The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 23

23 April 2008

Moderator:

Dr. Don Nichols, DVM, Diplomate ACVP

CASE I – 07-253 (AFIP 3065807).

Signalment: 1-year-old, male, *Petaurus breviceps,* sugar glider

History: A 1-year-old, male, sugar glider was presented to the Emergency Service at MJR-VHUP for weakness and lethargy. The owner reported the acquisition of a new sugar glider during the previous week. The animal was emaciated and dehydrated. He vomited during the physical exam and died while the doctor was placing an IV catheter.

Gross Pathology: On gross examination, the animal was emaciated with a poor hair coat. The liver was firm, diffusely red tan with disseminated light tan foci ranging from 1 to 4 mm in diameter.

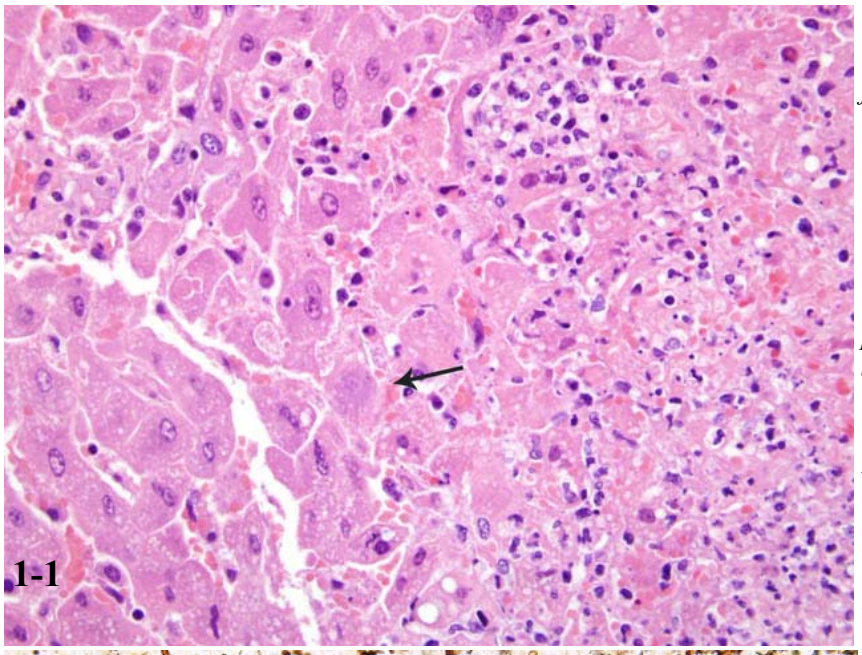
Laboratory Results: A small amount of blood obtained immediately postmortem revealed low blood glucose.

Histopathologic Description: Histology revealed multifocal random hepatocellular coagulative necrosis with associated neutrophils, fibrin and hemorrhage. Hepatocytes occasionally contained 5 to 10 micron long filamentous basophilic bacteria, which were haphazardly arranged within the cytoplasm **(Fig. 1- 1)**. No lesions were observed in the heart, and the gastrointestinal tract was severely autolyzed.

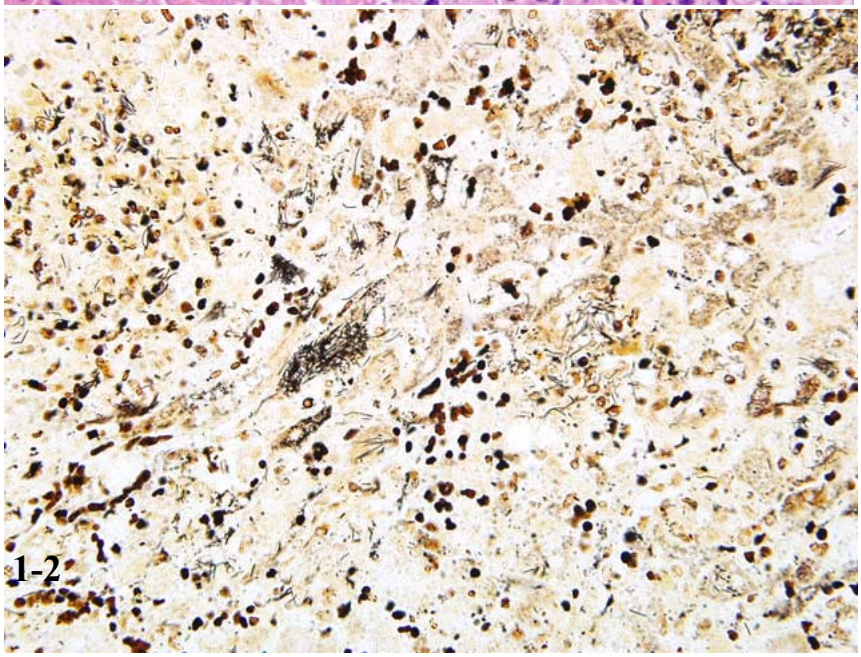
Contributor's Morphologic Diagnosis: Severe multifocal random acute necrotizing hepatitis with hemorrhage and hepatocellular cytoplasmic filamentous bacteria consistent with *Clostridium piliforme*.

Contributor's Comment: Tyzzer's disease is caused by *Clostridium piliforme*, a gram negative, filamentous, obligate intracellular bacterium. Clinical infection results in 1) hepatic necrosis, 2) myocardial necrosis, and 3) enterocolitis (specifically the distal ileum, cecum and proximal colon)[5] resulting in the "triad within a triad" lesion distribution. Although infection has been reported in many species (dogs[5], cats[5], horses[4], bovids[4], white tailed deer[1], cotton-top tamarins[10], Australian possums[3], snow leopards[12], muskrats[19], grey foxes[15], a red panda[6], a rainbow lorikeet[9], a serval[8], a cockatiel[11], a koala[3], a wombat[3], a dasyurid[3], a raccoon[20], and two coyotes[7]), it has not, to our knowledge, been reported in a sugar glider. Clinical disease often occurs in young (neonatal) animals or in immunocompromised individuals. The first reported occurrence of *C. piliforme* infection in a human was a subcutaneous infection in an immunocompromised HIV patient.[13]

Tyzzer's disease is common in laboratory animals including mice, rats, guinea pigs, and rabbits. *C. piliforme* is



1-1. Liver, Sugar Glider. Immediately adjacent to areas of necrosis, there are few to moderate numbers of hepatocytes that contain numerous, intracytoplasmic, 1-2 um diameter, long, filamentous bacilli. (HE 400X).



1-2. Liver, Sugar Glider. Numerous argyrophilic, 1 x 7 um, filamentous bacilli that occur singly or arranged in parallel or perpendicular sheaves, haystacks or bundles. (Warthin-Starry 400X).

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considered a commensal organism in the rodent intestinal tract which may spread to the liver via the portal circulation.[5] In laboratory mice systemic infection is often subclinical with fulminate disease occurring sporadically. Susceptibility varies among mouse strains. Likewise, virulence varies among *C. p iliforme* isolates. Factors involved in the immunity and virulence have been investigated in mice.[16-18] Infection of both susceptible and resistant strains of mice with either toxigenic (virulent) or non-toxigenic *C. p iliforme* isolates resulted in strain-independent and isolate-independent elevations in serum IFN γ and IL-6 from day 1 to 14 and in serum TNF α from day 1 to 28.[16,17] All mice had serologic evidence of inflammation; however, only mice infected with the toxigenic bacterial isolate had histologic lesions in the liver (day 7 to 14). A similar experiment revealed that IL-12 levels were significantly higher in resistant mice than susceptible mice.[18] The importance of IL-6 and IL-12 in mediating the immune response to *C. piliforme* was also demonstrated in these experiments; histopathologic lesions in the liver were more severe if polyclonal antibodies against these cytokines were injected immediately prior to infection with the bacteria.[17,18]

The possibility of latent infections has been debated. Presence of bacterial DNA in hepatocytes from animals with elevated serum cytokines and normal liver histology suggests that the infection may persist in a latent state for long periods of time (at least 28 days).[16]

Special stains to identify the organisms are occasionally required. Warthin-Starry, Giemsa, Gomori Methamine Silver (GMS), and gram stains are commonly used for these purposes.[5] In our laboratory, Warthin-Starry demonstrated the organisms **(Fig. 1- 2)** as well as occasional short rods. This second infectious organism may represent a secondary bacterial infection, possibly one that ascended from the gastrointestinal tract.

AFIP Di agnosis: Liver: Hepatitis, necrotizing, acute, random, severe, with fibrin, hemorrhage, and hepatocel-

lular intracytoplasmic bacilli, etiology consistent with *Clostridium piliforme*, sugar glider (*Petaurus breviceps*), marsupial.

Conference Comment: *Clostridium piliforme*, first described in 1917 in Japanese waltzing mice[10] was originally classified as *Bacillus piliformis*, but based on 16S rRNA analysis, has since been reclassified as a *Clostridium* sp.[18] Unlike other Clostridial species, *C. piliforme* is an obligate intracellular pathogen and will consistently stain gram negative.[18]

Transmission of Tyzzer's disease is presumed to occur through ingestion of contaminated feces, with colonization of the intestine followed by hematogenous spread to the liver via the portal circulation.[4] The mechanism of attachment and entry into the host cell is not currently known.[4,5] *C. piliforme* is considered difficult to grow on artificial media and requires eggs or cell culture to proliferate.[5,14] Diagnosis of infection is dependent on identification of the organism within the cytoplasm of degenerate and apparently healthy cells at the periphery of areas of necrosis. The bacteria have often been described as being arranged in characteristic sheaves, haystacks, or bundles. Immunohistochemistry, immunofluorescence, and PCR have all been used to aid in identification.[14]

Susceptibility to infection and disease progression has been linked to genetics, immune status, age, and bacterial virulence factors. T lymphocyte, natural killer cell, neutrophil cell function and cytokine responsiveness have all been linked to disease progression.[10,18] The pathogenesis of *C. piliforme* infection does not appear to be clearly dependent on toxin production. Some strains do produce cytotoxic proteins and these isolates are generally more virulent than the non-toxic isolates.[2,16]

Contributor: University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology and Toxicology
http://www.vet.upenn.edu/departments/pathobiology/pathology/

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CASE II – 03-399 (AFIP 2941570).

Signalment: 12-year-old, female, Pigtail macaque, *Macaca nemestrina*

History: The animal was part of a small research colony housed in outdoor/indoor gang cages. Following an outbreak of diarrhea, she had tested positive for fecal culture of *Yersinia pseudotuberculosis* and treated successfully with Enrofloxacin 8 years previously. During another diarrhea episode 2 years ago, she tested positive for *Balantidium coli* and was treated successfully with metronidazole. She recently developed diarrhea in which *Balantidium coli* was again identified. She was treated with metronidazole, but continued to have diarrhea that began to contain some fresh blood. Gentamycin and fluid therapy were quickly added to treatments, but she developed retching of food and continued to have diarrhea and dehydration until it was decided she should be euthanized approximately 10 day after onset of the initial signs.

Gross Pathology: At necropsy there was loose stool adhered to the perineal region and signs of dehydration based upon skin turgor and dryness of subcutaneous tissues. The stomach contained liquid contents, the small and large intestines contained pale greenish mucoid stool. There were petechial hemorrhages in the mucosa of the small intestine and paintbrush type hemorrhages on the mucosa and also serosa of the large intestines.

Laboratory Results: *Shigella flexneri* type IV was isolated from large intestines.

Contributor's Morphologic Diagnosis: Acute hemorrhagic purulent colitis - Shigellosis

Contributor's Comment : Shigellosis represents a "classic entity" of nonhuman primates, an infection long associated with exposure to humans.[2] For this reason, it is still a disease threat to captive primates and a good example of the need for employee health monitoring of the animal caretakers. This outbreak was associated with renovation of the gang cages by outside contractors. Pub MED currently lists over 12,500 citations for *Shigella* and 255 citations for *Shigella* and primates.

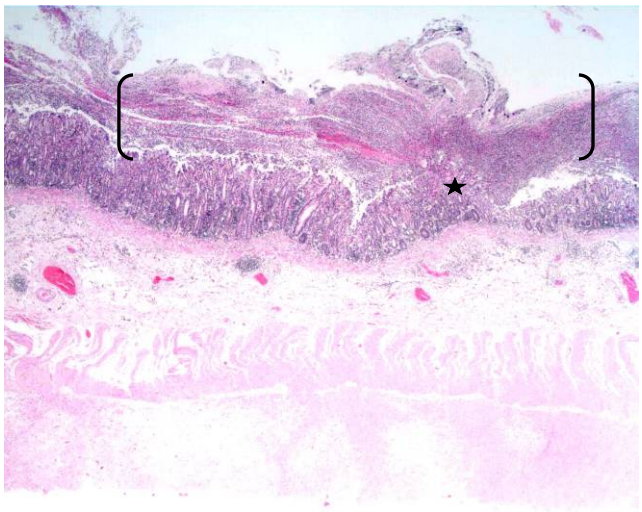
Shigella flexneri, *Yersinia enterocolitica*, *Strongyloides fulleborni*, adenovirus, *Campylobacter coli* and *C. jejuni* were recently reported to be associated with chronic diarrhea in Rhesus macaques.[7] Enteric agents not associated with chronic diarrhea within the same population included *Balantidium coli*, *Giardia lamblia*, *Trichuris trichiura*, *Enterocytozoon bieneusi* and enteropathogenic *E. coli* carrying the eaeA intimin or Stx2c Shiga toxin virulent genes. Chronic diarrhea was associated with increased CD69+ cells and CD4+ T lymphocytes as well as upregulation of IL-1-a, IL3, and TNF-a cytokine genes. Chronic diarrhea is the leading cause of morbidity requiring veterinary care in captive primates and is not currently a criterion of specific pathogen-free colonies. The enteric infections and associated modulation of the immune system is a complicating variable in the study of AIDS and SIV in the primate models.

AFIP Diagnosis: Colon: Colitis, fibrinonecrotic, subacute, diffuse, severe with edema and pseudomembrane **(Fig. 2- 1)**, Pigtail macaque (*Macaca nemestrina*), primate.

Conference Comment: Shigellosis is a common disease in non-human primates, caused by a gram negative, nonmotile, nonspore forming, bacilli.[2] The most common isolate is *Shigella flexneri*, although *S. sonnei*, *S. dysenteriae* and *S. boydii* have also been less frequently identified.[2,3,5] Clinical signs of shigellosis may vary depending on the species of Old World monkey infected. Rhesus macaques generally present with either an acute foul-smelling, watery diarrhea with frank blood, or a more chronic intermittent semi-soft diarrhea with occasional episodes of frank blood. Tamarins and marmosets, on the other hand, will primarily demonstrate lethargy, depression, dehydration, and dried blood around the anus, rather than diarrhea.[3]

Infection and disease does not provide immunity. Animals with shigellosis may be chronically reinfected, and colonies endemically infected my not demonstrate overt clinical disease unless challenged by a stressful event or compromised immune system.[3]

Upon ingestion of the bacteria, it is proposed that inva-



2-1. Colon, Pig-tailed macaque. Overlying and focally contiguous (star) with the mucosa, there is a thick fibrinonecrotic and suppurative exudate, or pseudomembrane (brackets). The submucosa and tunica muscularis are expanded by edema. (HE 20X).

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sion of the M cells overlaying lymphoid follicles within the colon, allows the bacteria to reach the basolateral pole of the epithelial cells, which is where they induce their entry.[5]

Shigellosis in humans is caused by both *Shigella* spp. and enteroinvasive *Escherichia coli*.[5] These bacteria contain a virulence plasmid that encodes most proteins directly involved in host cell entry, bacterial dissemination, and induction of apoptosis in infected macrophages.[4] This region encodes a type III secretion apparatus (Mxi-Spa TTS apparatus), translocators (IpaB and Ipa C), effectors (IpaD, IpgB1, IpgD and IcsB), their dedicated chaperones (IpgA, IpgC, IpgE and Spa15), and two transcriptional activators (VirB and MxiE). The current theory suggests that upon contact of the bacterium with the host cell, translocators are inserted into the host cell membrane which forms a pore, effectors are then transmitted through this pore and enter into the host cell cytoplasm.

Genes encoding a type III secretory apparatus have been identified in a number of mammalian and plant pathogens including enterohemorrhagic and enteropathogenic *Escherichia coli* , *Shigella*, *Salmonella*, *Yersinia*, *Chlamydia*, *Bordetella*, *Pseudomonas*, *Xanthomonas*, *Ralstonia*, and *Erwinia* spp.[6]

Contributor: Penn State Milton S. Hershey Medical Center, Penn State College of Medicine, Department of Comparative Medicine, H054, 500 University Drive, Box 850, Hershey, PA 17033-0850
http://www.hmc.psu.edu/comparative_medicine

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CASE III – 05-14824 (AFIP 2984543).

Signalment: Female, 11-year-old, Taita falcon (*Falco fasciinucha*).

History: This animal was one of seven captive Taita falcons (*Falco fasciinucha*) that died within 3 weeks during the breeding season of 2005. The falconer had 7 breeding pairs. Four of his females and three of his males died after being lethargic and anorexic for few hours to one day. Three of the four female animals died about one day after laying an egg.

Gross Pathology: The animal was in a good to fair post-

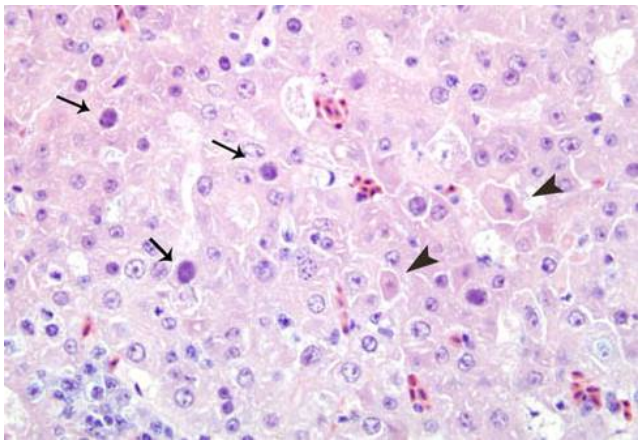
mortem preservation state. It was anemic and had marked hemorrhage into the reproductive tract. The liver was diffusely beige. The spleen was mildly to moderately enlarged and had multiple beige well demarcated foci, up to 2 mm in diameter.

Laboratory Results: The animal was negative for adenovirus-specific antibodies in 2002. Nucleic acid of falconid adenovirus was detected by PCR in the liver. The liver was negative for dependovirus (parvovirus) nucleic acid, herpesvirus nucleic acid, polyomavirus nucleic acid and chlamydial nucleic acid. Aviadenoviral nucleic acid was also detected in the liver by *in situ* hybridization. Adenoviral particles were present in the intestinal content. Few non-hemolytic *E. coli* were isolated from the liver by aerobic culture.

Histopathologic D escription: The nuclei of numerous hepatocytes contained one large basophilic inclusion body **(Fig. 3-1)**. Occasionally, a halo was present around the intranuclear inclusion and the nuclei membrane was hyperchromatic. A low to moderate number of necrotic individual hepatocytes were scattered within the parenchyma. Multiple portal fields were infiltrated by a moderate number of lymphocytes, plasma cells and macrophages.

Contributor's Morpho logic Diag noses: 1. Hepatitis, necrotizing, multifocal, moderate with abundant hepatocellular intranuclear inclusion bodies.
2. Hepatitis, portal, lymphoplasmacytic and histiocytic, mild to moderate.

Contributor's Comment: Primary histological differential diagnosis in this case included herpesvirus hepatitis and adenovirus hepatitis. The clinical presentation and finally the results of electron microscopy, *in situ* hybridization and PCR supported and confirmed a diagnosis of adenovirus hepatitis. Recently, a falconid adenovirus has been identified.[3] This virus may be fairly widespread in indigenous species, such as peregrine falcons in which it does not seem to cause clinical disease.[2] However, if naive tropical falcon species become infected with the virus, it may cause fatal peracute disease.[2,3,5]



3-1. Liver, falcon. Several hepatocytes contain large, deeply basophilic, intranuclear inclusions that often peripheralize the chromatin (arrows). There is scattered individual hepatocyte necrosis characterized by shrunken, deeply eosinophilic cytoplasm with pyknotic nuclei (arrowheads). (HE 400X).

AFIP Dia gnosis: 1. Liver, hepatocytes: Degeneration and necrosis, single cell, random, moderate, with basophilic intranuclear inclusion bodies, Taita falcon (*Falco fasciinucha*), avian.
2. Liver: Hepatitis, portal, lymphoplasmacytic, multifocal, moderate.

Conference Comment: The recently identified falcon adenovirus, most closely related to fowl adenovirus type

Major groups of the Aviadenoviruses, adapted from Schrentzel et al.[3] and Tomaszewski et al.[4]

Name		
Fowl adenovirus 1 (FAV-1)	Chickens	Includes chicken embryo lethal orphan strain (CELO). Tropic for hepatocytes, pancreatic acinar cells, and gizzard epithelium
Hemorrhagic enteritis virus (HEV)	Turkeys	Non-pathogenic in pheasants; Produces severe enteritis in turkeys and guinea fowl
Egg drop syndrome (EDS)	Chickens	Little or no disease in waterfowl; Reproductive abnormalities in chickens
Falcon adenovirus	Falcons	Necrotizing hepatitis and splenitis

1 and 4, has been associated with disease in a variety of falcons, with a particularly severe course of infection within falcons originating from more isolated island/ tropical regions or within the smaller falcon species such as kestrels and merlins.[2,3] The disease appears to be widespread within the Peregrine falcons (*Falco peregrinus*) population which are considered to be a potential reservoir species for the falcon adenovirus.[1,2,4,5]

There are four genera within the family Adenoviridae (mastadenovirus, Aviadenovirus, Atadenovirus, and Siadenovirus).[3] Aviadenovirus is further subdivided into subgenera and groups based on shared neutralizing epitopes.

Characteristic histopathological findings include a multifocal, necrotizing hepatitis and splenitis, with hepatocytes, biliary epithelium, and mononuclear cells resembling lymphocytes containing intranuclear basophilic inclusion bodies that frequently marginate the chromatin.[1,3,4] Electron microscopic evaluation will demonstrate a 70-90nm nonenveloped icosahedral viral particle occasionally arranged in paracrystalline arrays.[3] In one report of infection in gyrfalcon and peregrine falcon hybrids, intranuclear inclusions were identified within renal tubular epithelial cells and not hepatocytes, suggesting that disease presentation may be species dependent.[5] Other lesions reported in different species include, vasculitis, heterophilic nephritis, myocardial necrosis, and hemorrhagic enteritis.[1-3,5]

Conference participants are encouraged to examine a similar case in a Northern aplomado falcon (*Falco femoralis septentrionalis*) that was presented in the 1996-1997 Wednesday Slice Conference number 29, Case 4, and subsequently published by Schrenzel et al.[3]

Contributing Institution: Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1333 Gortner Ave, St. Paul, MN 55108, USA

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CASE IV - 061127/061129/061130 (AFIP 3073388).

Signalment: Adult, female and male, thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*)

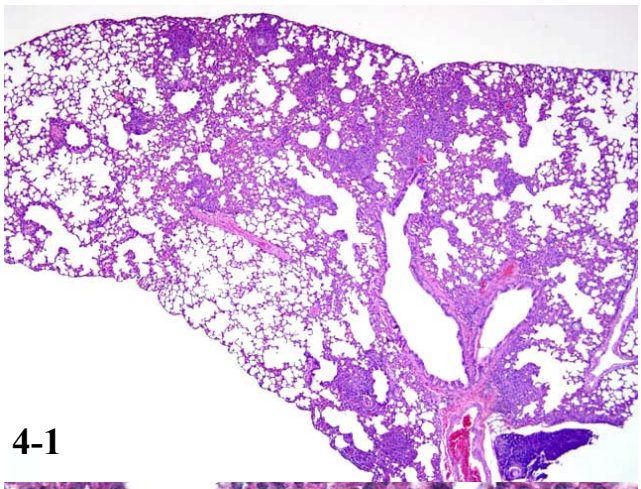
History: Selected tissue samples were collected from approximately fifty wild rodents as part of a plague surveillance project in Colorado during a plague outbreak in prairie dogs in July 2006. The animals were humanely trapped, euthanized, and selected fresh tissue samples were collected for PCR, ECL, microbial culture, histopathology, and immunohistochemistry.

Gross Pathology: No gross lesions were observed during necropsy.

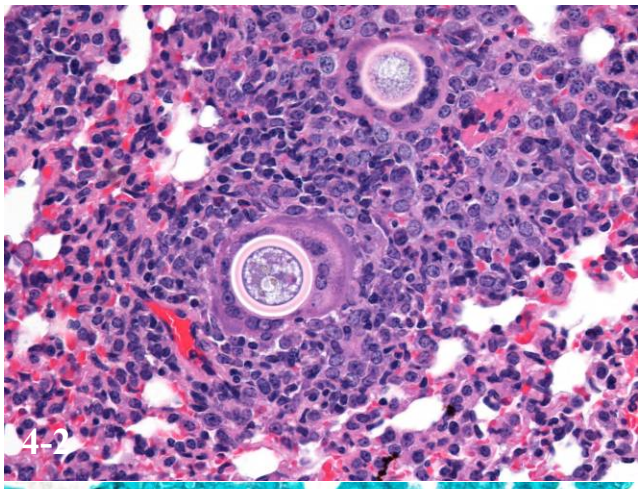
Laboratory Results: Immunonegative for *Yersinia pestis*

Histopathologic Description: Scattered throughout all lung lobes are coalescing pyogranulomas that disrupt and efface up to 20% of alveolar septae and bronchioles. The pygranulomas **(Fig 4- 1)** are up to 1mm in diameter and each is centered upon an adiaspore which is surrounded by degenerate and nondegenerate neutrophils, epithelioid macrophages, and foreign body and Langhans multinucleate giant cells in varying proportion. Lymphocytes and plasma cells are rare. The inflammatory cells extend into and expand adjacent alveolar septae. The adiaspores are round, up to 250 µm in diameter with a 20-30 µm thick refractile non-staining cell wall **(Fig. 4- 2).** The center contains amophophilic globular material. The cell wall stains dark purple with the Periodic Acid-Schiff (PAS) stain **(fig. 4-3)** and black with Grocott's Methenamine Silver (GMS) **(Fig. 4-4)** .

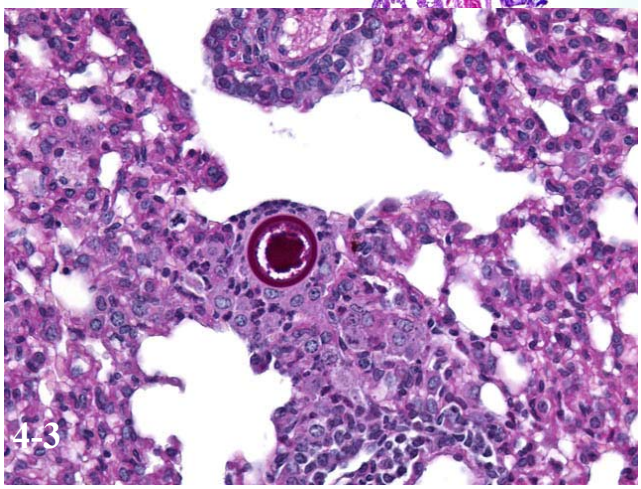
Contributor's Morphologic Diagnosis: Lung: Bronchopneumonia, pyogranulomatous, multifocal, mild, with fungal conidia (adiaspores), etiology consistent with *Chrysosporium parvum* (adiaspiromycosis).



4-1



4-2



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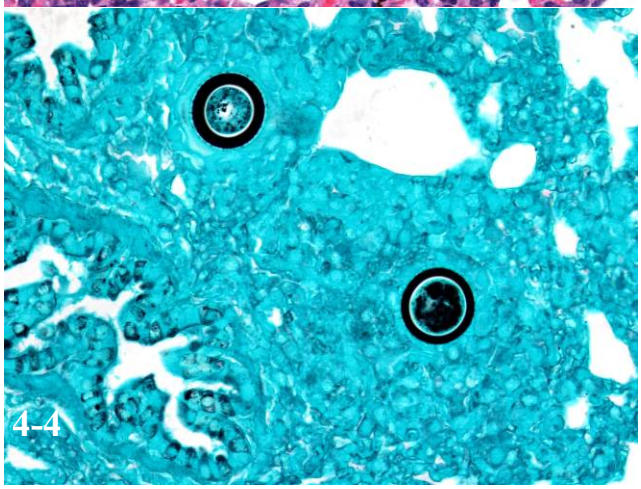

4-4

4-1. Lung, ground squirrel. Multifocal to coalescing granulomatous inflammation. (HE 20X).
4-2. Lung, ground squirrel. Multifocally, often within multinucleated giant cells and bounded by high numbers of macrophages and fewer lymphocytes and neutrophils, there are approximately 100-150 micron diameter adiaspores. (HE 400X)
4-3. Lung, ground squirrel. Adiaspores stain purple with periodic acid-Schiff staining method . (PAS 400X)
4-4. Lung, ground squirrel. Grocott's methenamine silver stain method further demonstrates the thick capsule of Chrysosporium parvum. (GMS 400X)

Photomicrographs (Fig. 4-2, 4-3, 4-4) courtesy of U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011 .

Contributor's Comment: Adiaspiromycosis is primarily a pulmonary fungal infection of small animals.[1] Infection occurs when the conidia (spores) are inhaled.[1] The name, adiaspore, refers to a spore that grows in size without replicating in tissues.[3] Therefore, the degree of infection is determined by the number of spores inhaled. The inhaled conidia simply enlarge in the lung tissue and are eventually removed by the immune system; thus, the disease is not contagious. [1,4] The most susceptible animals are those that live in close contact with soil that contains the saprophytic stage of the fungus, such as burrowing rodents.[1]

Two varieties of the fungal genus *Chrysosporium*, formerly known as *Emmonsia*, cause adiaspiromyosis: *C. parvum* var. *parvum* and *C. parvum* var. *crescens*. The latter has the widest host range and distribution.[1] *C. parvum* var. *crescens* is a dimorphic fungus with spores that are 2-4µm in the environment. They enlarge to 200-400µm when inhaled and incubated at body temperature.

At lower temperature (20-30°C) the spores will develop into a mycelial form.[4]

The typical pathologic feature of the infection is the granuloma or pyogranuloma which appear grossly as gray white nodules in the lungs.[1] The lung is the only organ known to be infected.[6] Histologically, these nodules contain a central adiaspore surrounded by varying degrees granulomatous to pyogranulomatous inflammation. Ruptured spores incite the most severe reactions.[1] Adiaspores range from 200-400µm in diameter for *C. parvum* var. *crescens* and 20-40µm *C. parvum* var. *parvum*.[2] *C. parvum* var. *crescens* has a characteristic thick cell wall that is up to 70µm thick.[4] The center of the adiaspore contains a mass of amphophilic to basophilic small globules.[3] There is a single nucleus in spores of *C. parvum* var. *parvum*, however *C. parvum* var. *crescens* develops multiple nuclei as it enlarges.[3] There is no budding or endosporulation.[1]

Differential diagnosis includes other fungi of similar size and morphology, such as *Coccidioides immitis* and *Rhinosporidium seeberi*. Morphologically, *C. parvum* (20-70µm) has a thick capsule while *C. immitis* (1–2µm) and *R. seeberi* (3–5µm) have relatively thin capsules.[4] The presence of endospores occurs with *C.immitis* and *R. seeberi*, but not *C. parvum*.[4] *C. parvum* infection does not produce hyphae, unlike *C. immitis*.[4] Histochemically, the capsules of all three stain with PAS and GMS.[4] The capsule of *R. seeberi* also stains with mucicarmine, unlike the other two.[4]

The infection has been reported in mice, moles, rats, rabbits, ground squirrels, weasels, martens, minks, armadillos, wallabies, skunks, opossums, dogs, cats, raccoons, and humans.[6]

The disease in immunocompetent animals (including humans) is typically benign, self-limiting, and confined to the lungs. However, clinical signs can occur with heavy infections.[1] Most infections are considered incidental findings during the course of histopathologic evaluation of the lungs – as in these ground squirrels.

Of the 20 ground squirrels examined during this study, adiaspiromycosis was detected in 13 of them (60.5%). Interestingly, only 1 of 18 (5.1%) prairie dogs in this study was infected. The reasons that two species of burrowing rodents from the same geographical area had such different prevalences of infection were not determined.

AFIP Diagnosis: 1. Lung: Pneumonia, pyogranulomatous, multifocal, moderate with fungal conidia, thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), rodent.
2. Kidney: Essentially normal tissue.

Conference Comment: Adiaspiromycosis caused by the dimorphic fungi *Emmonsia parva* (=*Chrysosporium parvum* var. *parvum*) or *Emmonsia crescens* (=*C. parvum* var. *crescens*) is a rare mycotic condition in small mammals and humans worldwide. In the literature, the names *Emmonsia* and *Chrysosporium* are considered synonyms with significant controversy occurring concerning their appropriate use. In 1962, Charmichael reclassified *Emmonsia* as a *Chrysosporium* and reduced the two species to a variety of *E. parva*.[9,10] This was later refuted by von Arx who retained *Emmonsia* as a single species with two varieties based on the reasoning that *Emmonsia* produces blastic conidia and adiaspores, and *Chrysosporium* produces thallic conidia and no adiaspore at elevated temperature.[9,10] To confuse taxonomic matters further, it has recently been found that *E. parva* is phylogenetically closer to *Blastomyces dermatitidis* (the anamorph of *Ajellomyces dermatitidis*) than it is to *Emmonsia crescens*.[7] At this time, both genera names continue to be used in the literature.

Inhaled dust-borne aleurioconidia (2-4 um) do not germinate in the host, but instead dramatically enlarge into thick-walled adiaspores. Clinically, infection may range from asymptomatic to severe necrogranulomatous pneumonia depending on adiaspore load and immunocompetence of the host.[5] Infection is not considered transmissible between individuals.[7,8]

Contributor: U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011.
http://www.usamriid.army.mil/

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