The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

CONFERENCE 18
8 March 2006

Conference Moderator: LTC Gary Coleman, DVM, Diplomate ACVP
Walter Reed Army Institute of Research
Washington D.C.

CASE I – 11353-04 (AFIP 2988322)

Signalment: 4-month-old, domestic shorthair cat, female, *Felis domesticus*

History: Several kittens in the litter died with lethargy and dyspnea and the mother had mild respiratory signs.

Gross Pathology: The kitten was somewhat thin. The lungs were diffusely pink-red and somewhat rubbery and edematous, had no consolidated areas, but they did not float well. The pericardial sac contained a small amount of gelatinous fluid. Other organs were grossly normal.

Laboratory Results: Chlamydophila PCR, liver and lung: positive
FeLV and panleukopenia fluorescent antibody: negative
Virus isolation: negative
Bacteriology, lung and liver: no bacterial growth
Giardia ELISA: negative

Histopathologic Description: The lungs have diffuse, severe, interstitial hypercellularity with an influx of histiocytes, a few small lymphocytes and early fibroblast proliferation. Some alveolar macrophages are shedding into the narrowed alveoli (interstitial pneumonia). Essentially no neutrophils are in the airways but there is diffuse type II pneumocyte hyperplasia. The liver has extramedullary hematopoiesis and occasional foci of acute hepatic necrosis and small foci of hepatocyte degeneration and individual cell necrosis. Rare packed clusters of minute intracellular basophilic organisms are consistent with *Chlamydophila*. They do not stain with Gram stain but do stain nonspecifically with a Giemsa stain. Some slides also contain a section of very congested spleen with some EMH and possible minute foci of necrosis and fibrin.

Contributor's Morphologic Diagnoses: *Chlamydophila felis* infection with 1) Severe, diffuse interstitial pneumonia and 2) Moderate, multifocal, hepatic necrosis with intracellular *Chlamydophila* colonies and extramedullary hematopoiesis.

Contributor's Comment: Chlamydophila infection is the cause of "Feline Pneumonitis." This is a particularly severe case as it usually causes conjunctivitis and upper respiratory infection (1). The organism has not been found in the liver previously in a natural infection but has been isolated in the liver and spleen in experimental infections with a report of a spontaneous case of peritonitis (2,3). Chlamydia (Chlamydophila) have also been found by EM and histochemical staining in the gastric mucosa of healthy colony cats (4). The interstitial pneumonia in this case is similar to that caused by feline calicivirus infection when the virus causes lower respiratory infection, rather than the usual upper respiratory tract infection (4). No virus was found by virus isolation in this case. This pattern, with foci of liver necrosis, would also make feline viral rhinotracheitis herpesvirus also a differential diagnosis. The liver involvement is suggestive of the lesions of *Chlamydophila psittaci* in birds and liver lesions were not found in experimental cases of feline chlamydiosis (3). We presume that the infection is due to *Chlamydophila felis*, previously *Chlamydia psittaci* or *C. psittaci felis.* Our PCR primer is probably "genus" specific (Chlamydia and Chlamydophila) rather than specific for *C. felis*. The old genus Chlamydia has been split in a new genus of Chlamydia that still includes *C. trachomatis*, with *C. suis* and *C. muridarum*, and a new genus of Chlamydophila that now includes *Cph. psittaci* (birds and humans) and the new species of *Cph. caviae* (guinea pig conjunctivitis), *Cph. felis*, *Cph. pecorum* (abortion, conjunctivitis, enteritis, pneumonia, arthritis in ruminants), and *Cph. pneumoniae* (koala, human, and horse respiratory disease). (1)

AFIP Diagnoses: 1. Lung: Pneumonia, interstitial, histiocytic, diffuse, moderate, with edema, domestic shorthair, feline.
2. Liver: Degeneration and necrosis, multifocal, mild.
3. Spleen, white pulp: Lymphocytolysis, multifocal, mild.
4. Liver; spleen: Extramedullary hematopoiesis, multifocal.

Conference Comment: As mentioned by the contributor, a recent taxonomic change in the family Chlamydiaceae created two genera, *Chlamydia* and *Chlamydophila*. The genera are distinguished by rRNA sequences and the detectable production of glycogen. The genus *Chlamydia* contains three recognized species, *C. trachomatis*, *C. muridarum*, and *C. suis*. The genus *Chlamydophila* contains six species, *C. pecorum*, *C. pneumoniae*, *C. psittaci*, *C. abortus*, *C. felis* and *C. caviae*.

Chlamydiaceae are obligate, intracellular, gram-negative organisms which contain DNA and RNA and form their own cell wall. They differ from bacteria in that they do not synthesize ATP; instead, they utilize ATP from host cell mitochondria. Chlamydia exist in two forms: elementary bodies and reticulate bodies. Elementary bodies are small (0.3um) particles with rigid cell walls that can survive outside the host cell but are metabolically inactive and incapable of replication. They attach to the host cell by adhesins on their surface and enter the host cell via phagocytosis. Elementary bodies shed their cell wall and grow larger (0.6-1.5um), forming reticulate bodies that replicate by binary fission. Reticulate bodies are metabolically active, utilizing ATP from host mitochondria, but are incapable of infecting other cells. Reticulate bodies condense and reform elementary bodies that are released during cell lysis to infect other cells.

Chlamydiae persist as commensal flora on the conjunctiva and respiratory, gastrointestinal, and genitourinary mucosae, often with no clinical signs. They are shed in saliva, milk, urine, and feces.

Although difficult to visualize on H&E stained sections due to their small size; special stains can help identify the organisms. Typically, Chlamydiae stain purple with Giemsa, blue with Castaneda and red with Macchiavello/Gimenez. Ultrastructurally, elementary bodies are round and dark with a bilayered cell wall and a unique, dense core of condensed chromatin (nucleoid). Reticulate bodies are larger with more dispersed chromatin. Intermediate forms (intermediate bodies) resemble reticulate bodies but contain a central electron dense core. All three forms occur together, within membrane-bound vacuoles (phagosomes). Host mitochondria are closely associated with these vacuoles.

Listed below are the Chlamydiaceae and the diseases they cause in animals and man:
Chlamydophila felis: endemic in cats; causes conjunctivitis with blepharospasm, hyperemia, chemosis, ocular discharge and rhinitis. Persistent genital and gastrointestinal infection may occur.
Chlamydophila psittaci: a common avian pathogen, a.k.a. Parrot fever, Ornithosis,
Chlamydophila abortus: is endemic in ruminants causing abortion, especially in ewes, a.k.a. Enzootic Abortion of Ewes (EAE). Also affects rabbit, guinea pig, mice and human.
Chlamydophila caviae: specific pathogen of guinea pigs causing conjunctivitis.
Chlamydophila pecorum: causes reproductive and urinary tract disease in Koalas.
Chlamydophila pneumoniae: a human pathogen causing bronchitis and pneumonia, also affects frogs and snakes.
Chlamydia trachomatis: sexually transmitted human disease. In women, can infect the cervix, urethra and fallopian tubes resulting in Pelvic Inflammatory Disease (PID); in men, causes epididymitis. Also causes inflammation of the rectum, throat,

and conjunctiva. Ocular infection in humans causes blindness and is called "trachoma". A problem in developing countries primarily, affects 84 million people worldwide (5).
Chlamydia muridarum: pneumonia in mice and hamsters
Chlamydia suis: causes conjunctivitis, enteritis and pneumonia in swine.

Contributor: Arkansas Livestock and Poultry Commission
www.arplc.org

References:
1. Hirsh DC and Biberstein, EL. Chlamydiaceae, Chap 39. In: Hirsh DE, MacLachlan NJ, Walker RL, eds. *Veterinary Microbiology*, 2nd Ed. 2004. Ames, IA: Blackwell Publishing; 2004: 235-239.
2. Davenport, JD. Bacterial and rickettsial diseases, Chap 18 In: Sherding RD, ed. *The Cat, Diseases and Clinical Management* 2nd Ed. 1994. Philadelphia: WB Saunders; 1994: 527-530.
3. Hoover, EA, Kahn, DE, and JM Langloss. Experimentally induced feline chlamydial infection (feline pneumonitis). AJVR. 1978; 39: 541-548.
4. Hargis, AM, DJ Prieur, and ET Gaillard. Chlamydial infection of the gastric mucosa in twelve cats. Vet Pathol 1983; 20: 170-178.
5. International Trachoma Initiative. 2005. Available at: http://www.trachoma.org/trachoma.php

---

CASE II – P05-171f (AFIP 2991413)

Signalment: 28-day-old, male, nursery pig, swine.

History: Three nursery pigs from a farrow-to-finish commercial farm located in central Taiwan were culled and submitted for pathological examination in July, 2005. The pigs were weaned at 28 days of age and moved to a nursery house. These submitted nursery pigs had moderate digestive distress with watery diarrhea and weight loss that had been unresponsive of a variety of antimicrobial agents. Of 100 pigs in the group, half were affected, and there was low mortality. The farmer had noticed diarrhea in suckling pigs since early May 2005, and diarrhea was found to affect nursery and growing pigs at the end of June 2005.

Gross Pathology: The piglet was in poor body condition and appeared dehydrated. The stomach contained some intact feed. The jejunum and ileum were distended

with yellow and frequently foamy fluid. The walls of the small intestine were thin and translucent. Large intestinal contents were pasty and lacked formed feces.

Laboratory Results: Coronavirus particles were demonstrated in intestinal contents of the pig by negative-contrast transmission electron microscopy.

Histopathologic Description: Extensive villous atrophy was present throughout the small intestinal mucosal circumference. The normal jejunal villous height: crypt depth ratio was reduced from 3:1 to 1:1. Epithelial cell necrosis of villi was accompanied by infiltration of inflammatory cells. The inflammatory cells were mainly lymphocytes, plasma cells and eosinophils. Immunohistochemistry testing for transmissible gastroenteritis virus (TGEV) was positive in infected-epithelial cells of villi.

Contributor's Morphologic Diagnosis: Enteritis, severe, acute to subacute, with villous atrophy, jejunum and ileum, swine. Etiology: TGE virus (a coronavirus)

Contributor's Comment: Transmissible gastroenteritis (TGE) is a highly contagious, enteric viral disease of swine characterized by vomiting, severe diarrhea, and high mortality (often 100%) in piglets less than 2 weeks of age. Although swine of all ages are susceptible to this viral infection, the mortality in swine over 5 weeks of age is very low. The disease is most frequently diagnosed and causes the most loss when occurring in herds at farrowing time [(1)]. TGE is recognized as one of the major causes of sickness and death in piglets. Swine producers are especially apprehensive about this disease because (1) mortality is high in newborn pigs; (2) there is no effective, practical treatment; (3) entrance of the virus into a herd in winter months is difficult to prevent because of the probable role of birds, especially starlings in the USA. The viral etiology, TGE, is in the genus *Coronavirus* of the family *Coronaviridae*. TGE is enveloped and pleomorphic, with an overall diameter of 60-160nm. It has a single layer of club-shaped surface projections that are12-25nm in length and widely spaced.

An epizootiologic feature of TGE is seasonal appearance. i.e., during the winter months, usually from the middle of November to about the middle of April in the USA. However, our laboratory found some cases of TGE in mid-summer in the past two years [(1)]. There is evidence for existence of TGEV in nonporcine hosts. Cats, dogs, and foxes have been suggested as possible carriers of TGEV from one herd to another since they can shed virus in their feces for variable period [(6)].

Porcine epidemic diarrhea virus (PEDV) is another coronavirus of pigs that causes a disease similar to TGEV; it is less severe, and newborn animals are not always affected; this disease has been documented only in swine in Europe, Asia (China, Japan) and Canada [(2, 5)].

TGEV is ingested, infects the mucosa of the small intestine, and causes a rapid and extensive loss of functional epithelial cells. The pathogenesis of diarrhea in TGEV-infected pigs includes altered sodium transport in the jejunum, resulting in accumulation of electrolytes and water in the intestinal lumen, and loss of extravascular protein. The ultimate cause of death is probably dehydration and metabolic acidosis coupled with abnormal cardiac function resulting from hyperkalemia. A marked shortening or atrophy of the villi occurs in the jejunum and to a lesser extent in the ileum, but it is often absent in the proximal portion of the duodenum. Both virus production and villous atrophy are greater in newborn pigs than in 3-week-old pigs [2, 3, 6].

Collection and preservation of appropriate clinical specimens is necessary for reliable diagnosis. Although villous atrophy is a consistent lesion in severely affected pigs, it frequently occurs in other enteric infections as well (rotavirus, PED, coccidiosis, and sometimes, *E. coli*). Laboratory diagnosis of TGE may be accomplished by one or more of the following procedures: detection of viral antigen (direct or indirect IF method), detection of viral nucleic acid (nucleic acid hybridization probes), microscopic detection of virus (negative-contrast transmission EM; immune EM), isolation and identification, or detection of a significant antibody response (VN test; ELISA test; indirect immunoperoxidase test adapted to detect immunoglobulin class-specific antibody; radioimmunoprecipitation; and a modified autoradiographic test) [3, 4, 5]. Antiviral agents have not yet been developed for the specific treatment of TGE. The only treatment presently available is to alleviate starvation, dehydration, and acidosis. The following measures are suggested: provide a warm (preferably above 32°C), draft-free, and dry environment and provide water or electrolyte or nutrient solution freely accessible to the thirsty TGEV-infected pigs. Such measure will tend to reduce mortality in pigs that are infected at more than 3-4 days of age. Antibacterial therapy might be beneficial in 2- to 5-week-old pigs, especially if there is a concurrent infection with pathogenic strains of *E. coli*. Cross-sucking, or putting infected or susceptible litters onto TGE–immune sows was found useful in various field outbreaks.

Diagnostic criteria for TGE includes: 1. Sudden onset with high mortality in sucking pigs, all ages of pig affected. 2. Severe atrophy of the villi in the small intestine. 3. Identification of the virus or of the viral antigen.

Differential diagnosis includes: 1. Colibacillosis 2. PED 3. Rotavirus infection 4. All other conditions causing an epidemic-like episode of diarrhea with or without vomiting.

AFIP Diagnosis: Small intestine: Villar blunting and fusion, diffuse, marked, mixed breed, porcine.

Conference Comment: Conference attendees discussed the possible mechanisms for the age-dependent susceptibility to TGE virus. Neonates normally have tall villi (villus height to crypt depth is normally 7:1 to 9:1) with mature differentiated enterocytes and short inactive crypts of undifferentiated epithelium, resulting in a large population of susceptible villus cells and crypts that are slow to repair. A second mechanism may be associated with gastric secretions. Milk buffers gastric acid in neonates, so this acid-labile virus is better protected in the less acidic environment of the neonate's stomach. In addition to the above, neonates are inherently more susceptible to dehydration, electrolyte imbalances, and hypoglycemia, making them more susceptible to the effects of this virus. (7,8,9)

Although all ages of pigs may be affected in a susceptible herd, TGE is generally a disease of high morbidity and mortality in pigs younger than 10 days of age, causing vomiting and profuse diarrhea. Differential diagnosis for diarrhea in young pigs includes *E. coli*, rotavirus, *Clostridium perfringens* type C, hemagglutinating encephalomyelitis virus, and coccidiosis. Enteric colibacillosis is a common cause of profuse diarrhea, without vomiting, in piglets less than 10 days of age with peak incidence at 3 days of age. Rotavirus causes disease in suckling and weaned pigs (1-5 weeks of age) with less severe villar atrophy than is seen in TGE. Clostridial enterotoxemia is a rapidly fatal disease of newborn piglets less than one week of age, causing bloody diarrhea. Hemagglutinating encephalomyelitis virus, another coronavirus, is the cause of vomiting and wasting disease. Vomiting and wasting disease affects pigs less than 10 days old and is characterized by vomiting and weight loss. Additionally, a number of affected pigs develop acute encephalomyelitis. In contrast to TGE, diarrhea is not severe. Coccidiosis causes diarrhea without blood in piglets 5-15 days of age, with peak incidence at 7-10 days of age. (10)

Grossly, TGE causes distension of the small intestine with gas, yellow frothy fluid, and flaccid, thin, transparent intestinal walls. Of the differentials listed above, the gross lesions that most closely resemble TGE are those of *E. coli* and coccidiosis. (7,8,9)

Below is a simple chart listing some of the coronaviruses of veterinary importance, species affected and diseases they cause:

CORONAVIRUSES:

Bovine coronavirus (winter dysentery)	Bovine	Gastroenteritis, thought to be a coronavirus – still some debate
Canine coronavirus	Canine	Enteritis
Feline coronavirus (FIP)	Feline	Peritonitis, pneumonia, meningoencephalitis, panophthalmitis; granulomatous vasculitis
Feline enteric coronavirus	Feline	Diarrhea in kittens
Mouse hepatitis virus (MHV)	Mouse	Hepatitis, enteritis, encephalomyelitis; syncytia formation
Porcine transmissible gastroenteritis (TGE)	Porcine	Gastroenteritis, usually piglets less than 10 days old
Porcine hemagglutinating encephalomyelitis virus	Porcine	Vomiting, wasting and encephalomyelitis (usually no diarrhea)
Porcine epidemic diarrhea	Porcine	Gastroenteritis (western Europe, similar to TGE)
Rat coronavirus	Rat	Rhinitis, tracheitis, pneumonitis in young
Rat sialodacryoadenitis virus	Rat	Sialodacryoadenitis, porphyrin released from damaged harderian gland, squamous metaplasia of ducts
Avian infectious bronchitis	Chickens	Tracheobronchitis, nephritis
Bluecomb (turkeys)	Turkeys	Enteritis, cyanosis of the comb
Rabbit coronavirus	Rabbits	Enteritis, myocarditis

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

References:

1. Alsop J, Harding J, Constantini V, Lewis P, Saif LJ. A trial to determine true TGE status in a multiplier herd. *Proceedings of the17th IPVS Congress*. Ames, Iowa, USA; 2002: 415.
2. Buergelt CD, Chu RM, Lee RCT. *An Atlas of Alimentary Tract Pathology*. Pig Research Institute, Taiwan; 1994: 139-142.
3. Chu RM, Yang PC, Chang WF. Neonatal diarrhoea of pigs in Taiwan: Aetiology, epidemiology and treatment. In: Tzipori S *et al*.,eds. *Infectious Diarrhoea in the Young*. Netherland. Elsevier Science Publishers B. V.1985: 151-155.
4. Grądzki, Winiarczyk S., Rutkowska-Pejsak B. An outbreak of severe dirrhea caused by concurrent infection with TGEV, Rotavirus and *E. coli* in large scale pig farm in Poland. *Proceedings of the 14th IPVS Congress*. Bologna, Italy; 1996: 120.
5. Park NY, Cho HS, Kim TJ, Kim YH. Differential diagnosis of porcine enteric pathogens by DNA chip. *Proceedings of the 18th IPVS Congress*. Hamburg, Germany; 2004: 316.

6. Walton JR. The abdomen, 1. the gastro-intestinal tract condition: transmissible gastro-enteritis. In: 3rd ed. *A Handbook of Pig Disease*. England, Liverpool University Press; 1993: 68-69.
7. Saif LJ, Wesley RD: Transmissible gastroenteritis and porcine respiratory coronavirus. *In*: Diseases of Swine, eds. Straw BE, D'Allaire S, Mengeling WL, Taylor DJ, 8th ed, pp. 295-325. Iowa State University Press, Ames, Iowa, 1999
8. Barker I, Van Dreumel A, Palmer N: The alimentary system. *In*: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 185-187. Academic Press, San Diego, California, 1993
9. Gelberg HB: Alimentary system. *In*: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 50-52. Mosby, St. Louis, Missouri, 2001
10. Radostits O, Blood D, Gay C, Hinchcliff K: Veterinary Medicine, 9th ed., pp. 1106-1114. W.B. Saunders, London, England, 2000

CASE III – 04-27 (AFIP 2983603 / 2952718)

Signalment: A 6 year old female owl monkey (*Aotus trivirgatus*)

History: This sample of lung was obtained from an 840g, 6 year old female owl monkey. The monkey was splenectomized and inoculated with various Plasmodium agents over a two year period. Aside from a reported history of mild anemia dating back to 10/03, the monkey was otherwise healthy. The monkey was found dead and no overt clinical signs were observed prior to death.

Gross Pathology: The lungs were multifocally consolidated. The remainder of the gross examination was unremarkable.

Histopathologic Description: The kidneys contained a moderate collection of lymphocytes, with glomerular adhesions, and tubular necrosis. Few mineralized calculi were found throughout the distal tubules. The liver contained lymphocytes and occasional eosinophils, and hepatocellular degeneration and necrosis was evident. Malarial pigment phagocytized by Kupffer cells was moderately distributed throughout the hepatic parenchyma. The lungs contained a mixed population of inflammatory cells. The alveolar walls were moderately thickened and malarial pigment was diffusely distributed throughout the lungs. The heart contained a focal collection of lymphocytes with occasional eosinophils. The stomach and intestinal tract were moderately autolyzed.

Contributor's Morphologic Diagnoses: 1. Kidney, interstitial lymphocytic nephritis, multifocal, moderate with minimal tubular necrosis, glomerular synechiae, few mineralized calculi, and intrahistiocytic and extracellular yeast.
2. Lung, mixed cell interstitial pneumonia, diffuse, moderate with fibrosis, pulmonary edema, phagocytized malarial pigment, and intrahistiocytic and extracellular yeast.
3. Liver, lymphoplasmacytic hepatitis, multifocal, mild with individual hepatocellular degeneration/necrosis, phagocytized malarial pigment, and intrahistiocytic yeast.
4. Heart, lymphocytic myocarditis, focal, minimal.

Contributor's Comment: On hematoxylin and eosin (H&E), the lungs, kidneys, liver, and lymph nodes contained macrophages distended with multiple round organisms measuring 2-4 μ in diameter. The organism also had a thin cell wall that enclosed a clear space with a single central basophilic structure. The organism was visibly stained with PAS and GMS. The morphologic characteristics of the organism were consistent with *Histoplasma capsulatum* var *capsulatum*, in which the cell wall appears as a distinct pale blue ring outlining the darker blue of the cell protoplasm. (2,3).

Histoplasmosis is a soil borne organism that is highly prevalent in certain regions of North and South America. There are two reported cased of systemic infections encountered in wild caught owl monkeys from South America. Generally, clinical signs are inapparent and diagnosis is based on histologic evaluation of intracellular and/or extracellular organisms in internal organs. (1,4).

AFIP Diagnosis: Lung: Pneumonia, interstitial, lymphohistiocytic, diffuse, marked, with edema, alveolar histiocytosis, hemosiderosis, and myriad intrahistiocytic yeast, etiology consistent with *Histoplasma* sp., owl monkey (*Aotus trivirgatus*), primate.

Conference Comment: *Histoplasma capsulatum* is a dimorphic fungus that grows as a nonparasitic, mycelial form in the soil and a parasitic, budding yeast in animals with body temperatures between 30 and 37°C. The mycelial form produces spherical microconidia ranging in size from 2-4 μm, and club shaped macroconidia which range from 8 to 14 μm. In tissue, *H. capsulatum* is a 2-4 μm spherical or oval yeast that reproduces by narrow-based budding. Histopathological processing often causes the cytoplasm of *H. capsulatum* to shrink from the cell wall, creating a clear halo.

Histoplasma infection is initiated when the microconidia are inhaled. Inhaled particles must be about 2 μm in diameter or smaller to reach the alveoli. Larger particles are trapped and removed from the respiratory tract by the mucociliary elevator. The small size of histoplasmal microconidia allows them to by-pass the mucociliary elevator and colonize the lung. Once in the alveoli, the microconidia convert to the yeast form and are phagocytized by pulmonary macrophages. In most instances, *H. capsulatum* causes a self-limiting pneumonia. In susceptible animals, however, the organism is spread by macrophages within the lymphatics and then the circulatory system. These animals develop a disseminated disease which can affect many organs including the central nervous system, intestine, adrenal glands, skeletal system, heart, and kidneys. It has also been hypothesized that the mycelia or conidia of *H. capsulatum* can infect the intestine after being ingested. (5)

Contributor: Centers for Disease Control and Prevention, Division of Parasitic Diseases, www.cdc.gov/ncidod/dpd/professional/default.htm

References:

1. Baskin GB. Disseminated Histoplasmosis in a SIV-infected Rhesus Monkey. J Med Primatol.1991;**20**: 251-252.
2. Butler T and Hubbard G. An Epizootic of Histoplasmosis Duboisii (African Histoplasmosis) in an American Baboon Colony. Lab Anim Sci 1991; **41**: 407-410.
3. Miller G and Owens J. Ultrastructural Characterization of the Etiologic Agent of Systemic Yeast Infection of Aotus Monkeys. Medical Mycology 1999; **37**:139-146.
4. Miller G, Feinsteine L, Azadegan A, Thomas M, and Bacher J. Systemic Yeast Infection in Owl Monkeys *(Aotus vociferans)*: Ante-mortem Screening and Diagnosis by Bone Marrow Aspirates; Lab Anim Sci.1998; **48**: 391-394.
5. Ettinger SJ, Feldman EC: Textbook of Veterinary Internal Medicine, 6th ed. vol. 1, pp.680-682. Elsevier Saunders, St. Louis, MO, 2005

CASE IV – NIAH #1 (AFIP 2988335)

Signalment: Six-week-old, female, chicken, white leghorn, *Gallus gallus* var. *domesticus*

History: Six-week-old specific-pathogen-free chickens were inoculated intranasally with 10^6EID_{50} highly pathogenic avian influenza virus (H5N1), and examined

pathologically to confirm the virus-induced lesions. The virus (A/duck/Yokohama/aq10/2003) was isolated from duck meat, which had been imported from China to Japan, at the animal quarantine laboratory. All the inoculated chickens died from 2 to 6 days post-inoculation (dpi). The tissue sample of a chicken that died at 5 dpi was presented.

Gross Pathology: Grossly, dead chickens showed subcutaneous congestion in the comb and wattle, mucosal congestion in the palpebral conjunctiva, and subcutaneous congestion in the tibiotarsal joint. In addition, necropsy findings included diffuse whitish foci in the spleen and petechial hemorrhage in the adipose tissue at the base of heart.

Histopathologic Description: The major histopathological changes in the chicken included severe necrotic myocarditis in the heart, moderate nonsuppurative meningoencephalitis in the brain, mild focal necrosis in the pancreas, and severe dermatitis in the comb. In addition, the chicken had multifocal hepatocytic necrosis in the liver; a large number of hemosiderin-laden macrophages in the lung and spleen; and moderate depletion of lymphocytes in the spleen, thymus and the bursa of Fabricius.

Immunohistochemical examination using monoclonal antibody against type A influenza virus matrix protein revealed virus antigen-positive cells, as follows (+ + +, + +, and + signify a large, moderate, and small number of viral antigen-positive cells, respectively): cardiocytes in the heart (+ +), macrophages in the lung (+), exocrine cells in the pancreas (+), endocrine cells in the adrenal gland (+), ganglion cells in the ganglion associated with the adrenal gland (+), neurons, glial cells, and ependymal cells in the brain (+ + +), epithelial reticular cells in the thymus (+), and epidermal cells, macrophages and vascular endothelial cells in the subcutis (+ + +). No viral antigen-positive cells were detected in the trachea, liver, spleen, kidney, sciatic nerve, or femoral bone marrow.

Contributor's Morphologic Diagnosis: Heart: Myocarditis, necrotizing, lymphohistiocytic, subacute, multifocal, severe, chicken.

Contributor's Comment: An H5N1 influenza virus, A/duck/Yokohama/aq10/2003, was isolated from duck meat processed for human consumption, imported to Japan from China in 2003 (1). This virus was antigenically different from other H5 viruses, including the Hong Kong H5N1 viruses isolated from humans in 1997 and 2003. Sequence analysis revealed that six genes (PB1, PA, HA, NA, M, and NS) of the virus showed 97% nucleotide identity with their counterparts from recent H5N1 viruses, but that the remaining two genes (PB2 and NP) were derived from other unknown viruses. This duck meat isolate was highly pathogenic to chickens upon intravenous or intranasal inoculation. The histopathological and

immunohistochemical analysis confirmed that the virus has properties of highly pathogenic avian influenza virus and pantropism in domestic chickens. The pathogenicity investigation of H5 influenza viruses for domestic ducks (2) indicated that A/duck/Yokohama/aq10/2003 caused neurological sings, grew in multiple organs more rapidly than A/chicken/Yamaguchi/7/04(H5N1) isolated from a dead bird during the HPAI outbreak in Japan (3). A/duck/Yokohama/aq10/2003 also replicated well in the lungs of mice and spread to the brain, but was not as pathogenic in mice as H5N1 human isolates. However, viruses isolated from the brain of mice previously infected with the virus were substantially more pathogenic and possessed some amino acid substitutions relative to the original virus. These studies show that poultry products contaminated with influenza viruses of high pathogenic potential to mammals are a threat to public health even in countries where the virus is not enzootic and represent a possible source of influenza outbreaks in poultry.

AFIP Diagnosis: Heart: Myocarditis, necrotizing, histiocytic, multifocal, moderate, white leghorn chicken, avian.

Conference Comment: Avian influenza is a contagious viral infection and/or disease of many avian species including poultry, ratites, shore birds and migratory waterfowl. The highly pathogenic form is characterized by severe depression, decreased egg production, edema, hemorrhage, frank necrosis and high mortality. Highly pathogenic avian influenza (HPAI) is reportable to the World Health Organization for Animal Health (OIE).

Avian influenza (Orthomyxoviridae) is a type A influenza virus. The type designation is based on envelope matrix (M) antigens and the nucleoprotein (NP) within the virus. Avian, swine, equine, and most significant human influenza are type A viruses. Subtypes are described based on envelope glycoproteins. Fifteen hemagglutinin (HA) and nine neuraminidase (NA) antigens are currently used. Each virus has only one type of hemagglutinin antigen and one type of neuraminidase antigen on its surface. Standard nomenclature for naming viruses includes virus type, host of origin, geographic origin, strain number, year of isolation, and subtype designation. For example: A/Ck/TX/309402/04(H5N2).

Migratory waterfowl (especially ducks) are relatively resistant to clinical disease and are considered an important reservoir for other species. Clinical disease is most important in turkeys and chickens. Transmission occurs from infected to susceptible birds through both direct and indirect contact (aerosol, contaminated fomites, feces) via the respiratory tract or conjunctiva. (4) After inhalation and attachment of influenza virions to cilia, virulent strains replicate in the respiratory

and intestinal epithelium and disseminate to the viscera and central nervous system. Hemagglutinin antigen (HA) is responsible for virus attachment to various sialic acid residues on the apical surface of respiratory epithelial cells. (5) Neuraminidase is responsible for release of the virus by cells via its action on neuraminic acid.

Influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species. For example, until 1998, only H1N1 viruses circulated widely in the U.S. pig population. However, in 1998, H3N2 viruses from humans were introduced into the pig population and caused widespread disease among pigs. Most recently, H3N8 viruses from horses have crossed over and caused outbreaks in dogs.

Avian influenza A viruses may be transmitted from animals to humans in two main ways:
•Directly from birds or from avian virus-contaminated environments to people.
•Through an intermediate host, such as a pig.

Influenza A viruses have eight separate gene segments. The segmented genome allows influenza A viruses from different species to mix and create a new influenza A virus if viruses from two different species infect the same person or animal. For example, if a pig were infected with a human influenza A virus and an avian influenza A virus at the same time, the new replicating viruses could mix existing genetic information (reassortment) and produce a new virus that had most of the genes from the human virus, but a hemagglutinin and/or neuraminidase from the avian virus. The resulting new virus might then be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or neuraminidase) not previously seen in influenza viruses that infect humans.

This type of major change in the influenza A viruses is known as antigenic shift. Antigenic shift results when a new influenza A subtype to which most people have little or no immune protection infects humans. If this new virus causes illness in people and can be transmitted easily from person to person, an influenza pandemic can occur.

It is possible that the process of genetic reassortment could occur in a human who is co-infected with avian influenza A virus and a human strain of influenza A virus. The genetic information in these viruses could reassort to create a new virus with a hemagglutinin from the avian virus and other genes from the human virus. Theoretically, influenza A viruses with a hemagglutinin against which humans have little or no immunity that have reassorted with a human influenza virus are more likely to result in sustained human-to-human transmission and pandemic influenza. Therefore, careful evaluation of influenza viruses recovered from humans who are

infected with avian influenza is very important to identify reassortment if it occurs. (6)

Contributor: Diagnostic Section, Department of Epidemiology, National Institute of Animal Health, Tsukuba, Ibaraki, 305-0856, Japan
http://niah.naro.affrc.go.jp/

References:

1. Mase M, Eto M, Tanimura N, et al.: Isolation of a genetically unique H5N1 influenza virus from duck meat imported into Japan from China. Virol (article in press), 2005
2. Kishida N, Sakoda, Y, Isoda N, et al.: Pathogenicity of H5 influenza viruses for ducks. Arch Virol 150: 1383-1392, 2005
3. Mase M, Tsukamoto K, Imada T, et al.: Characterization of H5N1 influenza A viruses isolated during the 2003-2004 influenza outbreaks in Japan. Virol 332: 167-176, 2004
4. Easterday BC, Hinshaw VS, Halvorson DA: Influenza. *In*: Diseases of Poultry, ed. Calnek BW, 10th ed., pp. 583-602. Iowa State University Press, Ames, IA, 1997
5. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Veterinary Virology, 3rd ed., pp. 79-80, 121-122, 466-468. Academic Press, San Diego, CA, 1999
6. Page found at http://www.cdc.gov/flu/avian/gen-info/transmission.htm, page last modified October 17, 2005

Signature Authenticated by Approve
Approved by: Carl I Shaia,
on:Monday, 10 April, 2006 at 16:14:10

Carl I. Shaia, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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