain high numbers of foamy macrophages with many acid-fast bacilli; granulomatous lymphangitis; atrophy of skeletal muscle and fat; and dependent intermandibular edema (bottle jaw). Aortic mineralization, when observed, is specific for Johne's disease in cattle. Hepatic microgranulomas sometimes occur. In contrast, sheep, goats, and deer form tuberculoid (caseating) granulomas with high numbers of epithelioid macrophages and low numbers of acid-fast bacilli. Additionally, granulomas in small ruminants can mineralize while caseation and mineralization is extremely rare in cattle.[6,8] The moderator added that massive serous atrophy of fat and dependent mandibular edema/bottle jaw are often seen in sheep with Johne's disease.

In horses, *Mycobacterium avium-intracellulare complex* (MAIC) can occasionally cause a proliferative enteritis similar to Johne's disease in cattle.[7] *Mycobacterium avium* subsp. *paratuberculosis* has been implicated in some cases of Crohn's disease in humans.[6,8]

Conference attendees briefly reviewed the classification of Mycobacteria. *Mycobacterium avium-intracellulare complex* (MAIC) includes *M. avium*, *M. intracellulare*, *M. scrofulaceum*, and *M. avium* subp. *paratuberculosis*. By convention, the term "tuberculosis" is reserved for infections caused by Mycobacteria in the *M. tuberculosis* complex (MTC) and include *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. "Atypical Mycobacteria" include all

Mycobacteria except those in the MAIC or MTC groups, *M. leprae*, and *M. lepraemurium*.[7]

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References:

1. Sigurdardottir OG, Press CM, Evensen O: Uptake of *Mycobacterium avium* subsp. *paratuberculosis* through the distal small intestinal mucosa in goats: An ultrastructural study. Vet Pathol 38:184-189, 2001
2. Perez V, Garcia Marin F, Badiola JJ: Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. J Comp Pathol 114:107-122, 1996
3. Stehman SM: Paratuberculosis in small ruminants, deer and South American camelids. Vet Clin North Am Food Anim Prac 12(2):441-455, 1996
4. Marsh IB, Bannantine JP, Paustian ML, Tizard ML, Kapur V, Whittington J: Genomic comparison of *Mycobacterium avium* subsp. *paratuberculosis* sheep and cattle strains by microarray hybridization. J Bacteriol 188:2290-2293, 2006
5. McAdam AJ, Sharpe AH: Infectious Diseases. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., p. 381. Elsevier Saunders, Philadelphia, Pennslyvania, 2005
6. Gelberg HB: Alimentary System. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 372-374. Mosby Elsevier, St. Louis, Missouri, 2007
7. Hines ME, Kreeger JM, Herron AJ: Special topic overview. Mycobacterial infections of animals: Pathology and pathogenesis. Laboratory Animal Science 45(4):334-351, 1995
8. 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. 247-252. Academic Press, Inc., San Diego, CA, 1993

CASE II - IVABS Massey University NZ628-2 (AFIP 3026727).

Signalment: 12-week-old, male, crossbred pig.

History: Small piggery with wasting syndrome in weaner pigs first identified in 2003. In previous 3 months the herd has lost approximately 3% of weaners and finishing pigs, mainly 8-12 weeks of age. The tissues presented are from a pig killed in extremis that had gross skin lesions which were representative of other affected animals.

Gross Pathology: The carcass was in poor general condition. All lymph nodes, and particularly those of the superficial inguinal and mandibular lymphocentres, were diffusely enlarged, sometimes nodular and those draining skin and subcutis had reddened subcapsular tissue. The skin was studded with innumerable 0.5-2.0cm roughly circular red to purple macules that often coalesced into plaques, some crusted. Oedema was present in dependent regions and over bony prominences. The lesions were distributed widely over the body but were most extensive over the hind quarters, ventral abdomen and head. The kidneys were diffusely swollen, somewhat pale and the capsular and incised cortical surfaces contained multiple petechial haemorrhages.

Laboratory Results:
Immunohistochemistry: IgG and IgM were demonstrated in glomeruli and renal effusions.

Insitu hybridization: Strong PCV2 DNA signals in histiocytes of follicle centres and splenic periarteriolar sheaths, weak signal in renal tubule epithelium.

Histopathologic Description: 1. Kidney: Most glomeruli in the section are distorted by fibrinous exudate that distends the urinary space or has formed in capillaries. Small numbers of neutrophils, sometimes degenerate are enmeshed in the coagula. Tubules are distended by intralumenal sero-haemorrhagic fluid and proteinaceous casts. The epithelial changes are of irregular distribution throughout the cortex and vary from necrosis (infrequent and often associated with neutrophil infiltrates), to hyaline droplet reabsorption, hyperplasia and hypertrophy, representing the acute to subacute responses to injury. Sparse focal aggregates of lymphocytes and plasma cells are present in the interstitium. Infrequent (one or two in the submitted sections) arcuate and interlobular arterioles have fibrinoid degeneration of the media accompanied by endothelial cell necrosis, thrombosis and leukocyte infiltration of the vessel wall and perivascular tissue.

2. Spleen: Many of the small arteries and arterioles are obliterated by fibrinoid necrosis, but transmural cellular infiltrates that often include degenerate neutrophils, are recognizable in some of the affected vessels. The periarteriolar sheath lymphoid cells are depleted, and there is a concomitant increase in macrophages, often with bi- and multi-nucleate forms.

Contributor's Morphologic Diagnosis: 1. Kidney: Glomerulonephritis, fibrinous, diffuse and severe with leucocytoclastic vasculitis, porcine (*Sus scrofa*).

2. Spleen: Arteriolitis, fibrinonecrotic, leucocytoclastic, random with periarteriolar lymphoid atrophy and histiocytic infiltration, porcine, (*Sus scrofa*).

Contributor's Comment: The diagnosis of porcine dermatitis and nephropathy syndrome (PDNS) was made in this and other affected pigs on this property. PDNS was first recognised the United Kingdom and has subsequently been reported in most major pig producing countries world-wide.[1,2]

The clinical and pathological features of the disorder are characterized by the appearance of haemorrhagic skin plaques and erosions that are followed by variable signs of pyrexia, anorexia, weight loss, depression and (usually) death.[2] In fatal cases the haemorrhagic renal and cutaneous lesions are the most characteristic features at necropsy, resulting from a necrotising vasculitis with microscopic and immunological features that are consistent with an immune-mediated process, most likely a type III hypersensitivity and/or direct cytotoxic T cell response. The latter is supported by the demonstration of immunoglobulins, complement fragments and CD8+ lymphocytes in the lesions.[3]

Although an inciting antigen has yet to be defined, it is widely suspected that porcine circovirus-2 (PCV2) is implicated.[4] PRRSV, another widely cited causal or contributory agent, does not occur in New Zealand.

AFIP Diagnosis: 1. Kidney: Glomerulonephritis, necrotizing, acute, diffuse, severe, with hemorrhagic and proteinaceous casts, glomerular fibrin thrombi, neutrophilic tubulitis, and tubular degeneration, necrosis, and regeneration, crossbred pig (*Sus scrofa*), porcine.

2. Spleen: Vasculitis, necrotizing, acute, diffuse, severe, with lymphoid depletion, and diffuse moderate granulomatous splenitis.

Conference Comment: PDNS is an emerging disease that primarily affects recently weaned and feeder pigs from 1.5-4 months of age.[2] Although the pathogenesis is not fully understood, both postweaning multisystemic wasting syndrome (PMWS) caused by porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (porcine arterivirus) have been implicated in the pathogenesis of PDNS.[3,4,5] In both infections, viremia can coexist with the presence of antibodies facilitating immune complex formation. Additionally, both viruses infect monocytes/macrophages and may indirectly affect the efficiency of the mononuclear phagocytic system in removing immune complexes from circulation.[6]

Typical gross lesions range from multifocal dermal petechiation and ecchymoses to dark brown to black thick crusts that are distributed primarily on the hind limbs and perineal area. With time, the skin lesions gradually fade and may leave scars.

Kidneys are enlarged, pale, and edematous with multifocal cortical petechiation in acute cases. In chronic cases, the kidneys are finely granular, shrunken, and contracted.[3,4,5,6]

Necrotizing vasculitis is a systemic feature of the disease, showing marked tropism for the skin and kidneys. Histologically, the skin lesions are characterized by marked dermal hemorrhages associated with a severe necrotizing leucocytoclastic vasculitis affecting small caliber blood vessels. Thrombosis and focal ischemic coagulative necrosis may also be present. Histopathological changes in the kidney include an exudative and occasionally necrotizing glomerulonephritis with fibrinoid deposits (immune complexes) in glomeruli that may be accompanied by an interstitial nephritis and fibrosis. Splenic vasculitis, thrombosis, and infarction are also frequently observed.[3,4,5,6]

Differential diagnoses for the skin & renal lesions seen in PDNS include:

1. Classical swine fever (porcine pestivirus)
2. *Erysipelothrix rhusiopathiae*
3. Salmonellosis
4. *Actinobacillus suis*
5. African swine fever (asfarvirus)

Conference attendees briefly reviewed causes of vasculitis in other species. The diseases that cause vasculitis in animals are summarized in a table below from Pathologic Basis of Veterinary Disease.[7]

Causes of Vasculitis in Animals

VIRAL

Equine viral arteritis (arterivirus), malignant catarrhal fever (gammaherpesvirus), hog cholera (porcine pestivirus), feline infectious peritonitis (coronavirus), bluetongue (orbivirus), African swine fever (asfarvirus), equine infectious anemia (lentivirus), bovine virus diarrhea (bovine pestivirus)

BACTERIAL

Salmonellosis, erysipelas (*Erysipelothrix rhusiopathiae*), *Hemophilus* spp. infections (*Hemophilus suis*, *Histophilus somni*, *Hemophilus parasuis*)

MYCOTIC

Phycomycosis, Aspergillosis

PARASITIC

Equine strongylosis (*Strongylus vulgaris*), dirofilariasis (*Dirofilaria immitis*), spirocercosis (*Spirocerca lupi*), onchocerciasis, elaeophoriasis (*Elaeophora schneideri*), filariasis in primates, aelurostrongylosis, angiostrongylosis

IMMUNE-MEDIATED

Canine systemic lupus erythematosus, rheumatoid arthritis, Aleutian mink disease (parvovirus), polyarteritis nodosa, lymphocytic choriomeningitis, drug-induced hypersensitivity

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References:

1. Thomson JR, Higgins RJ, Smith WJ, Done SH: Porcine dermatitis and nephropathy syndrome. Clinical and pathological features of cases in the United Kingdom. (1993-1998). J Vet Med 49:430-437, 2002
2. Chae C: A review of porcine circovirus 2-associated syndromes and diseases. Vet J 169:326-336, 2005
3. Thibault S, Drolet R, Germain M-C, D'Allaire S, Larochelle R, Magar R: Cutaneous and systemic necrotizing vasculitis in swine. Vet Pathol 35:108-116, 1998
4. Wellenberg GJ, Stockhofe-Zurwieden N, de Jong MF, Boersma WJA, Elbers ARW: Excessive porcine circovirus type 2 antibody titres may trigger the development of porcine dermatitis and nephropathy syndrome: a case-control study. Vet Micro 99:203-214, 2004
5. Cameron R: Diseases of the Skin. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 196-198. Blackwell Publishing, Ames, Iowa, 2006
6. Drolet R, Dee SA: Diseases of the Urinary System. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 203-205. Blackwell Publishing, Ames, Iowa, 2006
7. Van Vleet JF, Ferrans VJ: Cardiovascular System. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 606. Mosby Elsevier, St. Louis, Missouri, 2007

CASE III –8005-1088 (AFIP 2986827).

Signalment: Adult female white tail deer (*Odocoileus virginianus*).

History: Several wild caught adult white tail deer were presented for necropsy following a short history of vague illness of being found dead. Some animals showed bloody diarrhea of very short duration, but many were without premonitory signs. Animals were caught from various regions in Mississippi.

Gross Pathology: This adult female white tail deer in good body condition presented for necropsy within minutes of death. The tail and perineum were soiled with a small amount of frankly bloody fecal paste and dark bloody fluid dripped from the anus. Tissues were dry and tacky. The spiral colon and terminal ileum

were dark with bloody contents and moderate gas; mucosal surfaces had loosely adherent fibrinous plaques. Mesenteric lymph nodes were dark and mushy. The terminal colon had a small amount of red brown fluid and formed no feces.

Laboratory Results: Culture of the spiral colon and ileum yielded heavy growth of Clostridium perfringens, Type A (negative for enterotoxin and beta-2 toxin by PCR).

Histopathologic Description: Sections of colon showed mucosal necrosis, hemorrhage, inflammation, and exudation with myriad bacterial rods. The mucosa shows areas of collapse due to loss of glands interspersed with glands lined with attenuated epithelial cells. The luminal surface is often covered by a layer of fibrin, hemorrhage, degenerating epithelial and inflammatory cells and myriad chaining bacterial rods. Inflammatory exudate includes many pale swollen neutrophils.

Contributor's Morphologic Diagnosis: Colon: Acute necrotizing and hemorrhagic colitis.

Contributor's Comment: Toxigenic strains of *Clostridium perfringens* are typed according to exotoxin production or, more commonly in contemporary practice, by the presence of toxin genes using PCR. Classical types include type A with alpha toxin; type B with alpha, beta, and epsilon toxins; type C with alpha and beta toxins; type D with alpha and epsilon toxins, and type E with alpha and iota toxins. Clostridial enterotoxin and/or beta-2 toxin can be present or absent in any of the classical clostridial types. *Clostridium perfringens* type A has been associated with severe intraluminal hemorrhage into the small intestine (hemorrhagic bowel syndrome) in mature cattle. As well, *Clostridium perfringens* causes gas gangrene, food poisoning and other forms of necrotizing enteritis. *Clostridium perfringens* alpha toxin is a Zn^{2+} phospholipase that hydrolyses choline-containing phospholipids (phospholipase C) and has sphingomyelinase activity causing a spectrum of membrane damage including hemolysis, myotoxicity, platelet aggregation and necrosis. Why *Clostridium perfringens* proliferates and secretes lethal amounts of toxin in the gut is often speculative but probably includes decreased oxygen concentration and decreased gut motility, two interdependent variables that may be influenced by a variety of factors (feed change, antibiotic administration, ileus, etc.).[1,2,3,5]

AFIP Diagnosis: Colon (per contributor): Colitis, necrotizing, acute, diffuse, severe, with hemorrhage, white tail deer (*Odocoileus virginianus*), cervid.

Conference Comment: The contributor provides a brief summary of the toxins associated with each clostridial type. *Clostridium perfringens* is a Gram-positive,

anaerobic bacillus that is a normal inhabitant of the alimentary tract of most species of warm-blooded animals and is ubiquitous in the environment.[1,4] *Clostridium perfringens* type A is the most frequently occurring clostridial species of mammals and birds. Below is a chart of the five types of *C. perfringens*, the toxins they produce, and the most important diseases they cause.[2,3]

Clostridium perfringens - Types, toxins and diseases					
Type	Toxin				Diseases
	Alpha	Beta	Epsilon	Iota	
A	+ +	-	-	-	Gas gangrene Food Borne Illness - Humans Necrotic enteritis - Chickens Gastroenteritis - Ferrets Yellow lamb disease - enterotoxemia, western US Colitis X in horses - unproven association
B	+	+ +	+	-	Lamb dysentery Hemorrhagic enteritis - Calves, foals, guinea pigs - UK, S. Africa, Middle East
C	+	+ +	-	-	Enterotoxic hemorrhagic enteritis - Neonatal lambs, goats, cattle, pigs Struck - Adult sheep, UK
D	+	-	+ +	-	Overeating disease/ pulpy kidney - Sheep, cattle, goats Focal symmetric encephalomalacia - Sheep
E	+	-	-	+ +	Enterotoxemia - Calves, lambs, guinea pigs, rabbits
Table adapted from Barker et al, 1993 p.237 & Jones et al, 1997 p. 421					

Enterotoxin and beta-2 toxin can be produced by all types of *C. perfringens*, but are not used in typing and are, therefore, not included in the chart. *C. perfringens* type A producing beta-2 toxin is associated with necrotic enteritis in piglets and typhlocolitis in horses.[6,7]

Conference attendees considered the contributor's diagnosis of clostridial enterotoxemia. However, the typical myriad clostridial organisms were not present in our H&E or Brown-Brenn stained sections. The moderator also commented that,

in his experience, there is typically more hemorrhage associated with enterotoxemia than present in this case. Conference attendees placed diseases that cause crypt necrosis, such as bovine viral diarrhea (bovine pestivirus) and coronavirus, higher in the differential diagnosis.

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References:

1. Timoney JF, Gillespie JH, Scott FW, Barlough JE: Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th ed., pp. 223-229. Cornell University Press, Ithaca, New York, 1988
2. Barker IK, Van Dreumel AA, Palmer Nigel: The Alimentary System. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 237-244. Academic Press, Inc., San Diego, California, 1993
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 420-423. Williams & Wilkins, Baltimore, Maryland, 1997
4. Gelberg HB: Alimentary System. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 365-366, Mosby Elsevier, St. Louis, Missouri, 2007
5. Aschfalk A, Valentin-Weigand P, Müller W, Goethe R: Toxin types of *Clostridium perfringens* isolated from free-ranging, semi-domesticated reindeer in Norway. Vet Rec 151:210-213, 2002
6. Songer JG, Uzal FA: Clostridial enteric infections in pigs. J Vet Diagn Invest 17:528-536, 2005
7. Herholz C, Miserez R, Nicolet J, Frey J, Popoff M, Gibert M, Gerber H, Straub R: Prevalence of β2-toxingenic *Clostridium perfringens* in horses with intestinal disorders. J Clin Microbiol 37(2):358-361, 1999

CASE IV - CASE 1 (AFIP 3026197).

Signalment: 4-year-old , Female, Holstein Friesian, Dairy cow, bovine.

History: The cow came from a herd with problems of chronic mastitis and reduction in milk production. One quarter of the mammary gland was collected at the slaughterhouse and sent for histological and cultural analysis.

Gross Pathology: The mammary gland had an increased consistency and, on cut section, was characterized by diffuse fibrosis, thickening and ectasia of mammary

ducts, catarrhal to interstitial inflammation with some areas characterized also by purulent exudate. The supramammary lymph nodes were diffusely and severely hyperplastic.

Laboratory Results: *Prototheca zopfii* was isolated in pure culture from the mammary tissue.

Histopathologic Description: The interlobular septal interstitium of the mammary gland is moderately to severely expanded by fibrosis and variable numbers (different areas) of mature small lymphocytes, macrophages, eosinophils and neutrophils. Occasionally, nodular areas of inflammation with a necrotic center surrounded by neutrophils, macrophages and lymphocytes can be seen. Acini and ducts are multifocally severely dilated and contain abundant necrotic debris associated with neutrophils, macrophages and poorly stained algal organism free or in the cytoplasm of inflammatory cells. Atrophy of the acinar portion is evident.

In the lumens of the dilated alveoli and ducts, PAS stain reveals the presence of variably abundant free or intracytoplasmic organisms. Organisms are round to oval, non budding, 5-20 micron in diameter with a thick, intensely PAS positive cell wall. Occasional minimal internal septation can be seen.

Contributor's Morphologic Diagnosis: Mammary gland: severe, diffuse, chronic pyogranulomatous and necrotizing mastitis with acinar atrophy and interstitial fibrosis with intralesional algal structures consistent with *Prototheca spp.*

Contributor's Comment: Protothecosis is an infectious condition caused by achlorophyllic algae of the genus *Prototheca* which affects domestic animals and man. The genus *Prototheca* belongs to the family of *Chlorellaceae*. Prototheca is a unicellular, oval to spherical organism that reproduces asexually by internal septation and irregular cleavage to produce between 2 and 20 sporangiospores within a hyaline sporangium. The sporangiospores are arranged in a characteristic morula configuration and upon rupture of the sporangium, are released to develop into additional endosporulating forms. Sporangiospores measure 3 to 30 μm in diameter and differ from *Chlorella* spp. since they lack chloroplasts and from fungi since they lack glucosamine in the cell wall.[1]

Prototheca spp. are ubiquitous and have been isolated from a variety of environmental sources, including plants, mud, sewage, different water sources and soil.[1,2] Protothecal organisms can also be found in the faeces of various domestic animals or wild animals.[1,2] Occasionally, they have been detected colonizing the human skin, fingernails, respiratory tract, and digestive system.[1] It is presumed that the mechanism leading to protothecosis is the traumatic inoculation of the etiologic agent (cutaneous form) and/or endogenous colonization (gastrointestinal,

respiratory or urogenital) followed by bloodstream invasion and algaemia.[1,3] Direct transmission from human to human or animal to human has not been demonstrated. *P.wickerhamii* and *P. zopfii* are the main etiologic agents of protothecosis. *P. wickerhamii* is predominantly responsible for human protothecosis. In man, the microorganism causes a variety localized to systemic diseases primarily in immunocompromised patients.[1,4,5] The three most common forms of protothecosis described in man are: cutaneous, olecranon bursitis and disseminated.[1,4,5]

Infections caused by *P. zopfii* are most frequently observed in domestic animals. *P. zopfii* may cause chronic colitis, dermatitis, ophthalmitis and systemic infections in dogs and mastitis in the bovine species. In dairy cows, protothecosis presents primarily as a severe clinical to subclinical, often therapy-resistant mastitis causing severe economic losses.[6] *P. zopfii* is biochemically and serologically differentiated into three different biotypes. Biotype II isolates are reported as the predominant infectious agents associated with bovine mastitis.[7] Algae seem to colonize the mammary compartment via the intracanalicular route and poor milking hygiene is considered the main predisposing factor for mammary protothecosis.[6] Algal infection generally triggers a chronic inflammatory response restricted to the mammary gland and regional lymph nodes. Inflammation is predominantly granulomatous with epithelioid and multinucleated giant cells, but it is also associated with necrosis and prominent infiltration of lymphocytes, plasma cells, eosinophils and neutrophils.[8] Algal structures may be also observed in macrophages and less frequently in neutrophils. Organisms are also found within the lumen and between the lining epithelium and basement membrane of the affected alveoli.[9] In macrophages both sporangiospores and sporangia have been observed, suggesting that, due to their ability to survive and replicate in these cells, the pathogen causes a persistent infection poorly responsive to therapy.

AFIP Diagnosis: Mammary gland: Mastitis, granulomatous and eosinophilic, chronic, diffuse, moderate, with myriad algae, Holstein-Friesian, bovine.

Conference Comment: The moderator provides an excellent overview of *Prototheca*. Tissue reactions induced by *Prototheca* and *Chlorella* can be granulomatous, necrotizing, or a combination of both. When a granulomatous reaction is incited, extracellular organisms are rare. *Prototheca* cannot be differentiated from *Chlorella* on H&E stained sections. However; the green color of the gross lesions, the light microscopic detection of PAS positive starch granules, and the ultrastructural detection of typical chloroplasts in lesions caused by *Chlorella* allow differentiation between the two algal infections.[11,12]

Conference attendees reviewed these other algae and fungi that reproduce by endosporulation:

1. *Chlorella* sp.
2. *Coccidioides immitis*
3. *Rhinosporidium seeberi*
4. *Batrachochytrium dendrobatidis*

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References:

1. Pfaller MA, Diekema DJ: Unusual fungal and pseudofungal infections of humans. J Clin Microbiol 43:1495-1504, 2005
2. Costa EO da, Melville PA, Ribeiro AR, Watanabe ET, Parolari MCFF: Epidemilogic study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis. Mycopathol 137:33-36, 1997
3. Mohabeer AJ, Kaplan PJ, Southern PM Jr, Gander RM: Algaemia due to *Prototheca wickerhamii* in a patient with myasthenia gravis. J Clin Microbiol 35: 3305-3307, 1997
4. Torres HA, Bodey GP, Tarrand JJ, Kontoyiannis DP: Protothecosis in patients with cancer: case series and literature review. Clin Microbiol Infect 9:786-792, 2003
5. Pascual JS, Balos LL, Baer AN: Disseminated *Prototheca wickerhamii* infection with arthritis and tenosynovitis. J Rheumatol 31:1861-1865, 2004
6. Buzzini P, Turchetti B, Facelli R, Baudino R, Cavarero F, Mattalia L, Mosso P, Martini A: First large-scale isolation of Prototheca zopfii from milk produced by dairy herds in Italy. Mycopathologia158:427-430, 2004
7. Roesler U, Hensel A: Longitudinal analysis of *Prototheca zopfii*-specific immune responses: correlation with disease progression and carriage in dairy cows. J Clin Microbiol 41:1181-1186, 2003
8. Benites NR, Guerra JL, Melville PA, da Costa EO: Aetiology and histopathology of bovine mastitis of spontaneous occurrence. J Vet Med B 49:366-370, 2002
9. Corbellini LG, Driemeier D, Cruz E: Immunohistochemistry combined with periodic acid-schiff for bovine mammary gland with protothecal mastitis. Biotech Histochem 76:85-88, 2001
10. da Costa EO, Ribeiro MG, Ribeiro AR, Rocha NS, de Nardi Junior G: Diagnosis of clinical bovine mastitis by fine needle aspiration followed by staining and scanning electron microscopy in a *Prototheca zopfii* outbreak. Mycopathologia 158: 81-85, 2004

11. Haenichen T, Facher E, Wanner G, Hermanns W: Cutaneous Chlorellosis in a gazelle (*Gazella dorcas*). Vet Pathol 39:386-389, 2002
12. Le Net JL, Ahmed MF, Saint-Martin G, Masson MT, Montois C, Longeart L: Granulomatous enteritis in a dromedary (*Camelus dromedarius*) due to a green algal infection. Vet Pathol 30:370-373, 1993

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