The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
Todd M. Bell, DVM

WEDNESDAY SLIDE CONFERENCE 2008-2009

Conference 5

8 October 2008

Conference Moderator:

Marc E. Mattix, DVM, MSS, Diplomate ACVP

CASE I – Case HN 2516 (AFIP 3105584)

Signalment: Whooper swan, *Cygnus cygnus*

History: This wild swan was found dead at the northern lake of Japan in May, 2008. The local veterinarian found the feces of this bird were influenza virus positive using a convenient test kit. The carcass was transported to our university and dissected within our P3 facility.

Gross Pathology: Diffusely the lungs showed severe congestive edema with edematous thickening of the pleura. Petechial hemorrhages were scattered on the pericardium and pancreas. Pericardial fluid was mildly increased and accompanied mild edematous thickening of pericardium and cardiac sac. The brain was congested.

Laboratory Results: Highly pathogenic avian influenza virus of H5N1 subtype was isolated from the brain, lungs, trachea, colon and pancreas of the birds. HA titers of the virus in each organ were between 32-256, and the titer of the brain was 128.

Histopathologic Description: Several glial nodules **(Fig. 1-1)** were scattered in the CNS. The nodules sometimes contained karyorrhexis or hyperchromatosis of nuclear wall of glial cells, rod cells, satellitosis to neuronophagia of nerve cells, and minute malacic foci. The karyorrhexis of glial cells and rod cells were also sparsely distributed throughout the CNS. The nuclei of perivascular cells (pericytes and astrocytes) were sometimes swollen, and perivascular inflammatory cell infiltration was indiscernible.

Imunohistochemistry using rabbit polyclonal antibody against highly pathogenic avian influenza virus of H5N2 subtype as primary antibody revealed viral antigens in the nuclei of astrocytes **(arrow head in Fig. 1-2 and 1-3)**, microglial cells **(arrows in Fig. 1-2)**, and nerve cells **(arrows in Fig. 1-3)** within and around the glial nodules.

Besides the brain, lymphocytic necrosis in the spleen, mild fibrinous bronchopneumonia and focal necrosis of exocrine pancreas were found with viral antigens in alveolar epithelial cells, bronchial epithelial cells and exocrine pancreatic cells. Inflammatory cell infiltration was minimal in these lesions.

Contributor's Morphologic Diagnosis: Nonpurulent encephalitis, diffuse, mild, influenza virus infection

Contributor's Comment: The threat of highly pathogenic avian influenza virus of H5N1 subtype to humans as well as domestic and wild birds is a great concern

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

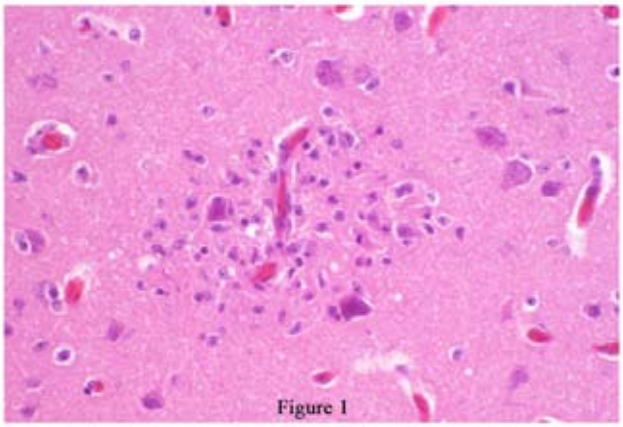


Figure 1

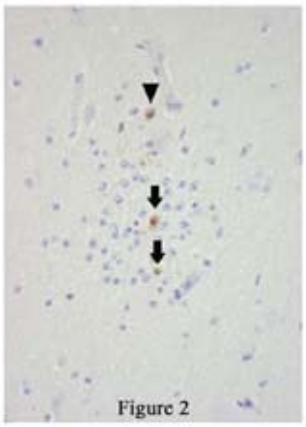


Figure 2

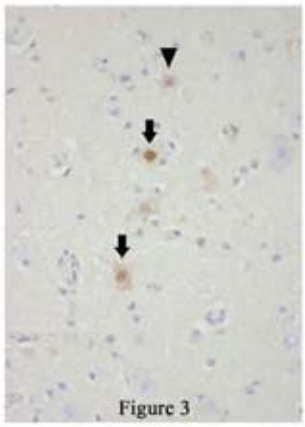


Figure 3

1-1. Cerebrum, Whooper Swan. Glial nodules within the white matter often surround blood vessels and rare degenerate or necrotic neurons. Photomicrographs courtesy of Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 North, Sapporo 060-0818, Japan.
1-2 and 1-3. Cerebrum, Whooper Swan. Immunohistochemistry using rabbit polyclonal antibody against highly pathogenic avian influenza virus of H5N2 subtype as primary antibody revealed viral antigens in the nuclei of astrocyte (arrowhead in Fig.1-2, 1-3), microglia cells (arrows in Fig.1- 2), and nerve cells (arrows in Fig.1- 3) within and around the glial nodules. Photomicrographs courtesy of Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 North, Sapporo 060-0818, Japan.

of human public health and fowl industry in worldwide magnitude.[1,10] The virus first emerged in 2003 in east and southeast Asian countries. Many human cases have been reported in Indonesia, Vietnam and China. Japan suffered the outbreaks of H5N1 infection in domestic fowl seven times from 2004 to 2007. All of these outbreaks were rapidly controlled by thorough culling. In April to May of 2008, a total of three Whooper swans were found dead in the northern lakes of Japan and highly pathogenic avian influenza viruses of H5N1 subtype were isolated from them. These birds were migrating from southern Asian countries to Siberia. The present case was one of the three cases. Genomic analysis on the isolated viruses revealed the sequence of these isolates were almost identical and were remote from those of the previous outbreaks in 2004 and 2007 in Japan.

Pathological changes of birds due to highly pathogenic avian influenza of H5N1 subtype are necrotic and hemorrhagic changes are centered in the CNS, pancreas, lungs, liver, adrenals, heart and lymphoid organs.[2-4,9,10] The CNS lesions in the present case were very mild and were at an early stage of encephalitis in comparison with previous reports on experimental or non-migratory birds.[2,4]

Birds infected with highly pathogenic avian influenza rapidly develop viremia, and then the virus infects and damages vascular endothelial cells[3,8,9] resulting in hemorrhagic and edematous changes in various organs and tissues including the skin and skeletal muscles. Necrotic and apoptotic changes of parenchymal cells of organs follow. In the CNS, the virus antigen first appears in vascular endothelial cells, then extends to astroglia and nerve cells.[8,9] In mice, the virus causes neither viremia nor endothelial damage. It invades the CNS via peripheral nerves.[5,7]

AFIP Diagnosis: Cerebrum: Neuronal necrosis, subacute, multifocal, mild with multiple glial nodules.

Conference Comment: Influenza viruses are important pathogens in both humans and animals, and their ability to cross species barriers is of major concern to medical professionals. Influenza viruses are in the family *Orthomyxoviridae,* encompassing the genera *Influenza A, B, and C,* and *Thogotovirus*. Viruses from the genus influenza A infect humans, horses, pigs, seals, birds, whales, and mink; influenza B viruses infect only humans; influenza C viruses infect humans and swine; thogotoviruses are tick borne viruses found mainly in Africa, Asia, and Europe.[6]

The reservoir for influenza A is waterfowl, and the virus causes an asymptomatic infection in these species with replication in the intestinal epithelium and subsequent fecal shedding. These problematic waterfowl often spread influenza via migratory routes, and the virus has a chance to exchange genes with other novel influenza viruses during these sojourns creating a potential for a new, virulent influenza virus.[6] Swine are important intermediate hosts because they can get both influenza A and C, and thus create an environment for viral genetic rearrangement. Influenza viruses can either undergo genetic drift, (point mutation) or

genetic shift (genetic segment reassortment) to create new strains of virus. Most combinations of influenza virus are non-pathogens, but when a drift or shift occurs creating a novel, virulent virus, pandemics such as the 1918 outbreak are the result. [6]

Highly pathogenic avian influenza in chickens and turkeys, also known colloquially as fowl plague, often causes death with little to no clinical warning. If birds survive the initial stage of disease they clinically present with severe respiratory distress along with cyanosis of the unfeathered skin to include the comb and wattles. [6] In birds, unlike mammals, influenza replicates in both the respiratory and gastrointestinal tracts. Virulent strains cause viremia with resultant necrosis of lymphoid and gastrointestinal tissue, pancreatitis, myositis, and encephalitis. Petechial hemorrhages are commonly found in the digestive, respiratory and cardiac tissues because of viral damage to endothelial cells. [6]

Contributing Institution: http://www.vetmed.hokudai.ac.jp/

References:

1. Kishida N, Sakoda Y, Isoda K, Matsuda K, Eto M, Sunaga Y, Umemura T, Kida H: Pathogenicity of H5 influenza viruses in ducks. Arch Virol **150**:1383-1392, 2005
2. Kobayashi Y, Horimoto T, Kawaoka Y, Alexander DJ, Itakura C: Pathological studies of chickens experimentally infected with two highly pathogenic avian influenza viruses. Avian Pathol **25**:285-304, 1996
3. Kwon YK, Joh SJ, Kim MC, Lee YJ, Choi JG, Wee SH, Sung HW, Kwon JH, Kang MI, Kim JH: Highly pathogenic avian influenza in Magpies (*Pica pica sericea*) in South Korea. J Wildlife Dis **41**:618-623, 2005
4. Le Gall-Recule G, Briand FX, Schmitz A, Guionie O, Massin P, Jestin V: Double introduction of highly pathogenic H5N1 avian influenza virus into France in early 2006. Avian Pathol **37**:15-23, 2008.
5. Park CH, Ishinaka M, Takada A, Kida H, Kimura T, Ochiai K, Umemura T: The invasion routes of neurovirulent A/Hong Kong/483/97(H5N1) influenza virus into the central nervous system after respiratory infection in mice. Arch Vorol **147**:1425-1436, 2002
6. Matsuda K, Park CH, Sunden Y, Kimura T, Ochiai K, Kida H, Umemura T: The vagus nerve is one route of transneural invasion for intranasally inoculated influenza A virus in mice. Vet Pathol **41**:101-107, 2004
7. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: *Orthomyxoviridae*. *In:* Veterinary Virology, 3rd ed., pp. 459-46. Academic Press, San Diego, California, 1999
8. Park CH, Ozaki H, Takada A, Kida H, Ochiai K, Umemura T: Primary target cells of virulent strains of type A influenza virus in chicken embryos. Avian Pathol **30**:269-272, 2001
9. Silvano FD, Yoshikawa M, Shimada A, Otsuki K, Umemura T: Enhanced neuropathogenicity of avian influenza A virus by passages through air sac and brain of chicks. J Vet Med Sci **59**:143-148, 1996
10. Teifke JP, Klopfleisch R, Globig A, Starick E, Hoffmann B, Wolf PU, Beer M, Mettenleiter TC, Harder TC: Pathology of natural infections by H5N1 highly patyogenic avian influenza virus in Mute (*Cygnus olor*) and Whooper (*Cygnus Cygnus*) swans. Vet Pathol **44**:137-143, 2007

CASE II – Case 07-13414-(AFIP 3101429)

Signalment: An 8-year-old neutered male domestic short hair cat

History: The cat presented to the referring veterinarian with fever, lethargy and excess salivation. Another cat in the household with similar signs had died the previous night. Both cats had been ill the previous year but had recovered. Both were indoor-outdoor cats that killed and ate wildlife. A FeLV/FIV test on this cat was negative.

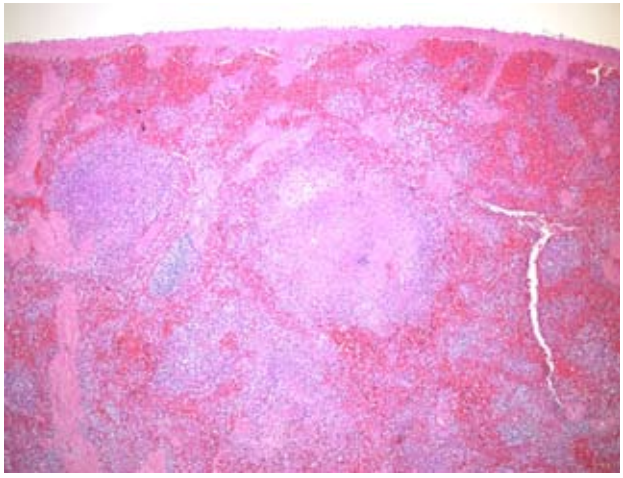
The referring veterinarian suspected tularemia due to positive cases in sheep in the same area the previous year.

Gross Pathology: The body was in good postmortem condition with good body condition. The spleen was enlarged and had pinpoint white foci throughout the parenchyma. The lungs had severe, diffuse, acute pulmonary edema; and the urinary bladder mucosa was reddened by multiple petechiae. Mesenteric lymph nodes were enlarged. The liver was slightly pale, but was considered to be grossly normal.

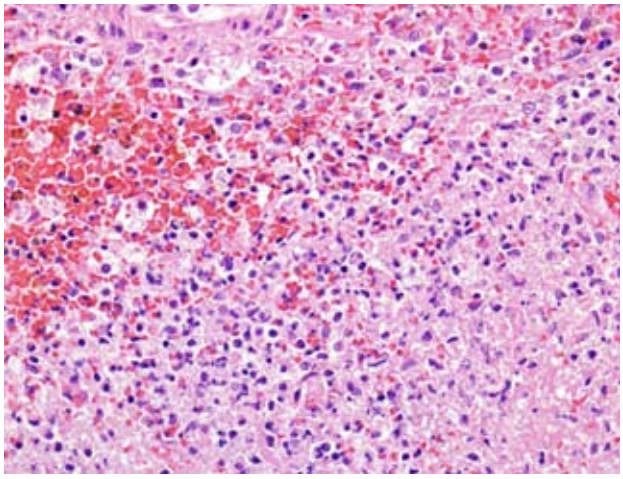
Laboratory Results: PCR for *Francisella tularensis* was positive on fresh spleen. The diagnosis was confirmed by culture in a BL-3 facility.

Histopathologic Description: Sections of liver and spleen were disrupted by multiple random foci of necrosis **(Figs. 2-1 and 2-2)**. Necrotic foci were composed of cellular debris admixed with moderate numbers of mononuclear cells and fewer neutrophils **(Fig. 2-3)**. Some foci were associated with hemorrhage. Brown and Hopp's tissue gram stain revealed small, gram negative coccobacilli both extracellularly and within macrophages.

Other lesions include large necrotic foci in the mesenteric lymph node similar to those seen in the liver and spleen



2-1 Spleen, cat. Multifocally and randomly are variably sized foci of lytic necrosis characterized by a central eosinophilic core bounded by a cellular infiltrate. (HE 20X).



2-2. Spleen, cat. Necrotic foci are bounded by moderate numbers of histiocytes, fewer neutrophils, lymphocytes and rare plasma cell admixed with hemorrhage and numerous erythrocytic laden macrophages (erythrophagocytosis). (HE 400X).

and smaller foci in the lung and bone marrow.

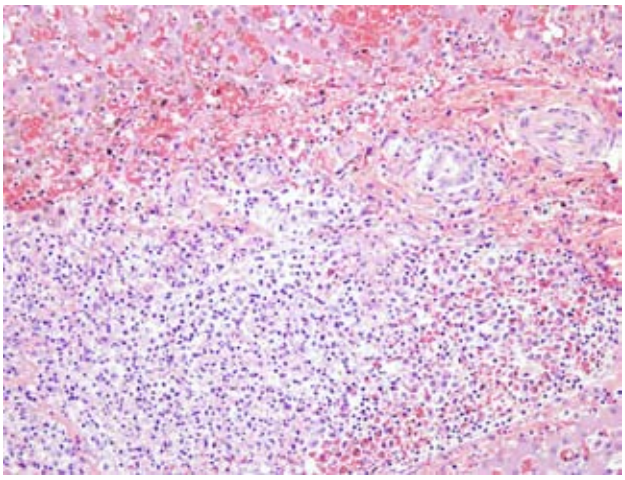
Contributor's Morphologic Diagnosis: Acute multi-focal necrotizing hepatitis and splenitis, compatible with tularemia

Contributor's Comment: Tularemia is an often fatal septicemic disease endemic in rodents primarily in the western US but distributed worldwide. [2] The disease is caused by infection with *Francisella tularensis*, originally named *Pasteurella tularensis* but later found to be unrelated genetically to other Pasteurella species. *F. tularensis* has 4 subspecies: tularensis, holartica, mediasciatica and novidia. It is the subspecies tularensis, present only in North America, which most often causes fatal zoonotic infections in other species, especially sheep and human beings. The subspecies holartica is distributed throughout the northern hemisphere and primarily causes disease in semi-aquatic rodents such as muskrats and beavers.

The organism is a small Gram-negative rod that is a facultative intracellular bacterium. It not only lives but proliferates in macrophages.[2] Transmission is by ingestion, inhalation, direct contact with skin or mucous membranes, or by arthropods, especially ticks and deer flies. Cats and dogs are thought to be somewhat resistant; however, this cat may well have been infected by ingestion of an infected rodent. Humans may be infected by direct contact or aerosols. Sheep are infected primarily by tick infestation. [4]

Clinical signs vary depending upon route of trans-mission. In humans the most common route of transmission is by direct contact in susceptible segments of the population (hunters, butchers, farmers, etc). [3] In these patients, the most common form of the disease is ulcerglandular, characterized by systemic illness with a skin ulcer at the site of infection and swelling and drainage of local lymph nodes. The increasing concern over weaponized bacteria for biological attack has brought renewed interest in the study of forms of tularemia caused by inhalation. The typhoidal form of tularemia is characterized by fever, prostration, and absence of lymphadenopathy. The pneumonia resulting from typhoidal tularemia can be severe and fatalities may reach 35%. The disease in domestic animals may be subclinical or may manifest as fatal septicemia.[4] Clinical signs previously reported in cats with tularemia include vomiting, weight loss and anorexia. [3]

The characteristic gross lesion of miliary foci of hepatic necrosis in rodents is not always visible in other species. Multifocal splenic necrosis, as seen grossly in this case, has been previously reported in other feline cases. [3] Histologic lesions of multifocal necrosuppurative inflammation in the liver, lung, spleen, and lymph nodes is characteristic of *Francisella tularensis*, but overlaps with other septicemic organisms such as *Yersinia pestis* and *Yersinia psuedotuberculosis*.[4] If possible, animals dying with a high suspicion of tularemia (signs of septicemia in an animal from an endemic area) should be necropsied under BL-2 conditions and culture should only be attempted in BL-3 facilities due to risks to laboratory personnel. In this case, characteristic gross and histologic lesions along with



2-3. Liver, cat. Predominately within the portal areas but also randomly, there are variably sized foci of necrosis characterized by loss of tissue architecture with replacement by high numbers of histiocytes, fewer neutrophils, lymphocytes and rare plasma cells admixed with karyorrhectic debris. (HE 400X).

positive PCR for *F. tularensis* allowed fresh tissues to be forwarded to an appropriate laboratory for confirmation of the diagnosis.

AFIP Diagnosis: Splenitis, necrotizing, random, multifocal, moderate with lymphoid depletion

Conference Comment: *Francisella tularensis* is a highly infectious zoonotic disease that is commonly found in the western United States. It has been reported in over 125 species of mammals, birds, reptiles, and fish. Tularemia gains access to its host by ingestion, penetration of the skin or mucous membranes, or injection by arthropods. The organism is engulfed by and multiplies within host macrophages, where it travels throughout the host via lymphatics and causes damage to vascular endothelium leading to vasculitis and thrombosis with subsequent necrotic lesions in the liver, spleen, lymph nodes, lung, and bone marrow. Cellular immunity is thought to be vital in fighting off this facultative intracellular bacteria. [1,4]

Rodents and lagomorphs are often found dead, but if found alive, they display signs of weakness and fever with lymphadenopathy. Tularemia causes a multifocal necrotizing hepatitis in rodents. Possible differential diagnoses for this lesion in rodents include Tyzzer's disease, (*Clostridium piliforme*), salmonellosis, *Listeria monocytogenes, Toxoplasma gondii, Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*. [1,4]

Contributing Institution: Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA (www.vetmed.wsu.edu/depts-vmp)

References:

1. Greene CE, DeBey BM: Tularemia. *In:* Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed., pp. 446-451. Saunders, St. Louis, MO, 2006
2. Sjdstedt A: Tularemia: epidemiology, physiology and clinical manifestations. Ann, NY Acad Sci **1105**:1-29, 2007
3. Tularemia. Institute for International Cooperation in Animal Biologics, The Center for Food Security and Public Health, Iowa State University, Ames, IA. www.cfsph.iastate.ed
4. Yalli VEO: Hematopoietic system. *In:* Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., pp. 297-298. Elsevier Limited, Edinburgh, UK, 2007

CASE III – R08-148 (AFIP 3103602)

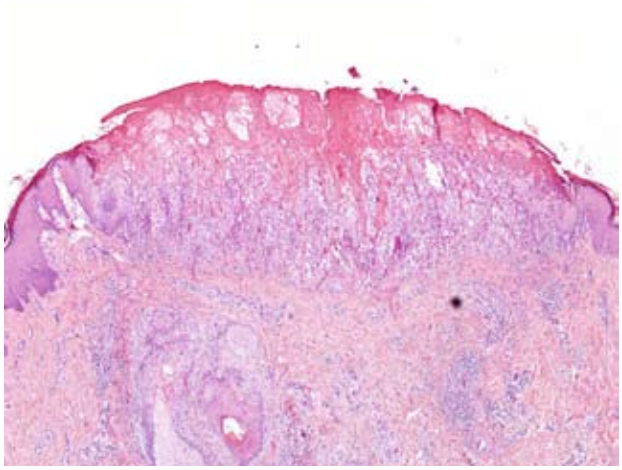
Signalment: Adult, female, crossbred goat

History: The owner of a goat/sheep ranch (open to the public) had previous history of contagious ecthyma (Orf). The owner's animals suffered from a pustular-like skin disorder with mortality around 50% during May to June of 2008.

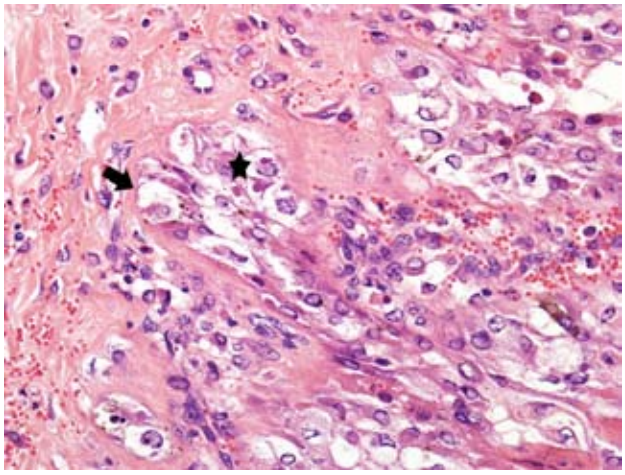
Gross Pathology: The goat submitted for necropsy showed good nutritional condition. The skin had slightly raised, 0.5~3 cm, white to red, round papules widely spread over sparsely haired areas. The mucocutaneous junction of the muzzle, nares, conjunctiva, lingual mucosa and vaginal mucosa had variably raised nodules with occasional ulcers. The lungs were discolored with a mottled, patchy, dark purple to dark grayish red appearance. Trachea and bronchi were filled with froth and white-tinged edema fluid.

Laboratory Results: PCR results were positive for capripoxvirus.

Histopathologic Description: The mucosal membranes and skin follicles consisted of thickened, hyperplastic epithelium with ballooning degeneration, vesicle formation and necrosis, where intracytoplasmic eosinophilic inclusion bodies were clearly visible **(Figs. 3-1 and 3-2)**. The superficial epidermal layers and the



3-1. Lip, goat. Focally extensive epidermal hyperplasia, ballooning degeneration, epidermal necrosis and intracorneal microvesicle formation. There is a moderate cellular infiltrate within the superficial dermis often surrounding adnexa. (HE 40X).



3-2. Lip, goat. Within the stratum basale there is hydropic degeneration (arrow) and low numbers of keratinocytes containing intracytoplasmic, 5-7 um, eosinophilic intracytoplasmic inclusion bodies (star). (HE 400X).

lingual ulcerated areas had fibrin, necrotic cellular debris, extravasated erythrocytes, and necrotic epithelium. The dermal layer was characterized by fibroplasia with a moderate infiltrate of lymphocytes, plasma cells, histiocytes, and some neutrophils. Capillaries were highly congested and some arterioles showed degeneration and perivascular infiltrates of histiocytic cells, lymphocytes, plasma cells, some neutrophils, admixed with fibroblastic cells. Intracytoplasmic eosinophilic inclusion bodies were also found in histiocytic cells and fibroblasts in the dermis in association with fibroplasia and vasculitis.

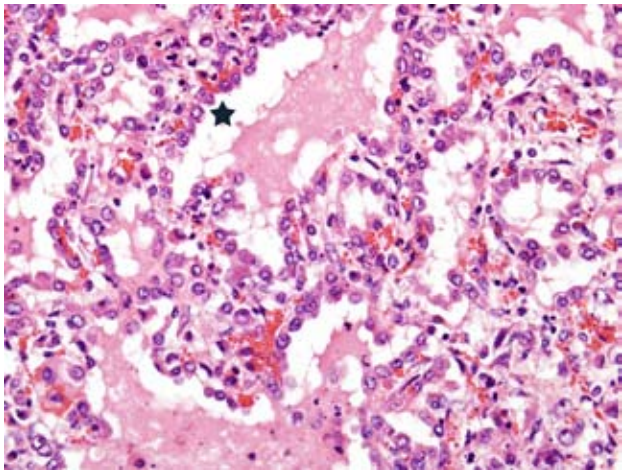
The bronchi, bronchioles, and terminal bronchioles showed varying degrees of epithelial hyperplasia, necrosis, and ballooning degeneration, with squamous metaplasia. In severely affected areas, alveolar septa were thickened as a result of pneumocyte hypertrophy. Alveolar spaces were commonly filled with fibrin, proteinaceous exudates, necrotic cellular debris, macrophages, edematous fluid with foci of alveolar septal necrosis, and septal vascular thrombi with vasculitis **(Fig. 3-3)**. Peribronchiolar and perivascular lymphocytic infiltrates were evident in some areas. Similar inclusion bodies were also detected in histiocytes, fibroblasts, epithelial cells and pneumocytes. There was lymphoid depletion along with histiocytosis in the spleen and lymph nodes, with necrosis and numerous inclusion bodies in histiocytic cells.

Contributor's Morphologic Diagnosis:
Skin: Dermatitis, hyperplasia, severe, multifocal, chronic, with vesicles, papules, ballooning degeneration, vasculitis and eosinophilic intracytoplasmic inclusion bodies

Lung: Bronchointerstitial pneumonia, hyperplasia, severe, multifocal, chronic, with ballooning degeneration, vasculitis, and eosinophilic intracytoplasmic inclusion bodies

Etiology: Capripox virus

Contributor's Comment: Capripoxvirus, the causative agent of sheep/goat pox, belongs to the family



3-3. Lung, goat. Multifocally, alveolar septa are expanded up to 5-7 times normal by predominately a lymphohistiocytic cellular infiltrate with low numbers of neutrophils and plasma cells and are lined by hypertrophied type II pneumocytes (star). The alveolar lumina contain edema, alveolar macrophages, low numbers of nondegenerate and degenerate neutrophils, and necrotic debris. (HE 400X).

Poxviridae. It is usually more severe in goats than in sheep. All goat age groups are susceptible to the virus. The disease is endemic in Africa, the Middle East, the Indian subcontinent, and much of Asia. This disease had never been reported in Taiwan. As a result, this will be the first confirmed sheep/goat pox outbreak in Taiwan.

Clinically, infected animals can have acute to chronic disease characterized by generalized pox lesions throughout the skin and mucous membranes accompanied with persistent fever, lymphadenitis, and often a focal viral pneumonia.

The differential diagnosis should include contagious pustular dermatitis (Orf), peste des petits ruminants, and bluetongue. Orf is an endemic disease in Taiwanese goat herds.

PCR for detecting Capripox or Orf virus by the primer pair CPVS, CPVA (413 bp) and OVS, OVA (708 bp) (Zheng M. et al) respectively was employed for final diagnosis of this case. The source of the outbreak, however, is inconclusive.

AFIP Diagnosis: Haired skin: Dermatitis, proliferative and necrotizing, subacute, focally extensive, moderate, with intraepidermal vesicles, ballooning degeneration, intracytoplasmic eosinophilic inclusion bodies, and periadnexal and perivascular histiocytic inflammation

Lung: Pneumonia, bronchointerstitial, proliferative, subacute, multifocal, moderate, with intraepithelial intracytoplasmic eosinophilic inclusion bodies

Conference Comment: The family Poxviridae is divided into two subfamilies: Chordopoxvirinae which infects vertebrates, and Entomopoxvirinae which infects insects. Poxviruses are double stranded DNA viruses that cause disease in numerous living organisms. Most poxvirus virions have a characteristic brick shape and are very large viruses measuring up to 250 x 200 x 200 um, with a complex structure with lateral bodies, an outer membrane, and are sometimes enveloped. The genera of the subfamily Chordopoxvirinae include: Orthopoxvirus, Capripoxvirus, Suipoxvirus, Leporipoxvirus, Molluscipoxvirus, Yatapoxvirus, Avipoxvirus, and Parapoxvirus. This table is a brief summary of some of these viruses.

GENUS	VIRUS	MAJOR HOSTS
Orthopoxvirus	Variola virus (smallpox) Vaccinia virus Cowpox virus Camelpox virus Ectromelia virus Monkeypox virus Seal poxvirus	Humans Humans, cattle, swine, rabbits Humans, cattle, cats, rats Camels Mice Humans, non-human primates Grey seals
Capripoxvirus	Sheeppox virus Goatpox virus Lumpyskin disease virus	Sheep, goats Goats, sheep Cattle, cape buffalo
Suipoxvirus	Swinepox virus	Swine
Leporipoxvirus	Myxoma virus	Rabbits
Molluscipoxvirus	Molluscum contagiosum virus	Humans, horses
Yatapoxvirus	Yabapox virus and tanapox virus	Humans, non-human primates
Avipoxvirus	Fowlpox virus	Chickens, turkeys
Parapoxvirus	Orf virus Pseudocowpox virus Bovine papular stomatitis virus	Sheep, goats, humans Cattle, humans Cattle, humans

Poxviruses are epitheliotropic viruses, and in some instances, such as with smallpox, sheeppox, goatpox monkeypox, or ectromelia, they can cause generalized, severe, or fatal disease. Grossly, poxvirus lesions progress from an initial macule, to a papule, to a vesicle, ending in pustule and and crust formation. Histologically, poxvirus infection often causes proliferation of cells within the stratum spinosum with ballooning degeneration and eosinophilic intracytoplasmic inclusions. [2,4]

Contributing Institution: Division of Animal Medicine, Animal Technology Institute Taiwan, P.O. Box 23, Chunan, Miaoli, Taiwan 350.

References:

1. Bhanuprakash V, Indrani BK, Hosamani M, Singh RK: The current status of sheep pox disease. Comp Immunol Microbiol Infect Dis **29**:27-60, 2006
2. Ginn PE, Nasell JEKL, Rakich PM: Skin and appendages. *In:* Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., pp. 297-298. Elsevier Limited, Edinburgh, UK, 2007
3. Kitching RP, Hammond JM, Black DN: Studies on the major common precipitating antigen of capripoxvirus. J Gen Virol **67**:139-148, 1986
4. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Poxviridae. *In:* Veterinary Virology, 3rd ed., pp. 277-291. Academic Press, San Diego, California, 1999
5. Parthiban M, Govindarajan R, Manoharan S, Purushothaman V, Chandran NDJ, Koteeswaran A: Comparative sequence analysis of diagnostic PCR amplicons from Indian sheeppox virus. Vet Arhiv **75**:203-209, 2005
6. Zheng M, Liu Q, Jin N, Guo J, Huang X, Li H, Zhu W, Xiong Y: A duplex PCR assay for simultaneous detection and differentiation of Capripoxvirus and Orf virus. Mol Cell Probes **21**:276-81, 2007

CASE IV – ND 1 (AFIP 3102366)

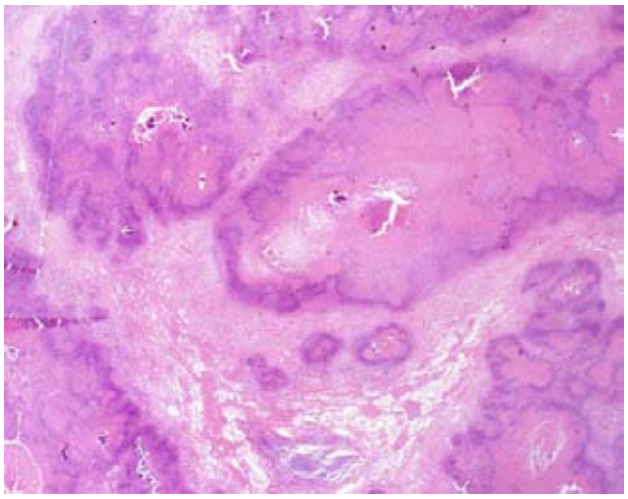
Signalment: 18-month-old female American bison (*Bison bison*)

History: The animal was sick for 10 days, gradually lost condition, developed diarrhea, and exhibited swelling in both carpal joints. Lameness led to difficulty walking. The animal eventually became recumbent and was euthanized.

Gross Pathology: Both lungs contained randomly distributed irregular, sometimes raised, variably sized foci of caseous necrosis. Both stifle joints were swollen, fluctuant on palpation, and, upon incision, oozed purulent to caseous exudate. There was marked inflammation of the joint capsules, synovial tissue, and tendon sheaths. There was a localized area of necrosis in the cervical musculature on the right side of the neck (injection site).

Laboratory Results: *Mycoplasma* spp. was cultured from lung tissue and joint exudate. This isolate was then identified as *Mycoplasma bovis* by PCR and immunohistochemistry. No other ruminant respiratory pathogens were identified in these tissues.

Histopathologic Description: Large areas of caseous necrosis and inflammation were noted in the pulmonary parenchyma **(Fig. 4-1)**. Affected areas were characterized by a distinct outer zone of variably dense fibrous connective tissue infiltrated with a mixture of inflammatory cells, a thinner middle zone consisting of a bilayer of activated macrophages and plasma cells (outer portion) and necrotic neutrophils (inner portion), and an expansive interior zone of caseous necrosis in which necrotic alveolar septa and bronchioles were seen. Normal lung adjacent to the outer fibrous connective tissue capsule had several areas of atelectasis, some accumulation of proteinic material within alveoli, and increased numbers of alveolar macrophages. Sections of synovium, joint capsule and tendon sheath had a marked necropurulent



4-1. Lung, bison. Multifocally affecting approximately 80% of the tissue section, there are large variably sized and irregularly shaped areas of lytic necrosis characterized by a deeply eosinophilic core which is often centrally mineralized and bounded by a rim of cellular infiltrate. Adjacent alveoli are compressed and atelectatic. (HE 20X).

synovitis, arthritis, and tenosynovitis respectively.

Contributor's Morphologic Diagnosis: Severe, multifocal to coalescing, caseonecrotic pneumonia with bronchiectasis and atelectasis

Contributor's Comment: *Mycoplasma bovis*-associated disease manifests itself in a variety of ways in cattle including pneumonia and tenosynovitis, arthritis, keratoconjunctivitis, otitis media, and mastitis.[2] A condition caused by *M. bovis* characterized by chronic pneumonia and polyarthritis has been recognized in feedlot cattle.[3] Coinfection with Bovine viral diarrhea virus (BVDV), and common bovine respiratory viruses appears to occur with some frequency. Previous studies utilizing IHC to examine the pattern of bacterial colonization in *M. bovis* infected lungs described staining in bronchiolar epithelial cells, inflammatory cells, and abscessed airways (naturally infected animals), random staining in areas of both coagulative and caseous necrosis with peripheral zones of purulent to pyogranulomatous inflammation (naturally infected animals), and staining in areas of coagulative necrosis and bronchiolar epithelium (naturally infected animals) and in inflammatory cells in alveoli and septal walls (experimentally infected animals).[3] Staining patterns in naturally infected animals were consistent with the type of staining seen in this animal. The most intense staining was observed in bronchiolar epithelial cells. This pattern of staining supports the proposed pathogenesis for this condition, which is early bronchitis leading to bronchiectasis that eventually coalesces into large zones of caseous necrosis.[1] Characteristics of this case indicate that bison are susceptible to severe infections with this pathogen, and that *M. bovis* is capable of causing primary disease in this species.

AFIP Diagnosis: Lung: Pneumonia, necrotizing, fibrinosuppurative, diffuse, severe with interlobular edema and fibrosis

Conference Comment: *Mycoplasma bovis* is of significant economic importance in both the United States and Europe. It is the cause of enzootic pneumonia in young calves and a cause of chronic pneumonia and polyarthritis in adult cattle. This disease in feedlot cattle mimics shipping fever, but infected cattle are often times refractory to antimicrobial treatment with continued clinical decline. *M. bovis* is spread via genital, nasal, and mammary secretions.[1] *M. bovis* is very effective at evading the host immune response by a variety of mechanisms too in-depth to discuss here, and subsequently this organism causes chronic infections despite valiant clinical intervention.

Grossly, *M. bovis* can cause striking lesions within the respiratory tract characterized by multiple, well demarcated, caseonecrotic nodules up to a few centimeters in diameter dispersed throughout the cranioventral lung lobes. Joint lesions are characterized by a reddened and reactive synovium and serofibrinous exudate within the joint capsule.[1] Otitis media is an additional sequelae in calves to *M. bovis* infection.[1]

Histologically, acute lesions begin in the airways and progress to areas of multifocal to coalescing caseonecrotic debris often containing mineral. Early in the progression of *M. bovis* infections, leukocytes are necrotic but retain their cellular architecture and contain hypereosinophilic cytoplasm and karyorrhectic nuclei. This is characteristic for respiratory lesions caused by *M. bovis*.[1] Foci of necrosis with this distinctive gross and histologic appearance are pathognomonic for *M. bovis* in cattle.[1]

Contributing Institution: North Dakota State University, Veterinary Diagnostic Laboratory, 1523 Centennial Boulevard, Fargo, ND, 58105, http://www.vdl.ndsu.edu/

References:

1. Caswell JL, Williams KJ: Respiratory system. *In:* Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., pp. 611-614. Elsevier Limited, Edinburgh, UK, 2007

2. Dyer N, Hansen-Lardy L, Krogh D, Schaan L, Schamber E: An outbreak of chronic pneumonia and polyarthritis syndrome caused by *Mycoplasma bovis* in feedlot bison (*Bison bison*). J Vet Diagn Invest **20**:369-371, 2008

3. Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL: Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. J Vet Diagn Invest **18**:29-40, 2006