

Joint Pathology Center
Veterinary Pathology Services



WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 1 5

18 January 2017

CASE I: WSC#2 (JPC 4066310).

Signalment: 28-year-old, male, American alligator, (*Alligator mississippiensis*).

History: This alligator was housed with 10 others at a rescue facility in the northeastern United States. During the winter months, the animals were kept indoors in a house, living on wood floors, with access to an unspecified water source; there was reportedly visible mold in this environment. The alligators were not induced to hibernate during this time, and were housed at an ambient temperature of 74°F (23°C). This alligator was found dead with no premonitory signs.

Gross Pathology: An adult, 70.5 kg male American alligator was presented in good nutritional condition with abundant fat stores. There was an extensive adhesion of the right lung to the right dorsal body wall which enclosed approximately 75 mL of pale tan, thin liquid with grey to pale tan particulates. The pleural surfaces of both lungs had discrete to coalescing areas of firm, brown to pale tan discoloration which were more extensive in the right lung and extended into the parenchyma. On cut section there were extensive regions of

parenchyma replaced by firm, white, crumbly material (caseous necrosis). Additionally, multiple subpleural cavitations and air spaces in the right lung were lined by white, slightly fuzzy, fungal mats. Numerous small, whitish foci up to 0.3 cm diameter were present in the liver, and similar individual foci were present in the heart, spleen, and kidney.



Lung and air sac, alligator. The pleural surfaces of both lungs had coalescing discrete areas of firm tan within the parenchyma. (Photo courtesy of Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory, College of Agriculture, Health and Natural Resources, University of Connecticut, <http://www.pathobiology.uconn.edu/>)

Laboratory results: Fungal culture and identification: A swab from the lung was initially plated on potato dextrose agar (PDA) and inhibitory mold agar (IMA) with and without antibiotics and then incubated at 30°C for 14 days; this yielded heavy growth of white fungal colonies on both plate types. On PDA, the bottom of the colony had an orange to pink tinge, whereas on IMA the bottom of the colony was red. Cultures were submitted to the Fungus Testing Laboratory, University of Texas Health Science Center in San Antonio, Texas for identification. Combined phenotypic characterization and DNA sequencing of ITS and TEF targets identified the fungus as *Beauveria bassiana*.

Histopathologic Description: Up to 80% of the parenchyma is effaced by necrosis that extends to the pleural surface. Necrotic areas are characterized by variable loss of architectural detail with accumulation of fibrin, hypereosinophilic cellular debris, edema, hemorrhage, and inflammatory cells composed predominantly of heterophils and



Lung and air sac, alligator. Multiple subpleural cavitations in the right lung were lined by white fuzzy fungal mats. (Photo courtesy of Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory, College of Agriculture, Health and Natural Resources, University of Connecticut, <http://www.pathobiology.uconn.edu/>)

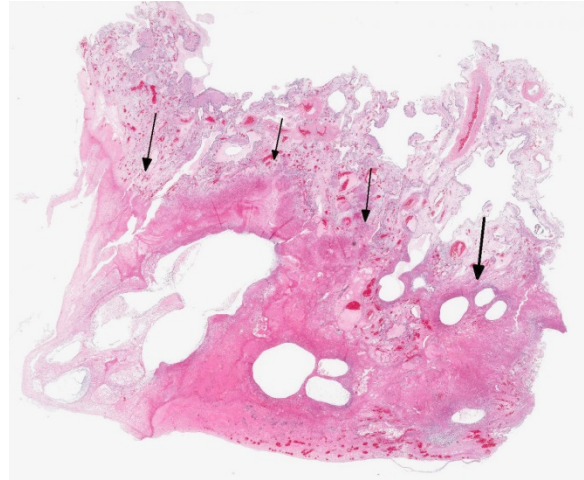
macrophages. Within the necrotic areas, there are numerous, lightly basophilic to transparent fungal hyphae that are 2-6 microns in diameter, septate, and parallel-walled with occasional right angle branching. At the air-tissue interface in a couple of large airways there are scattered hyphae that give rise to dense clusters of ampulliform conidiogenous cells measuring up to 3 microns in diameter, each with a single to several terminal round conidia measuring 1-3 microns in diameter. Large numbers of bacteria are also frequently admixed. Rarely, in areas of dense fungal growth, there are a few translucent, variably shaped, anisotropic crystals (oxalate crystals). Frequently, vessels are occluded by fibrin thrombi, and their walls contain necrotic debris, fibrin, moderate numbers of degenerate heterophils and occasional fungal hyphae (vasculitis). The parenchyma adjacent to necrotic areas is variably expanded by edema, mixed inflammatory cells, and reactive fibroblasts.

Contributor's Morphologic Diagnoses:

Lung: severe, subacute, multifocal to coalescing, fibrinonecrotizing and heterophilic pneumonia and pleuritis with vasculitis, fibrin thrombi and intralesional bacteria and fungal hyphae and conidia, consistent with *Beauveria bassiana*.

Contributor's Comment: This is a case of mycotic pneumonia in an American alligator caused by *Beauveria bassiana*. Identification of this organism was based on the morphology of the fruiting bodies (conidiogenous cells and conidia) on H&E; its phenotypic characteristics in culture; and DNA sequence analysis, all of which differentiated it from other common agents of fungal pneumonia, particularly *Aspergillus* species. *Beauveria bassiana* is a ubiquitous soil saprophyte that is entomopathogenic, i.e. pathogenic to insects

due to an affinity for chitinous exoskeletons. As such, it has been widely used for more than 100 years as biocontrol of pest insects.⁵ Though widespread in the environment, its upper temperature limit is around 30°C, and it is inactivated within hours or days when exposed to sunlight.⁹ Due to the temperature limitations, *B. bassiana* rarely causes infections in mammals but is an opportunistic pathogen of reptiles, with previous reports in captive American alligators,² chelonians,^{3,6} and in cold-



Lung, alligator. Approximately 75% of this section exhibits coagulative necrosis (arrows demonstrate line of demarcation from viable tissue.) (HE, 5X)

stunned Kemp's Ridley sea turtles.⁶

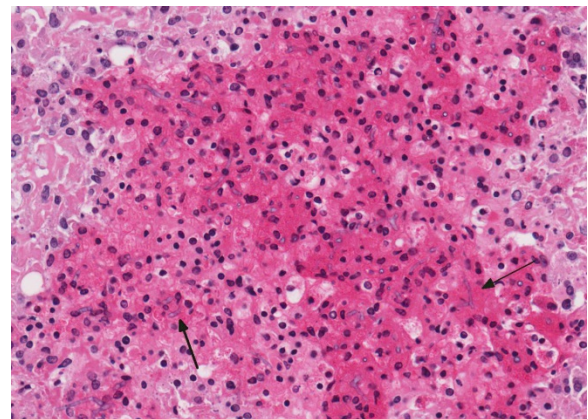
Exposure to low temperatures is often implicated as a contributing factor in these infections. Two previous cases in alligators occurred after a prolonged hibernation and brief heating system failure.² In the current case, the temperature of the indoor enclosure was reportedly kept at 23°C, well within the temperature range of *B. bassiana*. High levels of fungus in the environment and poor ventilation were also probably involved in this case, as mold was reportedly visible in the enclosure where this group of alligators was housed. Other predisposing factors for fungal pneumonia in captive reptiles include additional husbandry-related issues, such as humidity, hygiene, and nutrition,

immunosuppression, overuse of antibiotics, and concurrent disease.⁵ Shortly after diagnosis of this case, a second alligator from the same group died naturally, but a necropsy was not performed.

Transmission is thought to occur from inhalation or ingestion of fungal spores from the environment, and the lung appears to be the primary site of infection. Hematogenous dissemination of the infection from the lung to other tissues, such as liver and spleen, occurred in this case as in previous cases.^{2,6} *Beauveria bassiana* produces several toxic compounds including oxalic acid, which promotes the formation of oxalate crystals within affected tissues;⁹ only a few crystals were seen in this case.

JPC Diagnosis: Lung: Pneumonia, necrotizing, multifocal to coalescing, severe, with innumerable fungal hyphae and large colonies of mixed bacilli, American alligator, *Alligator mississippiensis*.

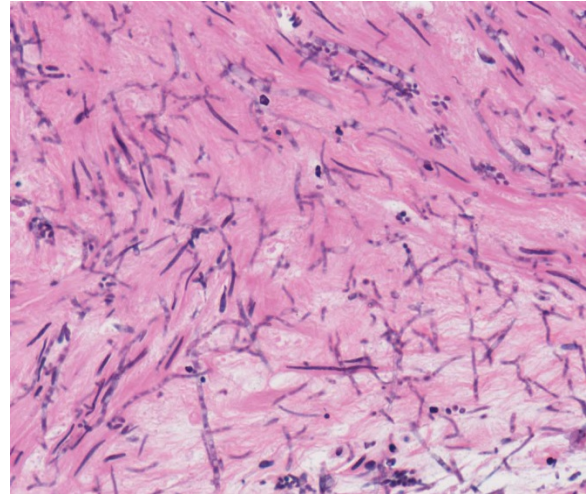
Conference Comment: This case provided conference participants the unique opportunity to describe lung pathology in an American alligator, an uncommonly seen species at the Joint Pathology Center. Prior



Lung, alligator. Heterophilic granulomas are scattered throughout the pulmonary parenchyma and contain numerous 2-4µm diameter septate fungal hyphae (arrows). (HE, 360X)

to the discussion of this case, the conference moderator led a review of the normal functional anatomy and physiology of alligator lungs, which was poorly understood until relatively recently.^{1,7} Research performed at the University of Utah indicates the external and internal morphology of alligator lungs is strikingly similar to the avian respiratory system, although in contrast to birds, alligators lack intra-abdominal air sacs.^{1,7} Alligators have a highly efficient unidirectional style of breathing, originally thought to be unique to avian species as a consequence of the high oxygen demands of flight.⁷ However, unlike birds, alligators use a diaphragm to pull air into the lungs. The air then travels one direction through bronchi which branch into numerous smaller parabronchi and continues further into alveolar-like spaces, called faveoli. Gas exchange then takes place within these faveoli, and the air then flows out of the lung via in a one-way loop and valve system.⁷ Unidirectional breathing is much more efficient than the mammalian bellows-style breathing because there is no alveolar mixing of inspired and expired air. Research is ongoing to elucidate the exact mechanism of unidirectional air flow in alligators and other reptiles, as it was thought that air sacs were necessary for unidirectional air flow breathing.^{1,7}

Reported cases of fungal pneumonia in reptiles caused by *Beauveria bassiana* are rare and typically involve extensive multifocal necrosis or granulomatous nodules with high numbers of fungal hyphae in the lungs with dissemination to the multiple abdominal organs, as present in this case.^{2,3,6} Infection occurs after inhaling or ingesting fungal spores from the environment and development of disease in reptiles has been associated with low environmental temperatures and poor husbandry of captive reptiles. As mentioned



Lung, alligator. Necrotic tissue is replete with proliferating fungi which often form dense mats. (HE, 400X).

by the contributor, the fungus will not grow at mammalian physiologic temperatures (37°C)^{2,3,6}, although it has been very rarely reported to cause fungal keratitis in people associated with contact lens wear and prior treatment with corticosteroid eye drops.⁴ The association of this fungus with low environmental temperatures and cold-shocked reptiles in previously reported cases prompted the conference moderator to discuss brumation in ectothermic animals.

Brumation is a time of dormancy in reptiles in response to colder winter weather (~21°C), and is similar, but not identical, to hibernation in mammals. During periods of brumation, reptiles have a markedly decreased metabolic rate, but do not fall into a deep sleep, and can regularly emerge to drink and bask during warm days. Additionally, reptiles typically do not eat during periods of brumation.⁸

Contributing Institution:

Department of Pathobiology and Veterinary Science
Connecticut Veterinary Medical Diagnostic Laboratory
College of Agriculture, Health, and Natural Resources

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CASE II: 15-5172 (JPC 4066860).

Signalment: Four-year-old, male, red tail boa constrictor, (*Boa constrictor constrictor*).

History: The patient initially presented on 10/29/2014 for a 4-5 month history of anorexia. At that time, there was atrophy of the epaxial muscles and a firm, but compressible swelling expanding the cranial cervical region. Ultrasound of this area revealed a soft-tissue mass, for which the tissue of origin was unclear. The mass did not appear to be associated with the trachea or within the esophageal lumen. The patient represented in January 2015 for continued anorexia, progressive lethargy, and recent regurgitation after forced feeding. The patient had lost a significant amount of weight and on examination, there was a large accumulation of necrotic material in the mouth. During this exam, a second mass was appreciated just caudal to the previously described cervical mass. At the owner's request, the patient was euthanized.

Gross Pathology: A 2 cm x 0.5 cm region of the hard palate is raised, irregularly surfaced, fleshy, and dark red. Extending from the esophageal wall and protruding into the lumen is a 2.5 cm x 1 cm x 1 cm, smooth, soft, pink and red mass, with a core of crumbly, brown-yellow material. Approximately 0.5 cm aboral to this mass is a 4.5 cm x 2.5 cm x 2.5 cm, smooth, firm, ovoid, pink and red mass that on section is composed of a central core of a crumbly, brown-yellow material (caseous necrosis) surrounded by a 0.7 cm wide rim of a fleshy, pink/red tissue. The corresponding esophageal serosa is firmly adhered to the local body wall. The esophageal mucosa, adjacent to the larger mass, has a 2 cm x 0.4 cm, poorly demarcated, mildly depressed, region composed of dozens of pinpoint red foci

(erosion). Within the lumen of the aboral esophagus, approximately 3.5 cm from the larger mass, are three rough, ovoid aggregates of a mottled brown, yellow, and green, crumbly material. This material is not adhered to the mucosa.



Oral cavity, boa constrictor: A 2x0.5 cm of the hard palate is raised, fleshy, and dark red. (Photo courtesy of Department of Biomedical Sciences, section of Anatomic Pathology, Cornell University, College of Veterinary Medicine.)

Laboratory results: N/A

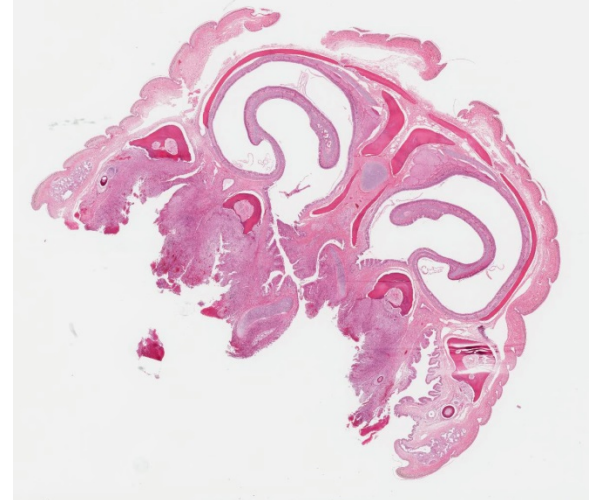
Histopathologic Description: ORAL CAVITY: Severely expanding the submucosa, confluent with a large region of epithelial ulceration, and obliterating the adjacent lamellar bone is a densely packed population of foamy macrophages with fewer granulocytes, lymphocytes, and plasma cells. Unilaterally the alveolar bone is disrupted and replaced by a similar inflammatory cell population and the islands of retained bone has irregular, scalloped

edges and are lined by numerous osteoclasts within distinct Howship's lacunae. The superficial osteoid matrix commonly contains a thin, irregular, basophilic line (reversal line). Similar inflammatory cells efface the dentin and invade the pulp of a tooth. The surface epithelium is entirely replaced by a band of necrotic cellular debris and fibrin.

BRAIN, NOS: Occasional neurons contain discrete, intracytoplasmic, 1-10 μ m diameter, glassy, eosinophilic inclusions.

NASAL CARTILAGE: Commonly, the respiratory epithelial cells and submucosal gland epithelial cells contain discrete, intracytoplasmic, 1-10 μ m diameter, glassy, eosinophilic inclusions. Scattered throughout the submucosa are scant numbers of lymphocytes and plasma cells.

ESOPHAGEAL MASSES (not submitted): Multifocally the esophageal wall is severely expanded by multiple unencapsulated, irregular masses composed of densely packed vacuolated macrophages mixed with

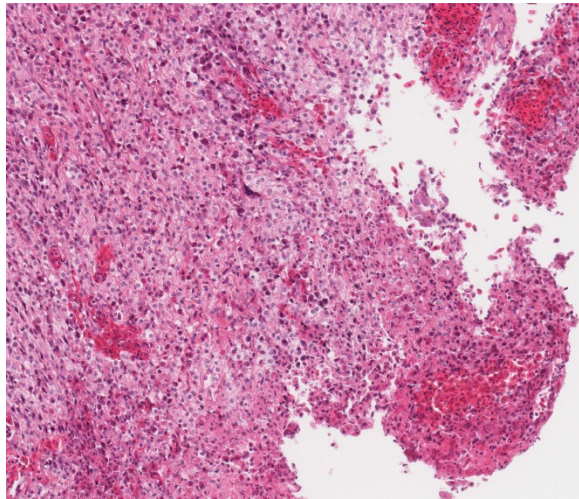


Oral cavity, boa constrictor: Large sections of the mucosa of the oral cavity is replaced by a densely cellular area of granulation tissue which extends through the submucosa and erodes underlying bone. (HE, 5X)

fewer small lymphocytes, plasma cells, granulocytes, and multinucleated nucleated giant cells. Lymphocytes are occasionally present in small, poorly defined islands and cells contain discrete, intracytoplasmic, 1-4 um diameter, glassy, eosinophilic inclusions. The overlying epithelial cells contain discrete, 2-6 um, glassy, eosinophilic, intracytoplasmic inclusions.

Additional findings include numerous discrete, 1-10um in diameter, glassy, eosinophilic, intracytoplasmic inclusions within multiple tissues including hepatocytes, biliary epithelial cells, gastric mucosa, intestinal epithelium, tracheal epithelium, bronchiole epithelium, and the retinal ganglion cells. Concurrently within the liver, there were small numbers of randomly distributed macrophages, lymphocytes, and plasma cells.

Contributor's Morphologic Diagnoses: Oral cavity: Severe, diffuse, chronic, granulomatous and ulcerative stomatitis with numerous, eosinophilic, intracytoplasmic inclusion bodies



Oral cavity, boa constrictor: Higher magnification of the granulation tissue within the oral cavity populated by large numbers of macrophages and fewer heterophils with an ulcerated surface (lower right). (HE, 188X)

Esophageal mass (not submitted): Severe, multifocal, chronic, granulomatous and ulcerative esophagitis with numerous, eosinophilic, intracytoplasmic inclusion bodies

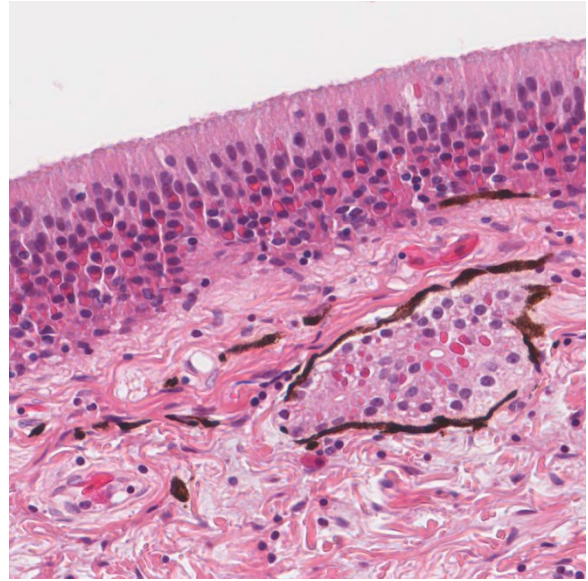
Stomach, intestine, trachea, lung, kidney, liver, nasal cavity, retina, and brain (not submitted): Severe, eosinophilic, intracytoplasmic inclusion bodies

Contributor's Comment: Inclusion body disease (IBD) is reported in multiple snake species, but most commonly within the family *Boidae* and *Pythonidae*. As the name implies, the characteristic finding in these cases are distinct, variably sized, eosinophilic, intracytoplasmic inclusions.² In one recent retrospective study, the prevalence of IBD in captive collections was approximately 19%. Although disease progression varies greatly between individuals and between species, the disease is classically associated with central nervous system signs, including head tremors, anisocoria, and opisthotonus.⁹ Commonly the animal succumbs to complications secondary to immunosuppression.¹⁵ In boas, the disease can have a more protracted progression of weeks to months⁹ and typically patients have a previous history of regurgitation.¹⁰ In addition, a proportion of boas can be subclinical carriers.⁷⁻¹⁰ Meanwhile, pythons tend to display a more aggressive disease course of only a few weeks,⁹ with a more profound inflammatory reaction.¹⁰ Interestingly, regurgitation tends not to be a part of clinical disease in pythons.¹⁰ Gold standard testing remains the histologic demonstration of intracytoplasmic inclusions in multiple organs, most notably the liver, stomach, and esophageal tonsils, however, their absence does not rule out the disease.¹⁰ Although with further characterization of the underlying etiology,

molecular testing and immunohistochemistry³ may be available in the future.

Although long considered to have an underlying infectious etiology, the cause of IBD has been elusive. Originally the disease was thought to be associated with a retrovirus, however, recently divergent arenaviruses have been implicated as the cause of IBD.^{2,4,8-10} Of late, *in vitro* Koch's postulates have been met linking arenavirus to the development of IBD, however, *in vivo* studies have not been reported.⁹ Arenaviridae are enveloped, negative sense, single stranded, bipartite RNA viruses.^{2,8} Arenaviruses have previously been thought to only affect rodents, with infrequent but possible transmission to other mammal species (e.g. humans and bats).² The viral genome is composed of a small (S) segment and a large (L) segment. The S segment encodes the viral nucleocapsid protein (NP) and the glycoproteins (GP1 and GP2) while the L segment encodes the viral RNA-dependent RNA polymerase and a small ring domain containing protein.^{2,9} The distinction intracytoplasmic inclusions consist of a unique 68KDa protein, that has been named "inclusion body disease protein" (IBDP).^{3,9,10} This protein has been demonstrated to be the arenaviral NP protein.⁶ Boid-associated inclusion body arenaviruses tend to be highly divergent.^{2,8,9}

Proposed theories as to the mode of transmission include direct contact, possible arachnid vectors (i.e. the snake mite, *Ophionyssus natricis*), mammalian vectors (i.e. live prey), and vertical transmission.⁸⁻¹⁰ As several other arenaviruses are able to cross the species barrier, it remains possible that the highly divergent boid inclusion body disease associated arenaviruses (BIBDAV) may as well. A recent report showed that BIBDAV was infective to tick cell lines (mite cell cultures were not available) and



Nasal cavity, boa constrictor: Respiratory and submucosal gland epithelium contain numerous 2-4um round to oval intracytoplasmic viral protein inclusions. (HE, 400X)

this data may support the role of *Ophionyssus natricis* infestation in disease transmission.⁸ Furthermore, maintenance of BIBDAV within mammalian (VERO E6) and boid cell lines appears to be temperature dependent, with strong growth at 30°C and inhibition of growth at 37°C. Thus, the authors propose that transmission from a mammalian host is possibly hindered by the higher mammalian body temperature.⁸

Boid snakes have large well-developed esophageal tonsils, which can be enlarged and abscessed. In this case, the large esophageal masses may represent severely inflamed esophageal tonsils.

JPC Diagnosis: 1.Oral cavity: Stomatitis, ulcerative and histiocytic, chronic, multifocal to coalescing, severe, with granulation tissue and bone resorption, red tail boa constrictor, *Boa constrictor constrictor*.

2. Epithelial cells: epidermis, salivary gland, and nasal cavity: Intracytoplasmic protein inclusions of viral origin, numerous.

Conference Comment: Boid inclusion body disease (BIBD) is considered by many to be the most important viral infection of captive boas and pythons, often causing progressive and rapidly fatal multisystemic disease.¹⁰ As mentioned by the contributor, the clinical presentation of BIBD is highly variable among individuals, especially in adult boas, where intracytoplasmic viral protein inclusions can be found in snakes without clinical signs.^{2,6,10,13} However, the disease is still considered to be fatal due to severe impairment of immune function of leukocytes and myelopoietic cells, resulting in death due to opportunistic infections.^{6,10} Interestingly, in this case, the conference moderator speculates that the extensive ulcerative stomatitis seen both grossly and histologically may be due to BIBD-induced starvation and regurgitation combined with the mechanical trauma from the reported forced feeding.

Typical gross findings associated with BIBD are usually limited to areas susceptible to secondary opportunistic infections, such as the oral cavity, gastrointestinal tract, lungs, liver, and kidney. Histologically, the hallmark of BIBD is the presence of numerous 1-4 um, pale, eosinophilic intracytoplasmic inclusion bodies in all major organs, especially the kidneys, liver, stomach, and brain. Inclusions are typically more prominent in the visceral organs of boas and central nervous system in pythons.¹⁰ In this case, inclusions are widely distributed throughout.

Conference participants discussed the composition of the highly distinctive inclusion bodies associated with this disease. The inclusions, which ultrastructurally are

cytoplasmic aggregates of electron-dense material, composed of an antigenically unique 68-kilodalton non-viral protein.^{2,3,6,15} Recently, a novel group of arenaviruses were isolated from snakes with BIBD. In an in-vitro cell culture model, this arenavirus induces the pathognomonic inclusion bodies and was discovered to predominantly consist of arenaviral associated nuclear protein. This finding led to the suggestion of the formation of a novel genus called the *Reptarenavirus* and placing the remaining arenaviruses in the *Mammarenavirus* genus. There is still some controversy surrounding the formation of a new genus given the lack of *in vivo* confirmation.^{3,9,11}

Conference participants discussed the importance of arenaviruses as zoonotic pathogens associated with rodent host species. In humans, arenaviruses, such as Lassa and Machupo (Bolivian hemorrhagic fever), cause outbreaks of rapidly fatal viral hemorrhagic fevers, not unlike the Ebolavirus.¹ Additionally, hamsters are the primary source of lymphocytic choriomeningitis (LCM) virus causing meningoencephalitis in humans and callitrichid hepatitis in New World primates.¹³ Arenaviruses generally produce only mild or subclinical disease in their natural host species.

Contributing Institution:

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Section of Anatomic Pathology

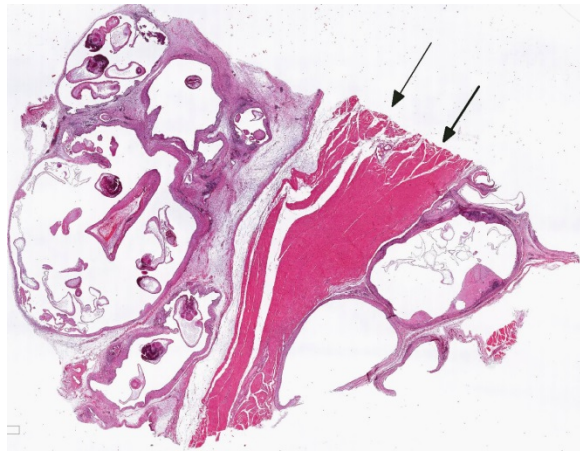
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- CASE III: G9428 (JPC 4085109).**
- Signalment:** 28-year-old, female, Nilgiri langur, (*Trachypithecus johnii*).

History: An adult female captive born Nilgiri langur (*Trachypithecus johnii*) from a zoological garden in Central Europe developed an edematous swelling of the left thigh, which persisted for several months and was associated with periods of a decreased general condition, depression, and anorexia. Sonographic examination of the thorax and abdomen revealed cardiomegaly as well as poor demarcation and cloudy appearance of the liver. The animal was finally euthanized due to a poor general condition, anorexia, and therapeutic resistance.



Skeletal muscle, langur. The skeletal muscle of the left thigh (arrows) is separated and compressed by several encapsulated cysticerci containing numerous cross-ad tangential sections larval cestodes. (HE, 7X)

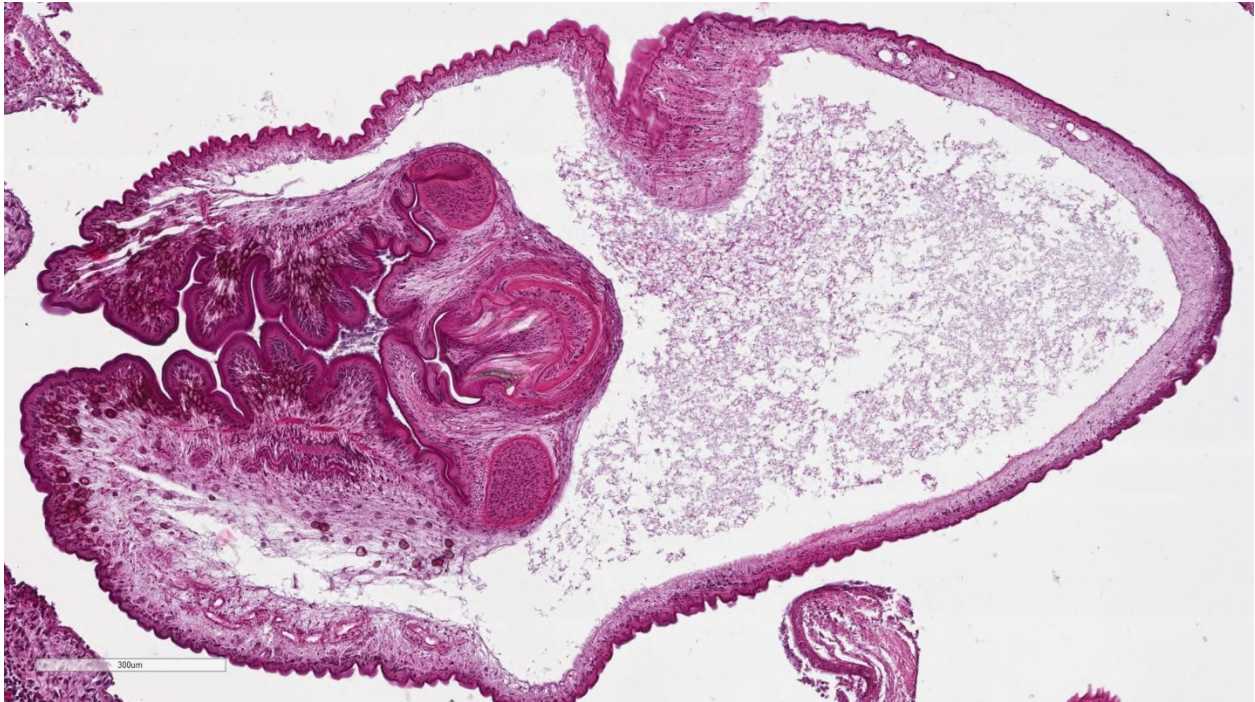
Gross Pathology: At necropsy, the animal was cachectic. The skeletal muscle of the left thigh was severely atrophic and replaced by fluctuant multilocular cysts containing numerous sand grain sized whitish structures. The left caudal lung lobe revealed a focal circumscribed area of atelectasis.

Laboratory results: PCR analysis of muscular metacestode tissue identified *Cysticercus longicollis*, the larval stage of *Taenia crassiceps*, as the etiologic agent.

Histopathologic Description: Within the skeletal muscle of the left thigh are multifocal extensive areas of fibrous connective tissue bearing multiple cystic structures with numerous larval cestodes (cysticerci). Cysts are surrounded by thick fibrous capsules that are multifocally infiltrated by plasma cells, lymphocytes, macrophages, and eosinophils. The inflammatory cells extend into the adjacent fibrous granulation tissue between muscle fibers that contains few multinucleated giant cells. Cysticerci are characterized by a 4 μ m thick, eosinophilic tegument, a fibrillar, eosinophilic parenchyma, numerous 5 μ m diameter, basophilic, calcareous corpuscles, and an invaginated scolex with muscular suckers and hooks.

Contributor's Morphologic Diagnoses: Skeletal muscle: Myositis, chronic, granulomatous and eosinophilic, multifocal, severe, with intralesional cysticerci, Nilgiri langur (*Trachypithecus johnii*), non-human primate.

Contributor's Comment: Non-human primates might act as aberrant hosts for a number of cestode species after oral infection and larval development in extra-intestinal locations.² *Taenia crassiceps* is a cestode parasite of the Northern hemisphere, whose life cycle includes canids as definitive hosts, most commonly the red fox (*Vulpes vulpes*) in Europe and the Arctic fox (*Alopex lagopus*) as well as the red fox in North America.⁶ Natural infection by *T. crassiceps* has also been reported in wolves (*Canis lupus*)⁵ and coyotes (*Canis latrans*)¹³ in North America as well as in wild cats (*Felis silvestris*)¹² and domestic dogs in Germany.³ Several rodent species and rabbits serve as intermediate hosts for the metacestode larval stage of the parasite, *Cysticercus (C.) longicollis*. However, the



Skeletal muscle, langur. At low magnification, larval cestodes are characterized by a ridged tegument and an invaginated scolex. (HE, 69X)

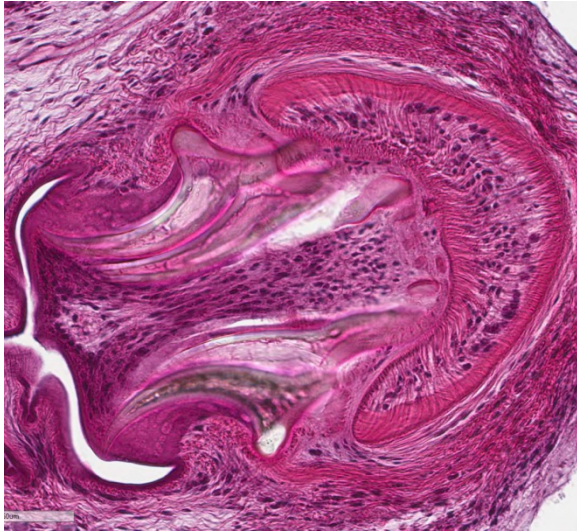
common vole (*Microtus arvalis*) is the predominant intermediate host in Europe.¹ Sporadic cases of clinical cysticercosis caused by *T. crassiceps* have been reported in humans and domestic animals such as dogs and cats, many of them in immunocompromised individuals.^{8,9,16} Infections by *T. crassiceps* may be particularly serious due to their proliferative nature. In contrast to cysticercosis associated with other *Taenia* species, *T. crassiceps* is able to proliferate by exogenous and endogenous budding. Exogenous budding may produce 1-6 daughter cysticerci at the absclex pole of the maternal cyst. Daughter cysticerci may bud off or remain attached by a stalk, form a scolex of their own, and bud again. Endogenous budding occurs less commonly and is seen in larger, older cysticerci. Such reproductive capability may result in extensive infections, most frequently involving the subcutis and pleural and peritoneal cavities. In humans, there are

occasional reports about intraocular manifestations of cysticercosis.⁴

In non-human primates, there are documented cases of *T. crassiceps* cysticercosis in a black lemur (*Eulemur macaco macaco*)⁴ and in a ring-tailed lemur (*Lemur catta*)¹⁰, both of them being prosimian species. *T. crassiceps* cysticercosis in an Old World monkey species like the Nilgiri langur has not been reported before. Interestingly, in the langur, metacestode tissue was not limited to the skeletal muscle, but could also be observed in the left caudal lung lobe, reflecting the proliferative and invasive nature of this parasite.

Other *Taenia* species causing cysticercosis in non-human primates include *Taenia solium*, *Taenia crocutae*, *Taenia hydatigena*, and *Taenia martis*.^{2,14} However, infections with the larval cestodes mainly occur in Old

World monkeys and apes, while reports of taeniid cysticercosis in New World monkeys and prosimians are sparse.



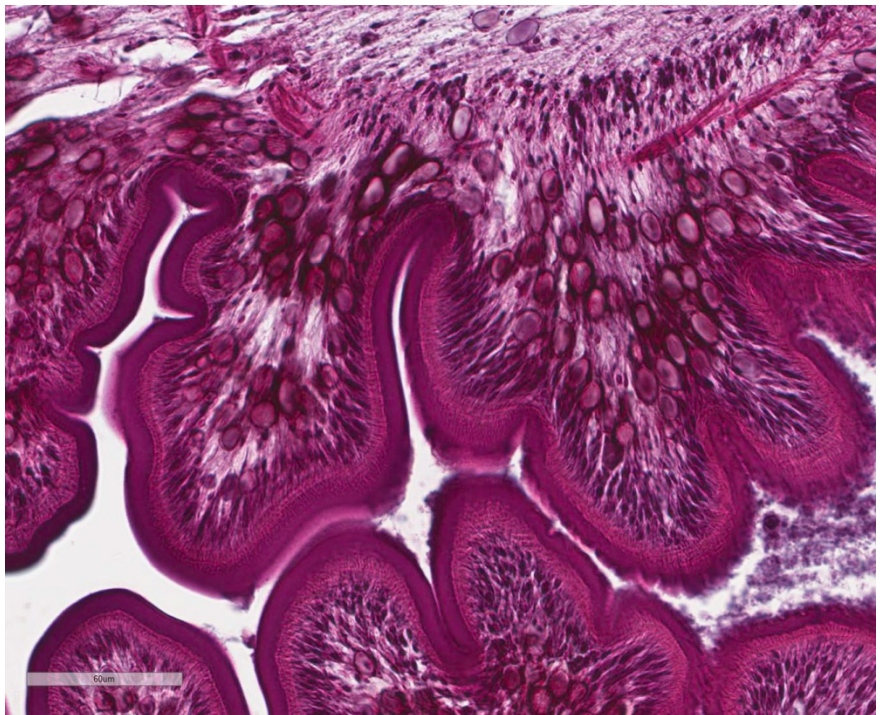
Skeletal muscle, langur: This cestode possesses an armed rostellum with birefringent hooklets. (HE, 400X).

Studies on the immune response elicited by *T. crassiceps* and its antigens in human and mice cells suggest a strong capacity of this parasite to induce a chronic Th2-type response that is primarily characterized by high levels of Th2 cytokines, a low proliferative response in lymphocytic cells, an immature and LPS-tolerogenic profile in dendritic cells, recruitment of myeloid-derived suppressor cells, and by activated macrophages.¹¹

JPC Diagnosis: Skeletal muscle: Cysticerci, multiple, with mild chronic granulomatous inflammation, Nilgiri langur (*Trachypithecus johnii*).

Conference Comment: The contributor provides a striking example of multiple

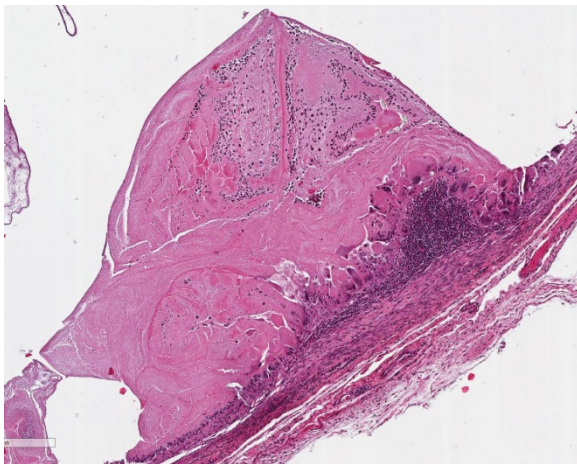
intramuscular cysticerci containing cross sections of taeniid metacestodes, the larval form of cestode tapeworms. The class *Cestoda* has two orders of veterinary importance. The first is *Pseudophyllidea*, comprised of *Diphyllobothrium* sp. and *Spirometra* sp.¹⁵ These parasites grow into extremely large adults, up to 15 meters in humans, lack suckers, and require two intermediate hosts, typically an aquatic copepod and fish. In contrast, the order *Cyclophyllidae*, which contains *Taeniidae*, *Mesocestoididae*, *Dipylidiidae*, *Anoplocephalidae*, and *Hymenolepididae*, require only one intermediate host, usually a land mammal or arthropod.¹⁵ Adult cestodes are normally present in the intestine, hepatic ducts, and/or pancreas of the final definitive host while the larval forms are present within the tissue or body cavities of intermediate hosts. Adult cestodes are broken into segments, called proglottids, which contain both female and male reproductive organs. Cyclophyllidae have four anterior suckers present in both larval and adult cestodes and birefringent armed hooks, depending on the species. The four



Skeletal muscle, langur. There are numerous oval calcareous corpuscles beneath the tegument and row of somatic cell nuclei. (HE, 400X)

anterior suckers may not all be visible histologically due to varying planes of section.^{7,15}

While adult tapeworms are usually of minor significance in their carnivorous definitive hosts, the larval form can migrate into various tissue and cause significant pathology in intermediate or paratenic hosts. Conference participants discussed the four different forms of larval cestodes in tissue section. These include cysticercus, present in this case, strobilocercus, coenurus, and the hydatid cyst.^{7,15} The cysticercus is thin walled, fluid filled, and contains a single larva with one inverted scolex and four suckers. The strobilocercus is later in development and contains an evaginated and elongated scolex and develops multiple segments, similar to the adult cestode. Coenurus is similar to cysticercus but contains more than one scolex, all of which can develop into an adult in the definitive host. Hydatid cysts, typical of the genus *Echinococcus* sp., have a bladder with large numbers of small protoscolices grouped into clusters called brood capsules.^{7,15}



Skeletal muscle, langur. A degenerate cestode is adherent to the wall of one cyst, where it has stimulated an inflammatory response characterized by prominent foreign body macrophages overlying an aggregated of lymphocytes and plasma cells inside the fibrous capsule (HE,63X).

The inflammatory response in this case is mild and composed of a mixed population of histiocytes, multinucleated giant cell macrophages, lymphocytes, and plasma cells with mild atrophy of the adjacent skeletal muscle bundles. The glycoprotein-rich wall of the cysticerci provokes little to no host reaction when intact; however, rupture of the cysticerci results in a severe granulomatous inflammation, fibrosis, and mineralization.¹⁵ Additionally, *Cysticercus longicollis*, the larval form of *Taenia crassiceps* present in this case, undergoes both endogenous and exogenous budding of the cysticerci leading to severe and disseminated infection.⁴

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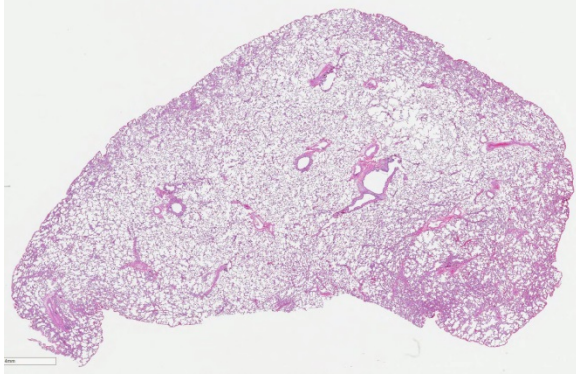
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CASE IV: 130055 (JPC 4083952).

Signalment: Adult male cynomolgus macaque (*Macaca fascicularis*).

History: This macaque was in a study to determine the efficacy of a novel therapeutic drug for treating Marburg virus (MARV) infection. All of the monkeys in this study were inoculated subcutaneously with MARV and then once daily intramuscular treatments with either saline (control group) or different doses of the therapeutic drug (experimental groups) began. This animal was in one of the experimental groups and it was found dead on Day 11 after viral challenge.



Lung, cynomolgus macaque. At low magnification, the submitted section of lung appears well-aerated with no gross lesion. (HE, 5x).

This monkey was part of a research project conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the 8th edition of the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Gross Pathology: The mucosa of the rectum and distal 20 cm of the colon was diffusely hemorrhagic. The liver was enlarged (~1.5 X), pale tan, and markedly friable. The spleen was also friable. Other organs were unremarkable.

Laboratory results: None

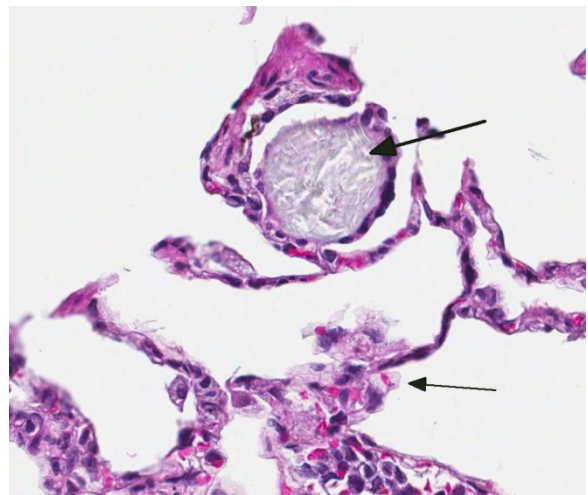
Histopathologic Description: Lung (right inferior lobe): Multifocally within alveoli and often attached to the alveolar septa, there are low numbers of multinucleated giant cells, measuring up to 100 µm in diameter, most of which contain intracytoplasmic aggregates of pale blue-gray amorphous to spicular refractile material. The interstitium also contains scattered aggregates of low numbers of histiocytes

containing intracytoplasmic brown-black finely-granular material. Many blood vessels contain numerous intraluminal mononuclear leukocytes (monocytes).

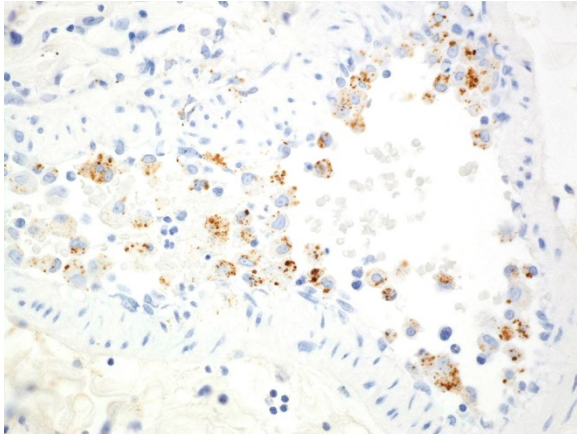
Contributor’s Morphologic Diagnoses:

1. Lung; intravascular leukocytosis (monocytic), moderate
2. Lung; multifocal histiocytic (multinucleated giant cell) alveolitis, mild, with intracytoplasmic crystalline foreign bodies
3. Lung; multifocal interstitial anthracosilicosis, minimal

Contributor’s Comment: The timing of this monkey’s death is within the usual interval (i.e. 7-11 days) that cynomolgus macaques die after experimental exposure to a lethal dose of MARV. There were histologic lesions in the liver, spleen, adrenal glands, tonsils, and lymph nodes of this monkey that were caused by MARV infection; these organs are considered “target organs” for the virus.⁷ Immunohistochemistry (IHC) revealed MARV antigen in every organ examined from this animal. The histologic findings and IHC



Lung, cynomolgus macaque. At high magnification, alveolar histiocytes and multinucleated giant cell macrophages contain vary amounts of a granular amphophilic crystalline protein within their cytoplasm. (HE, 400X)



Lung, cynomolgus macaque. Immunohistochemistry demonstrates that many of the monocytes in the lumen of a pulmonary artery contain MARV antigen (brown stain) within their cytoplasm. Immunoperoxidase stain with hematoxylin counterstain. (anti-MARV, 400X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD <http://www.usamriid.army.mil/>)

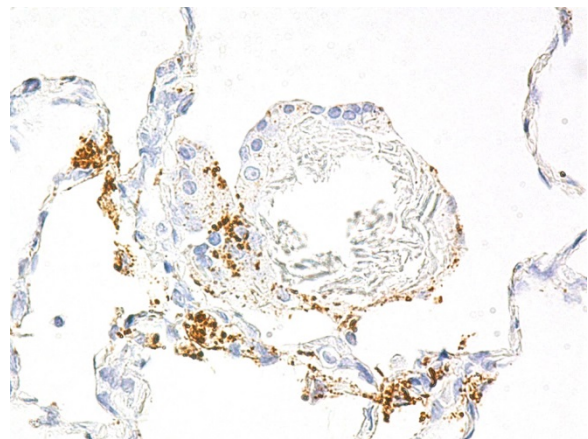
results confirmed that this macaque died from a disseminated MARV infection. Although the exact cause of the intestinal bleeding noted at necropsy was not determined, this was most likely associated with MARV-induced coagulopathy; disseminated intravascular coagulopathy (DIC) occurs commonly in primates (including humans) infected with viruses in the family *Filoviridae* (i.e. ebolaviruses and MARV).^{6,7}

The monocytic leukocytosis noted within pulmonary blood vessels of this monkey is attributable to the viral infection. IHC revealed abundant MARV antigen in many of these monocytes. Cells of the monocyte-macrophage system are infected very early during the course of filovirus infection and are primarily responsible for disseminating the viruses throughout the body.^{5,7}

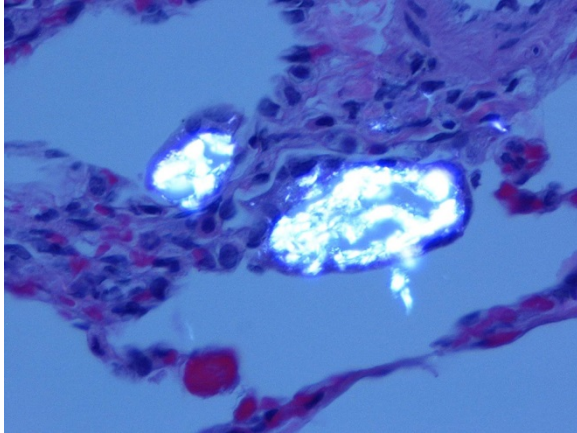
The presence of multinucleated giant cells within alveoli and/or attached to alveolar septa of this monkey was an unexpected finding and was unrelated to the MARV

infection. This lesion was seen in the right inferior lung lobe but not in the other lung lobes that were examined histologically. These giant cells were an inflammatory response to the presence of intra-alveolar crystalline foreign material; the foreign material was initially phagocytized by macrophages that then fused to form large multinucleated cells.¹ IHC revealed that some of the giant cells also contained intracytoplasmic MARV antigen.

The composition of the crystalline foreign material, which is anisotropic in polarized light, is unknown. However, a review of the medical records for this monkey revealed that approximately one month before the initiation of the MARV study, this animal had been administered an oral suspension of Pepcid® once a day for three consecutive days. It is possible that some of the suspension was aspirated into the right inferior lung lobe (which is a dependent lung lobe in a primate). The active ingredient in the Pepcid® suspension is



Lung, cynomolgus macaque. . Immunohistochemistry demonstrates MARV antigen within the cytoplasm of interstitial fibroblasts, alveolar macrophages, and a multinucleated giant cell that also contains crystalline material. Immunoperoxidase stain with hematoxylin counterstain. (anti-MARV, 600X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD <http://www.usamriid.army.mil/>)



Under polarized light, the crystalline foreign material within alveolar giant cells is anisotropic Hematoxylin and eosin stain. (HE, 400X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD <http://www.usamriid.army.mil/>)

famotidine, which is a crystalline compound, and inactive ingredients include microcrystalline cellulose.² Overall, the foreign-body alveolitis was a very mild and clinically insignificant lesion that did not affect the pathogenesis or outcome of the MARV challenge. Anthracosilicosis is a common finding in adult macaques and is usually an incidental lesion (as in this case).

Note: Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

JPC Diagnosis: Lung: Alveolitis, histiocytic, multifocal, mild with low numbers of multinucleated giant cell macrophages and abundant intracytoplasmic crystalline protein, cynomolgus macaque, *Macaca fascicularis*.

Conference Comment: This interesting case was submitted by the conference moderator and presented participants with a diagnostic challenge to identify the origin of the amphiphilic, anisotropic, and crystalline

material within macrophages and multinucleated giant cells in this section of lung. Most favored the diagnosis of pneumoconiosis, which is a lung disease secondary to inhalation of inorganic particulate material, such as asbestos or silica.⁴ Readers are encouraged to review Wednesday Slide Conference [2015 Conference 3 Case 4](#) for a review and fascinating discussion of silicate pneumoconiosis in a horse from California. Silica dusts typically generate a granulomatous inflammatory response with fibrosis, not seen in this case. Additionally, asbestos fibers in the lung are linear and beaded with globoid ends, also not a feature of this case.⁴

There have been sporadic reports of kaolin aspiration in nonhuman primates causing similar lesions to this case.^{3,8,9} Kaolin is a common crystalline compound found in antidiarrheal medication as well as a variety of other products, such as toothpaste, ceramics, soap, and paint. Initially, the terminal bronchioles and alveoli of animals exposed to aspirated or inhaled kaolin are acutely inflamed, but by day seven post exposure, there is only mild mononuclear inflammation, type II pneumocyte hyperplasia, and aggregates of anisotropic dust-laden macrophages and multinucleated cells.⁹ This is in contrast to silica inhalation, which induces a progressive granulomatous and fibrotic response.^{4,9} The route of exposure of most reported cases in nonhuman primates is aspiration of oral antidiarrheal medication.⁸ Kaolin can also cause granulomas containing numerous macrophages filled with birefringent crystals if delivered subcutaneously.³

To this author's knowledge, there have been no reported cases of famotidine aspiration causing aspiration alveolitis in humans or animals; although the pathogenesis posited by the contributor is plausible. Unfortunately, given strict regulations on

tissue handling of Marburg (MARV)-infected animals, a tissue block was unable to be submitted for further chemical analysis. Regardless of the origin of the crystalline proteinaceous material, conference participants agreed that this lesion is likely unrelated to MARV infection and is an incidental finding.

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