The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2006-2007

CONFERENCE 12
10 January 2007

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CASE I – 03-0212 (AFIP 2986822).

Signalment: 9-month-old female African Grey parrot (*Psittacus erithacus*).

History: This parrot was found dead in a commercial aviary and was submitted for postmortem examination.

Gross Pathology: At necropsy, general body condition was poor; the serosal membranes including air sacs were turbid, wet and covered by few fibrin tags. The liver was enlarged, markedly firm, and had patchy multifocal pale firm areas (Fig.1). The spleen was enlarged and markedly congested.

Laboratory Results: PCR for *Chlamydophila psittaci* was positive on liver and spleen.

Histopathologic Description: Microscopically, there was multifocal hepatocellular individualization, loss and necrosis. Necrotic hepatocytes had pyknotic nuclei and hypereosinophilic cytoplasm and contained myriad intracytoplasmic basophilic inclusions or clusters of organisms (cocci, 1 micron in diameter and stained red against a green background with PVK (Pierce-van der Kamp) stain (Figs 2 and 3). Similar organisms were present in the cytoplasm of the splenic reticuloendothelial cells. Serous membranes were edematous and infiltrated with high numbers of histiocytes (containing similar organisms) and fewer heterophils. Based on the aforementioned lesions and the presence of typical organisms, a tentative diagnosis of avian chlamydiosis was made and confirmed by identification of *Chlamydophila psittaci* (formerly *Chlamydia psittac*i) using PCR.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, lymphocytic and plasmacytic, necrotizing, subacute with myriad intrahepatocellular organisms typical of *Chlamydophila* spp.

Contributor's Comment: *Chlamydophila psittaci* (*C. psittaci)* is an obligate intracellular bacterium that affects a wide variety of birds including psittacines, turkeys, waterfowl, and many species of pet birds causing lesions that range from mild sinusitis and conjunctivitis to severe necrotizing multisystemic disease.[1,2] Chickens are relatively resistant and the incidence of epidemics in commercial breeds is rare.[2] Transmission of chlamydiae is by inhalation and ingestion of contaminated materials. *C. psittaci* is a serious zoonotic pathogen that infects humans by inhalation of contaminated materials causing severe pneumonia (Ornithosis). Avian chlamydiosis is an immediately notifiable disease in many countries and many laboratories require that the handling of infected materials and postmortem be done in a biosafety level III lab. Differential diagnosis for necrotizing hepatitis in parrots should include bacterial septicemia and the infection with psittacid herpesvirus (Pacheco's disease), avian polyomavirus, adenovirus, avian circovirus (Psittacine beak and feather disease virus), and avian reovirus.[1]

AFIP Diagnoses: Liver: Hepatitis, necrotizing, random, moderate, with intrahepatocellular bacteria, African Grey parrot (*Psittacus erithacus*), avian.

Conference Comment: The family Chlamydiaceae has been reclassified into two genera, *Chlamydia* and *Chlamydophila*. Under the new classification, *Chlamydia* includes three species (*C. trachomatis*, *C. muridarum*, and *C. suis*) and *Chlamydophila* includes six species (*C. psittaci*, *C. abortus*, *C. felis*, *C. caviae*, *C. pneumoniae*, and *C. pecorum*).[2]

All chlamydiae are gram-negative; however, Gram stains are of no practical value in identifying chlamydiae. In some cases, the organisms may be seen in Giminez, Machiavello, Giemsa, or Castañeda stained impression smears of liver, spleen, or air sacs. Electron microscopy is a good method for definitive diagnosis of *Chlamydophila*. There are three morphologically distinct forms:[1,2,3]

1. Elementary body (EB) – infectious form; 0.2-0.3 um diameter characterized by a highly electron dense nucleoid at the periphery of the EB clearly separated from an electron dense cytoplasm
2. Reticulate body (RB) – intracellular, metabolically active form; 0.5-2.0 um diameter; lacy or reticular nucleus, "hour-glass" profiles when undergoing binary fission
3. Intermediate body (IB) – 0.3-1.0 um diameter; central electron dense core with radially arranged individual nucleoid fibers surrounding the core;

cytoplasmic granules tightly packed at the periphery of the IB separated from the core by a translucent zone

Typical gross findings in birds include hepatomegaly, splenomegaly, fibrinous air sacculitis, pericarditis, and peritonitis.[2]

Typical light microscopic findings in birds include multifocal hepatic necrosis, lymphoplasmacytic portal hepatitis, intrahepatocellular bacteria, multifocal splenic necrosis, splenic histiocytosis with intrahistiocytic bacteria, splenic reticuloendothelial cell hyperplasia, and a fibrinous air sacculitis with heterophils and macrophages.[1,3]

Chlamydophila psittaci affects a wide range of hosts. A modified chart of diseases caused by *C. psittaci* in various species is included below.[3]

Disease caused by *C. psittaci*

Psittacosis (ornithosis)	Humans, birds
Sporadic bovine encephalitis	Cattle
Polyarthritis	Cattle, sheep, horses
Enzootic bovine abortion	Cattle
Enzootic ovine abortion	Sheep
Abortion	Horses, swine
Pneumonia	Cattle, sheep, goats, horses, dogs, rabbits
Conjunctivitis	Sheep, cats, guinea pigs, hamsters
Enteritis	Cattle, pigs, muskrats, snowshoe hares

There was some variation among slides with some sections containing a focus of nodular histiocytic inflammation.

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References:

1. Schmidt RE, Reavill DR, Phalen DN: Pathology of Pet and Aviary Birds, pp. 68-82. Iowa State Press, Ames, Iowa, 2003
2. Anderson AA, Vanrompay D: Avian Chlamydiosis (psittacosis, ornithosis). In: Diseases of Poultry, eds. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, 11th ed., pp. 863-879. Iowa State Press, Ames, Iowa, 2003
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 402-410. Williams & Wilkins, Baltimore, Maryland, 1997

CASE II – XN3076 (AFIP 2988331).

Signalment: 3-year-old, male, red-tailed boa constrictor, snake, *Boa constrictor*.

History: Two captive bred 3-year-old boa constrictors (*Boa constrictor*) were purchased by a reptile collector in the United Kingdom in October 2004, housed together in a vivarium and fed killed thawed mice, rats and day-old chicks. The male of the pair had radiologically confirmed spondylosis involving vertebral segments over a length of 25 cm at mid-body level. This snake exhibited regurgitation and developed stomatitis one week after purchase. It was treated with 10 mg/kg enrofloxacin (Baytril 2.5% Oral Solution, Bayer) administered by daily gavage for one week and appeared to recover. It developed anorexia in early February 2005 and was found dead on 9 March 2005, four months after purchase.

Gross Pathology: At postmortem examination, the male boa constrictor was 1.6 m long, weighed 2.9 kg and had adequate reserves of body fat. The lungs contained frothy greenish brown fluid and there was oedema around the lungs and heart.

Laboratory Results: Bacteriology: Profuse growths of *Salmonella enterica* serovar San Diego were recovered from the lungs and intestine at postmortem examination.

Histopathologic Description: Histologically, the snake had a proliferative pneumonia, with papillary expansion of interconnecting trabeculae lined by ciliated or non-ciliated columnar epithelial cells, supported by fibrovascular connective tissue. There were mild multifocal infiltrates of lymphocytes and plasma cells in the interstitial tissue and mild individual degeneration of epithelial cells. Large numbers of small bacilliform bacteria were present in the lumen of the lung, but there were few inflammatory cells in the luminal exudate. Numerous single or occasionally multiple, ovoid, eosinophilic cytoplasmic inclusion bodies, 1 to 5 mm in diameter, were detected in epithelial cells in the lungs. Similar inclusion bodies were also detected in the kidneys, pancreas, stomach and intestine. Pigment deposits and interstitial fibrosis were evident in the kidneys. There was mild individual degeneration of renal tubular epithelial cells and occasional sloughing of cells into tubule lumina. Diffuse vacuolation of hepatocytes, with ballooning degeneration and moderate numbers of inclusion bodies, was evident in the liver. Inclusion bodies were also detected in neurons and glial cells in the brain in association with mild meningoencephalitis. There was lymphoid depletion in the spleen, with fibrosis and numerous inclusion bodies in lymphoreticular cells.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, proliferative, diffuse, severe, with eosinophilic cytoplasmic inclusion bodies, snake, *Boa constrictor*.

Contributor's Comment: Boid inclusion body disease (IBD) is an important transmissible disease of captive snakes that occurs worldwide.[1,2,3] The disease occurs primarily in boids and pythons (Family *Boidae*), including the boa constrictor (*Boa constrictor*). It has also been diagnosed in colubrids (Family *Colubridae*) and viperids (Family *Viperidae*), but appears to be less common in these groups of snakes.[2,4] Boid IBD is characterised clinically by anorexia, regurgitation, weight loss, lethargy and neurological signs, including disorientation, incoordination, head tilting, "star gazing", tremors, convulsions and flaccid paralysis.[1,2,3] Affected snakes usually die or are euthanased after a prolonged clinical course. The disease appears to be immunosuppressive, permitting the development of secondary disease, such as bacterial pneumonia and stomatitis. Histopathological changes in affected snakes include demyelinating encephalomyelitis, interstitial pneumonia, hepatopathy with vacuolation and ballooning degeneration of hepatocytes, pancreatic atrophy and nephrosis.[1,2,3] Lymphoid depletion is also evident. Eosinophilic inclusion bodies are present in the cytoplasm of epithelial cells in the lungs, gastrointestinal tract, kidneys and pancreas, as well as in hepatocytes, neuroglial cells in the brain and lymphoreticular cells in the spleen.[1,2,3]

The aetiology of boid IBD is currently a matter of controversy. Retroviruses have been isolated in cell culture and detected by electron microscopy in tissues from affected snakes.[1,5] However, these may be endogenous retroviruses that are not aetiologically associated with boid IBD.[6] Ophidian paramyxoviruses (OPMV), of which more than 18 types have been recognized, are associated with necrotising or proliferative interstitial pneumonia, meningoencephalitis and mortality in viperids and colubrids.[7,8,9] Eosinophilic inclusion bodies, along with occasional multinucleate syncytia, are usually produced in the cytoplasm of infected cells. The degree to which boids are susceptible to OPMV is uncertain. OPMV type 7 has been isolated from a reticulated python (*Python reticulatus*) with respiratory disease in the UK.[10] High antibody titres against OPMV were detected by haemagglutination inhibition in serum from an unaffected reticulated python following an outbreak of disease in viperids in the USA.[9] *In situ* hybridisation was positive for paramyxovirus sequences in the brain of a Boelen's python (*Morelia boeleni*) with meningoencephalitis and eosinophilic cytoplasmic inclusions in glial cells in the brain but not in other tissues.[11] There is currently insufficient evidence to implicate OPMV in the aetiology of boid IBD, despite the pathological similarities. The cytoplasmic inclusion bodies in boid IBD are widely distributed in epithelial, nervous and lymphoreticular tissues.[1] Infection with OPMV may produce inclusion bodies in the lungs, brain, liver and kidneys, although they are less numerous, multinucleate syncytia may be present and there is usually a more pronounced suppurative and necrotising pneumonia.[8,9,11]

The proliferative pneumonia in the affected snake may be attributable to boid IBD, since there were only mild lymphoplasmacytic inflammatory infiltrates in the lungs,

limited necrosis, minimal exudation of heterophils and no evidence of multinucleate syncytia. A profuse growth of *Salmonella enterica* serovar San Diego was obtained from the lungs at postmortem examination and large numbers of bacteria were present in the pulmonary exudate. The *Salmonella* isolate may have been an opportunistic colonist of the lungs in an immunocompromised snake secondary to boid IBD, possibly related to regurgitation and inhalation of gastrointestinal contents.

AFIP Diagnosis: Lung: Bronchointerstitial pneumonia, proliferative, heterophilic and lymphoplasmacytic, diffuse, moderate, with edema, fibrin, and hemorrhage, numerous epithelial eosinophilic intracytoplasmic inclusion bodies, Gram negative bacilli and Gram positive cocci.

Conference Comment: The contributor provides a thorough overview of boid inclusion body disease (IBD) and compares and contrasts it with ophidian paramyxoviruses (OPMV). The controversial association of a type C retrovirus and IBD was discussed during conference. Ultrastructurally, the inclusions appear as electron dense structures that may vary in size and shape. The inclusions may represent previral material or some type of storage material from a dysfunctional cell. In one study, an antigenically distinct 68-kilodalton protein was isolated and characterized from nonviral inclusions in IBD-infected Boa Constrictors.[12]

As pointed out by the contributor, IBD affects both boids and pythons. Boas may be inapparent carriers. However, the severity of the disease is significantly worse in pythons, which have a rapid clinical course that progresses to a fatal CNS disturbance. There is no treatment for IBD and infected snakes die. Therefore, boas and pythons should not be mixed in the same collection. The snake mite, *Ophionyssus natricis*, is suspected as a vector associated with the spread of disease. Other modes of transmission include direct contact and venereal spread.[13]

Gross lesions are frequently limited to changes associated with secondary bacterial infections, such as pneumonia, stomatitis, and bacterial granulomas within the liver and kidneys. In some species, such as Boa Constrictors, fibrous changes and splenic atrophy may be observed.[12]

Some conference participants favored OPMV. The moderator preferred a diagnosis of IBD since the inclusions were widespread in other organs and there was a lack of a pronounced suppurative and necrotizing pneumonia as pointed out by the contributor. Additionally, in his experience, it is uncommon to see so many inclusions with OPMV infections. He also added that the syncytial cells seen in

OPMV infections are typically striking and the inclusions are more pleomorphic similar to those seen with canine distemper virus.

Gram stains performed at the AFIP revealed myriad Gram negative bacilli as well as chains and pairs of Gram positive cocci within the pulmonary exudate and were most likely opportunistic pathogens in this debilitated snake.

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References:

1. Schumacher J, Jacobson ER, Homer BL, Gaskin JM: Inclusion body disease in boid snakes. J Zoo Wildl Med 25:511-524, 1994
2. Carlisle-Nowak MS, Sullivan N, Carrigan M, Knight C, Ryan C, Jacobson ER: Inclusion body disease in two captive Australian pythons (*Morelia spilota variegata* and *Morelia spilota spilota*). Aust Vet J 76:98-100, 1998
3. Orós J, Tucker S, Jacobson ER: Inclusion body disease in two captive boas in the Canary islands. Vet Rec 143:283-285, 1998
4. Raymond JT, Garner MM, Nordhausen RW, Jacobson ER: A disease resembling inclusion body disease of boid snakes in captive palm vipers (*Bothriechis marchi*). J Vet Diagn Invest 13:82-86, 2001
5. Jacobson ER, Orós J, Tucker SJ, Pollock DP, Kelley KL, Munn RJ, Lock BA, Mergia A, Yamamoto JK: Partial characterization of retroviruses from boid snakes with inclusion body disease. Am J Vet Res 62:217-224, 2001
6. Huder JB, Boni J, Hatt JM, Soldati G, Lutz H, Schupbach J: Identification and characterization of two closely related unclassifiable endogenous retroviruses in pythons (*Python molurus* and *Python curtus*). J Virol 76:7607-7615, 2002
7. Franke J, Essbauer S, Ahne W, Blahak S: Identification and molecular characterization of 18 paramyxoviruses isolated from snakes. Virus Res 80:67-74, 2001
8. Jacobson E, Gaskin JM, Page D, Iverson WO, Johnson JW: Illness associated with paramyxo-like virus infection in a zoologic collection of snakes. J Am Vet Med Assoc 179:1227-1230, 1981
9. Jacobson ER, Gaskin JM, Wells S, Bowler K, Schumacher J: Epizootic of ophidian paramyxovirus in a zoological collection. Pathological, microbiological, and serological findings. J Zoo Wildl Med 23:318-327, 1992
10. Manvell RJ, Drury SE, Geach M, Lewis JC: Isolation of ophidian paramyxovirus type 7 from a reticulated python in the UK. Vet Rec 147:696, 2000
11. West G, Garner M, Raymond J, Latimer KS, Nordhausen R: Meningoencephalitis in a Boelen's python (*Morelia boeleni*) associated with paramyxovirus infection. J Zoo Wildl Med 32:360-365, 2001

12. Schumacher J: Inclusion body disease virus. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 836-840. Saunders Elsevier, St. Louis, Missouri, 2006
13. Bennet RA, Mehler SJ: Neurology. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 248-249. Saunders Elsevier, St. Louis, Missouri, 2006

CASE III – S 2674/00 R (AFIP 2788661).

Signalment: 1 year old, male, Chinchilla (*Chinchilla lanigera f. domestica*)

History: The chinchilla had been kept as a single animal by a private owner. The animal suffered four days from neurological signs characterized by seizures, biting into the cage wire and problems swallowing. Therapy with antibiotics and electrolytes did not improve the condition. The animal was euthanized.

Gross Pathology: Except for a moderate unilateral purulent rhinitis no gross lesions were detected.

Contributor's Morphologic Diagnosis: Chinchilla, brain: Multifocal acute, moderate, lymphocytic meningitis and severe, acute, diffuse encephalitis with intraneuronal eosinophilic inclusion bodies.

Contributor's Comment: The histological findings in the brain of this case were indicative of a virus infection consisting of a lymphocytic leptomeningitis and an acute encephaltis with perivascular cuffing and extensive neuronal damage. The distribution of lesions was bilateral in the cerebral hemispheres, basal ganglia and hippocampus, whereas the rhinencephalon was affected more unilaterally. Other parts of the brain including the trigeminal ganglia and the optic chiasm showed only minor or no lesions. Predominantly in neurons, intranuclear inclusion bodies were present either as eosinophilic inclusions surrounded by a clear halo and marked margination of chromatin along the nuclear membrane or homogenous amphophilic inclusions occupying the whole nucleus.

Ultrastructural examination of brain tissue post-fixed with glutaraldehyde revealed enveloped virus particles of 120-140 nm in diameter with morphology consistent with a herpes virus. Immunohistochemistry was performed using polyclonal antibodies for human herpes simplex virus 1 and 2 resulting in extensive labeling of viral antigen. Native brain samples were used for virological culture in vero cells. A rapidly growing virus causing a severe cytopathogenic effect was isolated. Two genome fragments encoding for replicative polymerase and glycoprotein B were amplified by PCR and subsequently sequenced. The comparison with known

sequences of other herpesviruses resulted in a 100% and 99.5% homology with the human herpes simplex virus type 1 (HSV1) and type 2 (HSV2), respectively. Histological examination of other tissues revealed a unilateral purulent rhinitis corresponding with the predominant unilateral affection of the rhinencephalon. In the cutaneous mucous membrane of the nasal vestibulum single erosive to ulcerative lesions with intranuclear inclusion bodies in epithelial cells were present. Additionally, occasional circumscribed necrosis was found in the adrenal glands. Only in the nasal mucosa was viral antigen demonstrated by immunohistochemistry.

The findings in the nasal cavity and the unilaterally predominant affection of the rhinencephalon are suggestive of a rhinogenic infection with HSV1. The mode of infection was probably close contact to a person with herpes labialis. Reports about virus infections in chinchillas are extremely rare. One case is described in Canada with a circumscribed brain stem encephalitis associated with necrotic foci in adrenal glands and spleen. Ultrastructurally, a herpes-like virus was detected, but the agent was not identified virologically. In other rodents, like rabbits, HSV1 infection has been well documented after spontaneous or experimental infections.

AFIP Diagnoses: Cerebrum: Meningoencephalitis, neutrophilic, lymphoplasmacytic and histiocytic, subacute, focally extensive, marked, with neuronal necrosis, gliosis, and eosinophilic intranuclear inclusion bodies, chinchilla (*Chinchilla lanigera f. domestica*), rodent.

Conference Comment: *Herpes simplex* is a double-stranded DNA enveloped virus, with an icosahedral capsid in the alphaherpesvirus subfamily. There are two serotypes, *H. simplex* virus type 1 associated with oral and conjunctival infections and encephalomyelitis in adults and H. simplex virus type 2 associated with genital and neonatal infections. Humans are the natural or reservoir host for the virus.[5]

Domestic rabbits have been used as an animal model for herpes simplex encephalitis for decades. Naturally occurring cases of herpes encephalitis have also been observed in pet rabbits and were most likely acquired from contact with human shedders of herpes simplex virus. Key histomorphologic lesions include a nonsuppurative meningoencephalitis with necrosis of neurons and prominent intranuclear inclusion bodies in neurons and astroglial cells.[1,4]

Human-to-monkey and monkey-to-monkey transmission have also been described. Lesions may be local or generalized. Oral vesicles and ulcers, conjunctivitis, encephalitis, and death may occur. Owl monkeys, tree shrews, lemurs, maromosets, and tamarins are susceptible to generalized disease. Infections in

gorillas, chimpanzees, gibbons and cebus monkeys are usually confined to the skin, oral cavity, external genitalia, and conjunctiva; however, fatal encephalitis may also develop in gibbons. Oral, lingual, labial, or genital vesicles and ulcers associated with conjunctivitis and keratitis are seen. Necrotizing meningoencephalitis may occur and focal necrosis can be found in the visceral organs. Histologically, multinucleated giant cells and intranuclear inclusion bodies can be seen adjacent to necrotic foci. *H. simplex* infection in owl monkeys, marmosets, and tamarins cannot be differentiated from herpesvirus T (*H. tamarinus*) infection grossly or microscopically. Squirrel monkeys are the natural host for herpesvirus T and a definitive diagnosis can only be made by virus isolation and identification, Immunohistochemistry, or molecular techniques. Although outbreaks of *H. simplex* infections can be devastating in owl monkeys, *H. simplex* infection is not common in any of the primate species.[5,6,7]

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References:

1. Weissenböck H, Hainfellner JH, Berger J, Kasper I, Budka H: Naturally occurring herpes simplex encephalitis in a domestic rabbit (*Oryctologaus cuniculus*). Vet Pathol 34:44-47, 1997
2. Goudas P, Gilroy JS: Spontaneous herpes-like viral infection in a chinchilla (*Chinchilla laniger*). J Wildlife Dis 6:175-179, 1970
3. Granzow H, Klupp BG, Fuchs W, Veits J, Osterrieder N, Mettenleiter TC: Egress of alphaherpesviruses: comparative ultrastructural study. J Virol 75:3675-3684, 2001
4. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., p. 254. Iowa State University Press, Ames, Iowa, 2001
5. Hunt RD: *Herpesvirus simplex* infection. In: Monographs on Pathology of Laboratory Animals Nonhuman Primates I, eds. Jones TC, Mohr U, Hunt RD, pp. 82-86. Springer-Verlag, Berlin, Germany, 1993
6. Mansfield K, King N: Viral diseases. In: Nonhuman Primates in Biomedical Research Diseases, eds. Bennet BT, Abee CR, Henrickson R, pp.10-11. Academic Press, San Diego, California, 1998
7. Mätz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, Verschoor E, Niphuis H, Kaup FJ: Fatal *Herpes simplex* infection in a group of common marmosets (*Callithrix jacchus*). Vet Pathol 40:405-411, 2003

CASE IV – Sn090/05 (AFIP 3026208).

Signalment: 2-year-old, crucian carp, male, fish, cyprinid.

History: In spring 2005 a local retailer, specialized in original Japanese koi, received a charge of two year-old crucian carps from a German wholesaler. Among these fishes he noticed one animal with a bilateral symmetrical swelling anterior to the dorsal fin. During the following months the fish was reared in one large glass aquarium together with all other fish of this shipment. Obviously the swelling was well tolerated by the fish although a slight increase in size was noticed. Due to the curious and unshaped appearance of the animal in contrast to the other fish the dealer refused to sell this fish. In autumn the bilateral swellings reached a size of 2.0 x 1.0 x 1.0 cm each. The skin became susceptible to superficial damages and intermittent small quantities of suppurative fluid derived from circumscribed ulcers at the top of the swellings. By reason of animal welfare the fish was euthanized and subjected to necropsy.

Gross Pathology: The main gross finding was a bilateral symmetrical swelling of the anterior part of the musculus laterodorsalis (each 2.0 x 1.0 x 1.0 cm in size, figure 1 and 2). The section revealed a cream-colored, pasty compound resembling pus (figure 3). A macroscopically visible demarcation of connective tissue or any inflammatory reaction was missing. The other organs were macroscopically without any pathological findings.

Histopathologic Description: The cream-colored, pasty compound turned out to be a mixture of necrotic debris and developmental stages of parasites within the muscular and connective tissue (figure 4: Native spores, X400; figure 5: Giemsa stain, X400). Intracellular stages of the parasites were hardly observed. Host reaction was limited to few lymphocytes and macrophages. At the border area eosinophilic degeneration of few muscle fibers was seen. The epidermis next to the focal ulceration was characterized by moderate mononuclear infiltrations and moderate to profound intracellular edema. Numerous parasitic spores were observed penetrating all dermal and epidermal layers.

Contributor's Morphologic Diagnoses:
1. Back muscles (Musculus laterodorsalis): Necrosis, focal, mild, with massive accumulation of myxosporidian spores in the connective tissue, consistent with *Myxobolus lentisuturalis*
2. Skin: Ulceration, focal, mild, with numerous myxosporidian spores, consistent with *Myxobolus lentisuturalis* (missing in slides)

Contributor's Comment: Up to now more than four hundred species of the genus *Myxobolus* are known to parasitize fish. In contrast, species with intramuscular (intracellular, histozoic) developmental stages are rare in cyprinid fishes.[1,2] Depending on spore morphology and tissue localisation we assume a severe

infection with *Myxobolus lentisuturalis* as described in *Carassius gibelio* by Dykova et al. from Lake Bao'an in Hubei Province, China.[1] This publication stated a close phylogenetic relation of *M. lentisuturalis* to *M. xiaoi* and a still unclassified species from *Catostomus commersoni* based on SSU rDNA sequence data whereas spore morphology and gross lesions resemble these of *M. carassii* and *M. kubanicus*. Mature spores of the myxosporidian genus *Myxobolus* are ellipsoidal, ovoid or rounded in valvular view and biconvex in sutural view. The two pyriform polar capsules with convergent anterior points contain an invaginated and coiled polar filament. Beside the binucleated sporoplasm two residual nuclei of the capsulogenic cells may be present.

Development of myxosporidians comprise an oligochaete and a vertebrate host.[3] Various actinosporeans, formerly supposed to be parasites of oligochaetes, are the infective stages to fish. The most common oligochaete seems to be *Tubifex tubifex*, however *Lumbriculus* spp. or *Branchiura* spp. can serve as hosts as well. For example, observations of the life cycle of *Myxobolus cerebralis* revealed the intrinsic function of actinosporeans as vehicles for the transmission between hosts. Infection of the fish host occurs by actinosporean perorally or percutaneously.[4] During clonal reproduction in the vertebrate host the sarcoplasm of infected cells is replaced by large masses of plasmodial stages, followed by enlargement of the host cells. Destruction of adjoining tissues due to pressure atrophy may be seen aside. Dispersal of mature spores occurs after rupture of the host cell wall into the connective tissue and epithelial structures like the mucous cells of the intestine and the upper layers of the skin. Thus a release of infective stages is given during the lifetime of the host. Infection of oligochaetes occurs by oral infection with mature spores. After release of the sporoplasma further asexual and sexual development takes place in the gut epithelium.[2]

As a result of evolutionary coexistence of myxozoans and their hosts there are only few humoral, cell or tissue responses of the host to histozoic plasmodia during asexual development. Normally the release of mature spores initiates a granulomatous inflammation including melanomacrophages and the replacement of parasite lesions by granulation tissue. Severe alterations of earlier stages are only seen in atypical tissues or hosts and are followed by elimination of the parasites before maturation. In addition, alteration of host tissue may be seen as pressure atrophy of surrounding tissues and reactive hypertrophy or hyperplasia respectively of infected internal and external organs.[5] In this case of *M. lentisuturalis* asexual division and development to mature spores occurs intracellularly in the muscle fibres of the musculus laterodorsalis. After degeneration of the host cell mature spores are released in the connective tissue of myosepta. Compared to other species of the genus *Myxobolus* only few cellular reactions caused by *M. lentisuturalis* are observed and despite wide dispersal of spores into other organs there is a lack of demarcation.[4]

In general, the genus *Myxobolus* is a member of the taxon Myxozoa. Nowadays myxozoans are classified as metazoans nested within the genus Cnidaria. Their main morphological features are the polar capsules, resembling nematocysts of cnidarians and the existence of desmosomes, tight junctions and collagen production demonstrated in different developmental stages. Present works concluded that the most reliable pattern to distinguish between special taxa of myxozoans is not spore morphology but rather development and tissue location. Molecular characterization becomes more popular although the data set might be confusing depending on molecular overlap and individual variability in between the 18S rDNA of different species.[4]

AFIP Diagnosis: Skeletal muscle: Myositis, necrotizing, subacute, multifocal, severe, with myriad myxosporidian spores, crucian carp, cyprinid.

Conference Comment: The contributor provides a thorough overview of *Myxobolus* to include characteristic histomorphologic features, life cycle, and recent reclassification as metazoans.

Myxozoans parasitize invertebrates (primarily annelids) and poikolothermic invertebrates with the vast majority infecting fish. The Myxozoa that infect fish are obligate parasites of either tissues (histozoic forms that reside in intercellular spaces, intracellulary, or in blood vessels) or organ cavities (coelozoic forms that reside primarily in the gall bladder, swim bladder, or urinary bladder). Most are intercellular parasites that are typically site and species specific. Key characteristics include a multicellular spore and the presence of one to six (usually two) polar capsules each of which contains a polar filament. Spores with polar capsules are pathognomonic for myxozoan infection. Polar capsules can be seen in fresh wet mounts but are more easily seen in Giemsa or Wright's stained smears. Spores are refractile and difficult to see in hematoxylin and eosin stained sections, but polar capsules stain intensely with Giemsa or toluidine blue.[6]

Most myxozoan infections of fish incite only a moderate host reaction; however, heavy infections can result in serious mechanical damage from pseudocysts or tissue necrosis and inflammation from trophozoite feeding. Young fish are usually most seriously affected and histozoic forms usually cause more serious disease. In many cases, tissue damage is most severe after death of the host when enzymes released by the parasites cause massive muscle liquefaction.[6]

Gross myxozoan lesions can look similar to other diseases that cause focal masses including microsporidians, *Ichthyopthirius multifilis*, lymphocystis (iridovirus), and dermal metacercariae. Internal lesions may resemble focal granulomas and

neoplasia. These can be easily differentiated by histopathology or by examining wet mounts.[6]

Other important fish diseases in which myxozoans are known or suspected to be involved include proliferative kidney disease, proliferative gill disease, and whirling disease/black tail (*Myxobolus cerebralis*).[6]

Some conference participants considered microsporidians as a differential for this case. In contrast to myxozoans, all microsporidians are intracellular parasites that form a characteristic thick-walled spore which contains a sporoplasm. Some microsporidians induce the formation of a markedly hypertrophied cell that, together with the parasite, forms a xenoma or xenoparasitic complex. Xenomas appear as whitish, cyst-like structures, up to several millimeters in diameter. Some species may form large pseudotumors comprised of many individual xenomas. The presence of spores that are small (2 to 10 um), egg-shaped to elliptical, with a prominent posterior vacuole is diagnostic for microsporidia. Spores have a polar tube (typically not seen with routine light microscopy) and, unlike the Myxozoa, have no polar capsule. Microsporidian spores are Gram-positive.

Conference participants discussed the reclassification of myxozoans as metazoans. The Myxozoa were grouped with the protistan taxa until the early 1900s. As early as 1899, Stolc claimed that myxozoans are not protists and should be included with the Metazoa since their spores are multicellular. In 1938, Weill suggested myxozoans are cnidarians since the polar capsules of myxozoans showed identical discharge properties to nematocysts. In 1995, Siddall et al. used molecular and morphological data to show that myxozoans nested within the cnidaria and were the first to note desmosomes, tight junctions, and collagen production. Siddall et al. also provided ultrastructural characterization of the development of myxozoan polar capsules finding it to be indistinguishable from the development of cnidarian nematocysts.[4]

There was some variation among slides with some sections containing scaled skin.

This case was reviewed in consultation with Dr. Chris Gardiner.

Contributor: http://www.vetmed.uni-leipzig.de/ik/wpathologie

References:

1. Dykova I, Fiala I, Nie P: *Myxobolus lentisuturalis* sp n. (Myxozoa: Myxobolidae), a new muscle-infecting species from the Prussian carp, *Carassius gibelio* from China. Folia Parasitol 49:253-8, 2002
2. Longshaw M, Frear PA, Feist SW: Descriptions, development and pathogenicity of myxozoan (Myxozoa: Myxosporea) parasites of juvenile cyprinids (Pisces: Cyprinidae). J Fish Dis 28:489-508, 2005

3. Gardiner CH, Fayer R, Dubey JP: An atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 14-15. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, 1998
4. Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallet SL, Lester RJG, Longshaw M, Palenzeula O, Siddall ME, Xiao C: Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48:395-413, 2001
5. Lom J, Dyková I: Protozoan Parasites of Fishes, pp. 159-215. Elsevier, London, England, 1992
6. Noga EJ: Fish Disease Diagnosis and Treatment, pp.173-193. Iowa State University Press, Ames, Iowa, 2000

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