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Conference Moderator: LTC Tom Larsen, Diplomate ACVP
Department of Pathology
USAMRIID, Fredrick, MD

CASE I – 030928-41 (AFIP 2941564)

Signalment: 16-year-old, female, rhesus macaque (*Macaca mulatta*), nonhuman primate.

History: This adult female rhesus monkey was originally acquired from the Delta Primate Center, Louisiana, in 1990 by the United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, Maryland and transferred to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1992. She served as a subject on several Ebola virus protocols, and most recently as a blood donor for a whole-blood transfer study conducted in May 2003. The monkey was clinically normal during the study. The only anomaly noted was a slight change in eating habits that developed in November 2002, described as being "very picky" in her choice of foods. Although her weight was normal during the short course of the study, she became noticeably thinner throughout the month. A routine blood draw and clinical exam in early June demonstrated a significant weight loss of three kilograms. By late June, the monkey was still active, but began losing hair, did not eat regularly, and occasionally vomited. On July 7, 2003 the monkey had difficulty standing, demonstrated abdominal discomfort, and was shaking. She was euthanatized the following day.

Gross Pathology: The carcass was very thin, with significant loss of muscle mass and only a small amount of subcutaneous fat over the abdominal region. The fat had a white, chalky appearance (interpreted as steatitis). There was marked alopecia and moderate flaking of the skin, primarily over the back and the back of the head. Mild hair loss was present over the remainder of the body. Within the abdominal cavity there were multiple strictures in the distal colon and distal ileum, decreasing the lumen by 60% and 85%, respectively. Both the jejunum and the

ileum proximal to the stricture were dilated up to five times normal and contained liquid fecal material (interpreted as reflux from the cecum and colon). The liver was yellow-brown and friable (hepatic lipidosis), and the gallbladder was distended by inspissated bile.

Gross Diagnoses
1. Whole body: cachexia of chronic disease, moderate.
2. Subcutaneous fat: steatitis, moderate.
3. Colon and ileum: strictures, multiple, marked.
4. Liver: lipidosis, diffuse, severe.
5. Gallbladder: inspissated bile, moderate.

Contributor's Morphologic Diagnoses: 1. Colon, serosa: Endometriosis, multifocal, mild to moderate, with low numbers of hemosiderin-laden macrophages. 2. Uterus; ileum; and jejunum, serosal surfaces: Endometriosis, with few hemosiderin-laden macrophages (not submitted).

Contributor's Comment: The cause of this adult female monkey's weight loss, anorexia, and vomiting over the period before euthanasia was severe strictures of the intestinal tract, especially the ileum, which significantly decreased the passage of ingesta. The pathogenesis of the intestinal strictures was attributed to chronic endometriosis. The intestinal strictures resulted from constant cyclical hemorrhage and menstruation from the ectopically located endometrial tissue within the abdominal cavity, followed by formation of abdominal adhesions. The endometrial tissue was present primarily on the serosa of the ileum, and to a lesser degree on the colon, uterus, and jejunum.

Endometriosis is the condition in which normal endometrial glands and stroma occur in abnormal locations outside the uterine cavity.[1] The ectopically located endometrial tissue physiologically responds to normal ovarian hormonal influences associated with the menstrual cycle. The aberrant endometrial tissue undergoes monthly desquamation and hemorrhage (menstruation), with the exception that the process occurs within the lower abdominal cavity rather than in the uterine lumen. The entrapped hemorrhagic menstrual fluid provokes an intense inflammatory reaction in the abdomen, often leading to fibrosis and adhesions among the pelvic organs. Infertility, abdominal pain, and occasionally bowel and/or urinary tract obstruction occur secondarily to the adhesions and strictures.[2]

Endometriosis occurs only in humans and animal species that menstruate.[2] In humans, endometriosis affects 10% of women and often causes dysmenorrhea, pelvic pain, infertility, and other problems; it is primarily a disorder of those in the active reproductive stage of life, especially during the third and fourth decades.[3]

Similarly, endometriosis is one of the most common reproductive disorders in Old World nonhuman primates, and has been proposed as a naturally occurring model of the disease in humans. While the disease occurs as a spontaneous condition, it is frequently a complication of repeated hysterotomies or caesarean sections.[2] As in humans, the most common clinical signs in affected monkeys and apes include abdominal discomfort and infertility; in some cases, the disease is asymptomatic. Other clinical signs include cyclical anorexia, depression, weight loss, and absence of feces for several days; there may be palpable masses within the abdominal and pelvic cavities.[1]

In nonhuman primates, endometriosis has been reported at various anatomical sites, although lesions most commonly occur in the pelvic cavity. Macroscopically, gross lesions often appear as soft, red-brown or white, masses of tissue adherent to the serosa of the pelvic organs, or masses of dense connective tissue containing fluid-filled cysts distended with brown menstrual blood ("chocolate cysts").[2] Common sites and organs involved include the ovaries, uterine tubes, urinary bladder, and, as in this case, the bowel. In some cases unilateral or bilateral hydroureter and hydronephrosis develop as a result of adhesions that develop among the lower abdominal organs which impinge upon the pelvic ureters. Infrequently, endometriosis may result in bowel infarction.[1]

Microscopically, endometriotic lesions consist of variably sized foci of normal appearing uterine glands surrounded by typical endometrial stroma and thick bands of fibrous connective tissue; scattered aggregates of hemosiderin-laden macrophages are often present throughout the bands of connective tissue.[2] In human pathology, the histological diagnosis of endometriosis is satisfied if two of the three following features are identified: endometrial glands; endometrial stroma; and hemosiderin pigment.[3] While present in a few locations, the number of hemosiderin-laden macrophages admittedly is less than overwhelming in the submitted histologic sections from this female monkey.

Although the pathogenesis of endometriosis is not understood, three potential but not mutually exclusive theories have been offered to explain both the origin and dispersion of the lesions:[3]

1. Regurgitation theory: retrograde menstruation or reflux of endometrial tissue through the fallopian tubes, with subsequent implantation and proliferation of viable endometrial fragments in the abdominal cavity.
2. Metaplastic theory: endometrial tissue arises directly from coelomic epithelium (itself the origin of the endometrium).
3. Vascular or lymphatic dissemination theory: this would explain the presence of lesions in the lungs and lymph nodes (described in both humans and nonhuman primates), which is not explained by the two previous hypotheses.

AFIP Diagnosis: Colon; mesentery: Endometriosis, multifocally extensive, Rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: The contributor provides a thorough overview of endometriosis in human and non-human primates. Although the gross lesions of endometriosis are often distinctive, other differentials to consider, especially in markedly fibrotic lesions, are adenocarcinoma and retroperitoneal fibromatosis.

In nonhuman primates, intestinal adenocarcinoma occurs most commonly in the cotton-top tamarin, which is the animal model for ulcerative colitis and associated carcinoma in humans. Approximately 50% of colony-maintained animals develop active colitis, with disease in 25-40% of those with active colitis progressing to colonic adenocarcinoma after 2-5 years of captivity. Grossly the lesions are nodular to annular, firm, gray-white, transmural, stenotic masses, often with proximal intestinal dilation or muscular hypertrophy. Histologically, there are four subtypes of adenocarcinoma based on the predominant cell type and growth pattern: papillary, tubular, mucinous, and signet ring. Although the pathogenesis is not completely understood, it is thought that chronic inflammation leads to hyperplasia and dysplasia, which may eventually progress to adenocarcinoma.[4]

Retroperitoneal fibromatosis is a disorder of macaques that primarily occurs in young animals (1-3 years of age), and is characterized by an aggressive proliferation of highly vascular fibrous connective tissue, usually involving the ileocecal junction. This disorder is associated with a gammaherpesvirus - Retroperitoneal fibromatosis-associated herpesvirus (RFHV), and with an oncovirus, Simian type D retrovirus (SRV-2). SRV-2 is unique in its ability to induce both Simian Acquired Immunodeficiency Syndrome (SAIDS) and retroperitoneal fibromatosis. Lesions may be localized or progressive. Gross lesions in the localized syndrome include 1-4 cm single to multiple, firm, pale nodules beneath the peritoneum. In the progressive syndrome, lesions may encircle the intestines and adjacent lymph nodes leading to obstruction. Histologically, there are proliferating fibroblasts arranged in ill-defined bundles with occasional interweaving patterns within a disorganized matrix of collagen and reticulum fibers.[5]

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

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* Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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CASE II – CASE 2 (AFIP 2942335)

Signalment: Female,1.5-year-old miniature donkey (*Equus asinus*).

History: The animal had a chronic history of crusty skin lesions near her vulva and on her flanks. A skin biopsy was taken (tissue submitted). A month after the biopsy, the animal was noted to be lame, with a painful, swollen knee. The animal was humanely euthanized and submitted for a necropsy.

Gross Pathology: At the postmortem examination the animal was in poor body condition with minimal fat stores. The skin was thickened, irregular and crusty with variably-sized areas of alopecia and hypotrichosis. When sectioned, the skin, subcutis and superficial fascia had hundreds of small pinpoint white granular organisms. The scleral conjunctiva and vaginal mucosa contained dozens of similar foci. The distal phalanges of the left and right forelimb and right hind limb were ventrally rotated 32, 20 and 40 degrees, respectively, from the hoof wall.

Contributor's Morphologic Diagnosis: Skin Biopsy: Locally extensive, chronic lymphoplasmacytic and granulomatous dermatitis with intradermal protozoal cysts (*Besnoitia bennetti*, presumptive).

Contributor's Comment: Throughout the dermis there are dozens of randomly distributed, 0.5 to 1 mm diameter protozoal cysts within greatly distended and flattened fibroblasts (vimentin positive, smooth muscle actin negative cells). The cysts have a 25 μm thick hyaline internal capsule and are engorged by hundreds of fusiform bradyzoites, 8-9 μm long and 1-2 μm wide. There are variable numbers of macrophages around the intact cysts and larger numbers of macrophages mixed with lymphocytes and lesser numbers of plasma cells surround ruptured cysts. The epidermis is multifocally ulcerated, with granulation tissue and necrotic cell debris mixed with lymphocytes and plasma cells. The superficial dermis is moderately and diffusely expanded by dense fibrous connective tissue, with a mild to moderate perivascular to interstitial infiltrate of lymphocytes, macrophages, plasma cells and rare eosinophils.

Cutaneous besnoitiosis is a serious skin condition of cattle and horses characterized by painful swellings and thickening of the skin with loss of hair. Besnoitiosis is a rare disease in equines. It has been reported only in animals with a history of individual or herd travel outside the United States. *Besnoitia besnoiti* has been reported from southern Europe, Africa, Asia, and South America, but it has not been reported in cattle in the USA. *Besnoitia bennetti* has been reported from Africa, southern France, and in two imported burros in the USA.[5] However, this is the second case in a donkey from the northeastern USA that our service has diagnosed in the last two months.

Besnoitiosis is a contagious protozoal disease of various domestic and wild animals, including horses, burros, cattle, rodents, goats, antelope, reindeer, caribou and mule deer.[1] It is caused by members of the genus Besnoitia, which are characterized by having cysts containing bradyzoites within fibroblasts. The apicomplexan parasites under the subphylum Sporozoa, class Coccidian, and within the family Sarcocystidae include four genera: Toxoplasma, Sarcocystis, Besnoitia and Hammondia. The genus Besnoitia has the following species: *B. bennetti* (horses and donkeys), *B. besnoiti* (cattle), *B. caprae* (goats), *B. oryctofelisi* (rabbits) *B. darlingi* (lizards), *B. jellisoni* (kangaroo and opossum), *B. tarandi* (reindeer and caribou), and *B. wallacei* (mouse).[1-2] The old name of the parasite was *Globidium* spp , which describes the characteristic large, thick-walled cysts filled with bradyzoites.[4] The life cycle involves a definitive host and an intermediate host. The cat has been identified as the definitive host for *B. besnoiti*, *B. wallacei*, and *B. darlingi*.[3] In the intermediate host, Besnoitia is found in the dermis, subcutaneous tissues and fasciae. The parasite produces characteristic thick-walled cysts containing bradyzoites within fibroblasts. It has been speculated that a biting

insect vector spreads Besnoitia between intermediate hosts, but this is not proven. Besnoitia tissue cysts are characterized by hypertrophy of the infected host cell.[2]

AFIP Diagnosis: Haired skin: Dermatitis, chronic-active and eosinophilic, diffuse, moderate, with numerous intradermal protozoal cysts, etiology consistent with *Besnoitia* sp., miniature donkey, equine.

Conference Comment: As mentioned by the contributor, *Besnoitia* sp. are apicomplexan parasites within the class Sporozoasida, order Eucoccidiorida, family Sarcocystidae. Other genera of the family Sarcocystidae include *Toxoplasma*, *Sarcocystis*, *Neospora*, *Hammondia*, *Cystoisospora*, *Frenkelia*, and *Atoxoplasma*.[6]

All members of the family Sarcocystidae have a motile stage with apical complex, have a simple resistant spore, and undergo both sexual and asexual reproduction. Sexual reproduction results in the production of oocysts with two sporocysts in the intestine of the definitive host, while asexual reproduction results in spore formation within the intermediate host.[6]

Gross lesions caused by organisms of this family vary, but infection often results in acute necrosis from migration and multiplication of the tachyzoites and little tissue damage in organs with cysts containing bradyzoites. However, if the cysts rupture, the organisms often incite a granulomatous response. Histologically, these organisms appear very similar and immunohistochemistry or electron microscopy is needed for a definitive diagnosis.

Ultrastructurally, bradyzoites are found in the cytoplasm within a parasitophorous vacuole, which constitutes the innermost cyst wall layer. This is lined by a thin granular layer, which often contains one or more host cell nuclei. The outermost layer (secondary cyst wall) surrounds the host cell. The structures which help identify the organism as an apicomplexan parasite include a conoid, rhoptries, and micronemes.[2] The presence or absence, number, electron density, and/or location of each of these, as well as other organelles, assist in identifying the organism to the genus level.

Contributor:
Department of Biomedical Sciences, Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Ithaca NY
www.vet.cornell.edu

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CASE III – 030926-44 (AFIP 2940303)

Signalment: Adult, female African green monkey (*Chlorocebus aethiops*), nonhuman primate.

History: This 4.3 kg female African green monkey (AGM) was procured from St. Kitts/Primate Products. The monkey was assigned to a protocol, but had not been exposed to any agent. Six weeks after arriving at the institute, blood was noted around the anogenital region by animal care technicians (day 1). Menses was suspected, but the condition was reported to Veterinary Medicine Division personnel after the animal had decreased appetite over the weekend.

Upon examination by a veterinarian, the suspected menses had persisted for 5 days (the normal menstrual period in AGMs is about 1-3 days, usually with scant discharge). Under ketamine sedation, the monkey had a body temperature of 100.7°F, pulse = 180, and respiratory rate = 28. The animal had lost 0.5 kg from the previous weight taken 7 days earlier. Formed stool with fresh blood and loose, stringy clots were noted in the catch pan beneath the cage. There was moderate periodontal disease with worn, stained teeth. Abdominal palpation was within normal limits. No vaginal bleeding was found; only rectal bleeding with decreased anal tone.

The differential diagnosis included stress-induced, dietary-related, or idiopathic inflammatory bowel disease; hemorrhoids, polyps, or neoplasia; and infection with

intestinal pathogens such as *Campylobacter*, *Shigella*, *Salmonella*, *Yersinia*, or enteropathogenic *Escherichia coli*. Although this monkey was never positive for intestinal parasites, she was previously housed in a room where one monkey was diagnosed with whipworms (*Trichuris* sp.), so all monkeys in the room were treated at that time.

For initial work-up, a complete blood count (CBC), serum chemistry, clotting times, and fecal exam were ordered (results below). Gingival and anal swabs were submitted for bacterial culture. A B-vitamin injection was given to stimulate eating, and high-water content fruit treats were offered along with the regular diet. A fruit-flavored, sweetened water solution (Prang) replaced the monkey's normal water supply.

On day 6, the monkey showed no signs of improvement. She remained anorexic and continued to pass blood, but no stool. Based on the unremarkable laboratory results, flat film abdominal radiographs and a proctoscopic examination were ordered. A possible right side colonic stricture was noted on radiographs. Endoscopy revealed an advanced, severe hemorrhagic colitis. Difficulty was encountered in trying to pass the endoscope 30-33 cm (12-13 inches) proximal to the anus. The monkey was administered a broad spectrum antibiotic (enrofloxacin), subcutaneous fluids and given another B-vitamin injection. Exploratory surgery was planned for the following day.

Exploratory abdominal surgery revealed segmental areas of hemorrhage, restricted to the colon, that were visible through the serosa. Within the proximal colon, a 10-13 cm (4-5 inch) section contained markedly thickened mucosa, with hemorrhage and sloughed pseudomembrane formation that completely occluded the lumen. Additional colonic and gingival culture swabs were obtained. The decision to euthanize the monkey was based on the monkey's clinical history, the fact that she had lost 10-12% of her body weight and continued to deteriorate, and the severity and extent of the lesion found at surgery.

Gross Pathology: The body presented for necropsy was that of an adult, female African green monkey (*C. aethiops*). The carcass was in good body condition with adequate subcutaneous and cavitary fat. There was moderate periodontal disease with gingivitis, gingival hyperplasia, and worn canines. The stomach was empty and the small intestine contained a small amount of gas. Colon walls were edematous with swollen rugae and there were segmental areas of hemorrhage with blood clots in the lumen. Other areas in the colon were ulcerated or had a sloughed, pseudomembranous mucosal lining with dry, adherent fecal material.

Gross diagnoses: 1. Colon: Colitis, ulcerative and hemorrhagic, segmental, moderate, with pseudomembrane.
2. Gingiva: Gingivitis, multifocal, moderate, with mild gingival hyperplasia.

Laboratory Results: Initial work-up (Day 5)
Serum chemistry panel values were within normal limits, except for slightly elevated blood urea nitrogen, alkaline phosphatase, and triglycerides; albumin was slightly low, but the total protein was within normal limits. CBC values were all within normal limits (no anemia and no inflammatory leukogram). Fecal exam revealed no significant findings. Clotting times were within normal limits. Bacterial culture results: Bacteria in the genus *Shigella* were isolated from the rectum, colon, and gingiva (growth was not speciated).

Contributor's Morphologic Diagnosis: Colon: Colitis, ulcerative and hemorrhagic, subacute, multifocal, moderate, with crypt abscesses and abundant luminal fibrinohemorrhagic and cellular debris (pseudomembrane), African green monkey (*Chlorocebus aethiops*), nonhuman primate.

Contributor's Comment: Histologically within the submitted section of colon, there are focally extensive areas of mucosal ulceration that are covered by a layer of sloughed mucosal epithelial cells, fibrin, hemorrhage, and necrotic debris. The lamina propria is expanded by many lymphocytes and plasma cells that widely separate crypts. Colonic crypts are often dilated and filled with many viable and degenerate neutrophils, mucus, and cellular debris (crypt abscesses). Subacute inflammation extends into the edematous submucosa where lymphatics are ectatic. Multifocally, similar inflammation and focal areas of hemorrhage are present within the tunica serosa.

By immunohistochemistry using a polyclonal anti-*Shigella* antibody, there is strong staining of necrotic mucosal epithelial cells and luminal necrohemorrhagic debris within affected sections of colon.

Shigella are gram-negative, non-motile, aerobic and facultatively anaerobic bacilli from the family Enterobacteriaciae.[1] *S. dysenteriae, flexneri, boydii,* and *sonnei* are highly infectious strains that can cause dysentery in humans with an ID_{50} of only 100-200 bacteria.[2] Nonhuman primates usually acquire the zoonotic infection from humans via a fecal-oral route and endemic infections can be maintained in monkey colonies via asymptomatic carriers.[1] Nonenteric *Shigella* infections in monkeys with gingivitis, air sacculitis, and abortion have also been reported.[1] The pathogenesis of diarrhea or dysentery among the strains is similar, with a typical incubation period of 1-4 days followed by watery and mucoid diarrhea mixed with blood. Although clinical disease usually requires a stressor in endemically infected monkeys, it is typically self-limiting in adults, requiring minimal supportive care.

Studies on the pathogenesis of *Shigella* have revealed unique methods of mucosal invasion that result in the lesions seen with infection. Because most lesions are often centered on gut-associated lymphoid tissue (GALT) and spread outward, it is suspected that the bacteria make their initial entry into the body through the normally phagocytic M cells overlying the lymphoid tissue.[2] Additional studies have revealed that through a complex process involving multiple genes found on both a large plasmid and on the *Shigella* chromosome, attachment of the bacteria to mucosal epithelial cells stimulates a structural alteration of the normally non-phagocytic epithelial cell cytoskeleton and actin filaments to cause uptake of the organism in a manner similar to phagocytosis. Once within the intracellular vacuole of the invaded cell, a hemolysin produced by *Shigella* causes release of the organism into the cytoplasm. The *Shigella* then rapidly multiply and migrate along polymerized actin filaments to reach the plasma membrane so that adjacent cells can be invaded.[3] Early in the course of disease, low numbers of *Shigella* organisms can be found by electron microscopy within mucosal epithelial cell vacuoles. As the disease progresses, though, fibrinous exudate replaces the dead epithelial cells.[4] Death of epithelial cells and sloughing of mucosa creates the ulceration, pseudomembrane formation, hemorrhage, and inflammatory response that typifies shigellosis.

An additional aspect of virulence involves the production of an exotoxin, shiga toxin, by *S. dysenteriae*. Released during host cell lysis, shiga toxin stops host cell protein synthesis by inactivating the 60S ribosomal subunit (similar to the method of action of the plant toxin, ricin). Shiga toxin exerts effects similar to enterotoxins, neurotoxins, and cytotoxins, and can induce apoptosis in epithelial cells. Shiga toxin also enhances the lipopolysaccharide-mediated release of cytokines, such as interleukin-1 and tumor necrosis factor-alpha, which likely contributes to the vascular damage leading to renal failure seen in a complication of shigellosis, hemolytic uremic syndrome.[2]

AFIP Diagnosis: Colon: Colitis, necrotizing, subacute, diffuse, moderate, with a fibrinohemorrhagic pseudomembrane, African green monkey (*Chlorocebus aethiops*), primate.

Conference Comment: There is variation in slides with some slides exhibiting ulceration of the colonic mucosa, while others are only eroded. In this case, immunohistochemistry reveals that most of the *Shigella* organisms are located within the pseudomembrane and along the epithelial border, with few organisms found in the submucosa. However, in other cases, it is not uncommon to find moderate numbers of organisms within the submucosa.

Lesions of enteric shigellosis, as in this case, are primarily in the cecum and colon. The intestinal walls are thickened and edematous with luminal contents varying from fluid mucus with fibrin and cellular debris to frank hemorrhage, multifocal ulcerations and pseudomembrane (diphtheritic membrane) formation. Nonenteric *Shigella* infections have been reported, including gingivitis, abortion, and air sacculitis. With gingivitis, the gums are swollen, hyperemic, with scattered yellow-white foci of necrosis. Severely affected monkeys may have gingival recession and root exposure.[1,4]

The differential diagnosis should include yersiniosis, salmonellosis, and *Campylobacter*-associated enteritis, as well as *Clostridium piliforme* and *E. coli*. Definitive diagnosis requires culture of the organism from a rectal swab or fresh stool specimen.[1,4]

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

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CASE IV – 04011035 (AFIP 2948689)

Signalment: 5-year-old, intact female, Shetland sheepdog.

History: (Per clinician) This canine patient was previously diagnosed with Ehrlichiosis and had been on a Imidocarb therapy regimen. Five months following the initial diagnosis of Ehrlichiosis, the dog presented with clinical signs of ataxia, vestibular signs, lethargy and pale mucus membranes. The patient exhibited anemia and thrombocytopenia. After a month, the neurological signs worsened and the dog started falling while walking. The PCV and platelet count decreased sharply. Horizontal, rotary and positional nystagmus appeared. Craniopropioceptive deficits were observed in the rear limbs and right front limb. Based upon a neurological examination, central vestibular disease, a cerebral cortical lesion, and a lesion between T2-T3 were suspected. Pulmonary radiographs revealed increased interstitial opacity. The dog continued to grow weaker with declining PCV and platelet count. The dog developed hypoalbuminemia (1.5 g/L) and a markedly distended abdomen. The dog died in respiratory distress.

Gross Pathology: At necropsy, the dog was in poor nutritional condition characterized by prominent bony protuberances and absence of visceral and subcutaneous adipose tissue. The mucous membranes and subcutis were mildly icteric. The subcutaneous tissue in the ventral caudal cervical and cranial thoracic region was focally wet and jelly-like (subcutaneous edema). Large volumes of serofibrinous effusions were present within the peritoneum (2L) and pleural cavity (1L). The liver contained multiple, randomly scattered nodules with pale yellow margins and indented, soft, friable (necrotic) centers. Multiple, discrete, variably-sized, slightly raised, dark brown necrotic foci were present on the capsular surfaces and in the parenchyma of spleen, renal cortices and pancreatic lymph node. There was mild cerebellar coning and the leptomeninges over the frontal lobes were multifocally cloudy.

Laboratory Results:
Clinical pathology findings: January 10 – January 17; anemia (PCV-18% to low of 13%), thrombocytopenia (15,000 to low of 13,000/mm^3); hypoalbuminemia (1.5g/dL).
Ehrlichia canis serum titer: 1:40000
Fungus Testing Laboratory, The University of Texas Health Sciences Center at San Antonio identified the fungus as *Ochroconis gallopava.*

Contributor's Morphologic Diagnoses:
Submitted tissue:
1. Liver and kidney: Necrosis, multifocal, subacute, severe with vasculitis and intralesional fungus, Shetland sheepdog, canine.
2. Liver and kidney: Plasmacytic perivasculitis, multifocal, chronic, moderate.
3. Kidney: Membranous glomerulopathy, multifocal, chronic, marked with mild proteinuria.

Tissue not submitted:
4. Brain, spinal cord, gall bladder, pancreas: Perivasculitis, plasmacytic, multifocal, chronic, moderate to severe.
5. Lung: Pneumonia, interstitial, chronic, moderate.
6. Liver, spleen, lung: Extramedullary hematopoiesis.

Contributor's Comment: This patient has two overlapping disease processes. The perivascular lymphoplasmacytic infiltrate in multiple organs, interstitial pneumonia, glomerulopathy, multiorgan microthrombosis, anemia, thrombocytopenia and hypoalbuminemia, which are consistent with the clinical diagnosis of Ehrlichiosis. An unexpected finding in this case includes the necrotizing lesions in multiple organs secondary to a systemic fungal infection.

Phaeohyphomycosis is a collective term for cutaneous and systemic diseases caused by several genera of black molds that develop in tissue in the form of dark-walled, septate mycelium. Phaeohyphomycotic fungi belonging to the genera *Ochroconis* (formerly *Dactylaria*) are known for their neurotropic potential and their predilection to cause severe necrotizing encephalitis in humans,[1] cats[2] and young birds. *Dactylaria gallopava* infection was first reported in 1962 in turkey poults in South Carolina.[3] Subsequent epidemics have been reported in young birds – chickens,[4] grey-winged trumpeters (*Psophia crepitans*),[5] Japanese quail (*Coturnix coturnix japonica*).[6]

The exact mechanism by which this fungus causes systemic disease is unknown. Respiratory exposure to spores has been shown to produce the disease experimentally in poultry.

Ochroconis is a thermophilic fungus and favors soil and decaying vegetation, which can undergo a composting phenomenon associated with the generation of heat and an acidic environment. It has been isolated from broiler house litter where similar environmental conditions prevail. It is also a contaminant of effluents of hot springs and nuclear reactors, thermal soils and self-heated coal waste piles.[7]

Though this fungus is more amenable to therapy, if not recognized and treated in time it can be a cause of significant mortality. Amphotericin B is considered the

antimycotic agent of choice for systemic phaeohyphomycosis, including ochroconiosis. In a case report by Kralovic and Rhodes in 1995, a human liver transplant patient developed *Ochroconis* sp. infection despite receiving prophylactic fluconazole treatment.

AFIP Diagnoses: 1. Kidney: Necrosis, focally extensive, with moderate pyogranulomatous inflammation, vasculitis, and dematiaceous fungal hyphae, Shetland Sheepdog, canine.
2. Liver: Necrosis, multifocal, with neutrophilic inflammation, vasculitis, and dematiaceous fungal hyphae.
3. Kidney: Glomerulonephritis, membranous, global, diffuse, moderate, with multifocal mild plasmacytic interstitial nephritis.

Conference Comment: As mentioned by the contributor, this animal had a high titer to *Ehrlichia canis* as well as related clinical pathology abnormalities. Histologically, a characteristic change is generalized perivascular plasma cell infiltration. Infiltrates are evident in the section of kidney; although increased numbers of perivascular plasma cells might be expected in the liver, the numbers on the slides examined by conference attendees are deemed within normal limits.

Ehrlichiosis is a tick-transmitted rickettsial disease affecting several species of animals and humans. In dogs, disease is caused by *E. canis*, *E. chaffeensis*, *E. risticii*, *E. ewingii*, *E. equi*, *E. phagocytophilia*, and *E. platys* and in horses by *E. equi* and *E. risticii*. *E. risticii* is the causative agent of Potomac horse fever and *E. platys* is the cause of canine cyclic thrombocytopenia.[8]

The arthropod vector of *E. canis* is the brown dog tick, *Rhipicephalus sanguineous*. Following a short incubation period, *E. canis* induces acute disease in which organisms infect monocytes and spread throughout the mononuclear phagocyte system. During this stage, the morula of *Ehrlichia* species may be noted on cytological examination in neutrophils, lymphocytes, and monocytes. Endothelial invasion follows, resulting in vasculitis. There is then a subclinical phase from which the dog either recovers or develops pancytopenic bone marrow failure. The chronic phase is characterized by pancytopenia with depletion of erythrocytic, granulocytic, and megakaryocytic cells, with a persistence of plasma cells within the bone marrow. Gross findings include widespread petechiae and ecchymoses, splenomegaly, lymphadenomegaly, and either hyperplastic (acute disease) or hypoplastic (chronic disease) bone marrow. Histologically there is a perivascular plasma cell infiltration, nonsuppurative meningoencephalitis, interstitial pneumonia, and glomerulonephritis in most dogs. Ehrlichiosis in German Shepherd dogs causes

a severe hemorrhagic disorder attributed to a depressed cell-mediated immune response to *E. canis* in this breed.[8]

Phaeohyphomycoses are uncommon opportunistic infections caused by a number of ubiquitous saprophytic and plant pathogenic molds with the characteristic of forming pigmented (dematiaceous) hyphal elements in tissue. The pigment is melanin and the fungus will generally stain with the Fontana Masson method. Other special histochemical stains commonly used to visualize the fungal morphology include Grocott's methenamine silver (GMS), or periodic acid-Schiff (PAS). With these stains, the fungal hyphae of *Ochroconis* (*Dactylaria*) *gallopavum* are characterized by thick, 2-4 μm wide, septate, non-parallel walls, with acute and right angle dichotomous branching, and yeastlike swellings.[9]

Contributor: Oklahoma State University, College of Veterinary Medicine, Department of Pathology, Rm. 250 McElroy Hall, Stillwater, OK
http://www.cvm.okstate.edu

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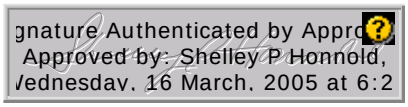

Shelley P. Honnold, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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