Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 16

24 January 2018

CASE I: F16-0079.3 (JPC 4101317).

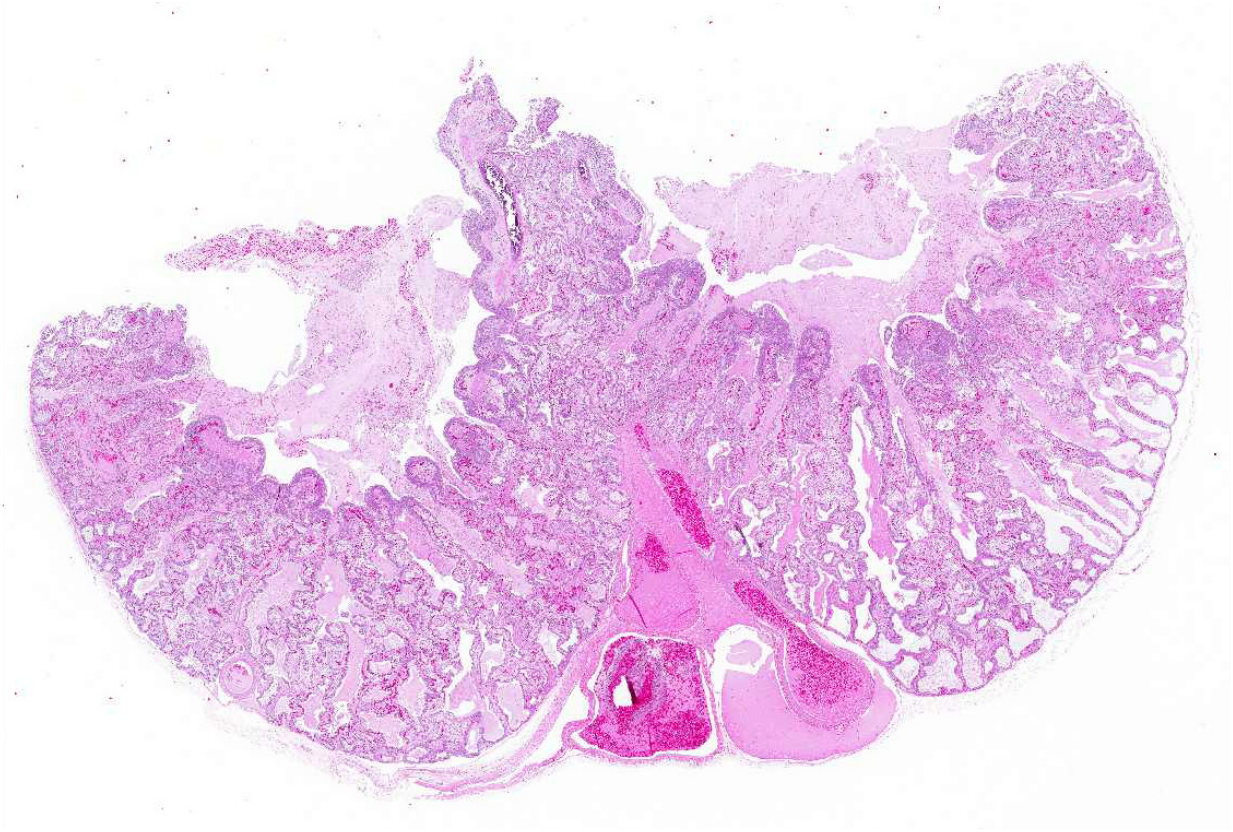
Signalment: 5-year-old, male, Green tree python, *Morelia viridis*, reptile.

History: The animal showed acute respiratory distress and discharge of mucus from the oral and nasal cavity. Two days after the onset of symptoms the animal was found dead.

Gross Pathology: A marked thickening of the lung parenchyma and a severe accumulation of clear mucus in the upper and distal airways (larynx, trachea, lung, air sacs) were noted at gross examination. The animal was in moderate body condition and the carcass was slightly dehydrated. All other internal organs showed no macroscopic changes.

Laboratory results: The presence of nidoviral RNA (Morelia viridis Nidovirus, "MVNV") was confirmed through RT-PCR of the affected lung tissue and intracellular nidoviral protein was detected via immunohistochemistry in respiratory and faveolar epithelial cells of the lungs. Infected epithelial cells were also found in the trachea and the nasal cavity. A markedly increased proliferative activity was noted in all epithelial cell layers of the diseased lung via a PCNA marker. Additionally, the immunohistochemical examination for the detection of active-caspase 3 revealed an increased number of apoptotic epithelial cells in the lung of the infected animals.

Microscopic Description: The two histologic hallmarks are the epithelial thickening in the entire lung and excess mucus in the lumen of lungs and airway. The multilayered respiratory epithelium covering the smooth muscle at the luminal end of the trabeculae displays increased cellularity with an increase in cell layers and irregular cell arrangement (hyperplasia). In the faveolar space, the epithelium is characterized by patchy to diffuse proliferations of cuboidal to columnar cells carrying short microvilli (type II pneumocytes) and rare flat cells with short

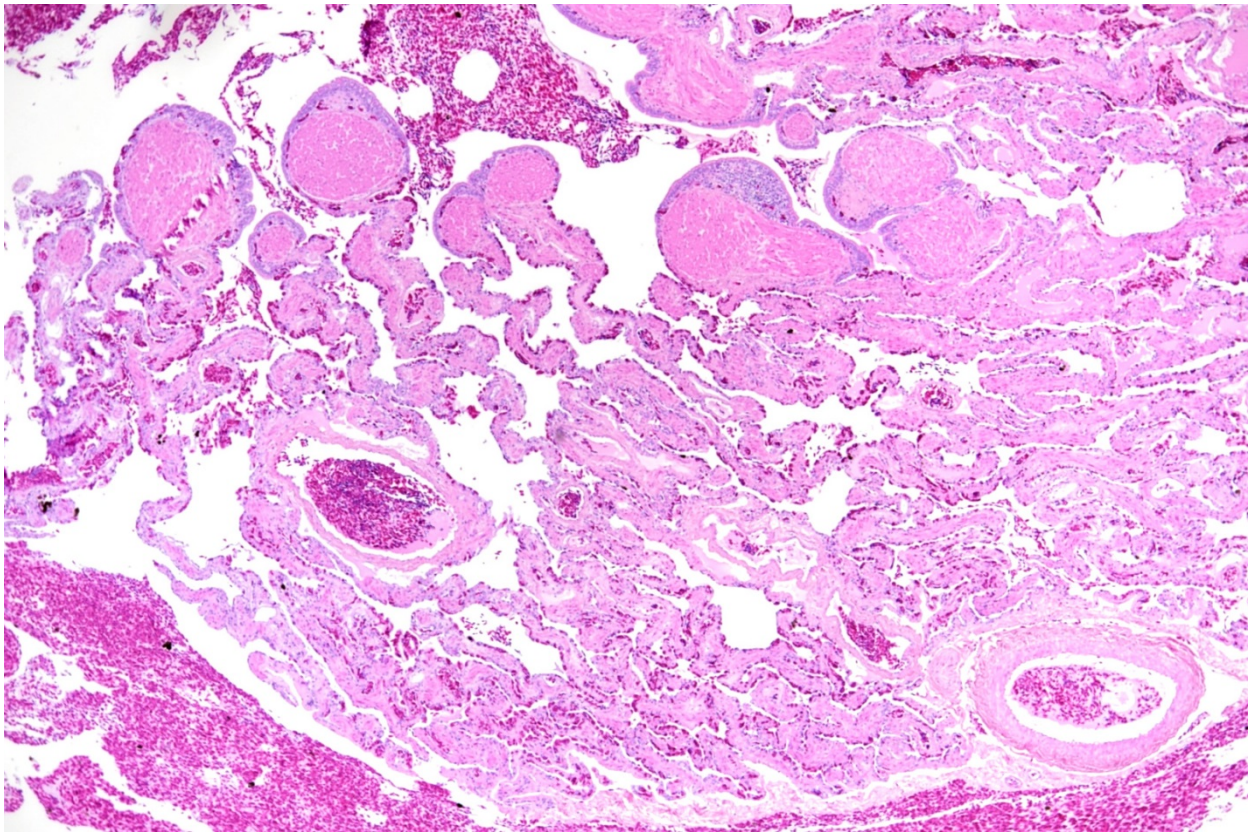


Lung, green tree python. Subgross image of the lung demonstrates marked edema within the airway and faveolae. Faveolar septa are markedly expanded and the respiratory epithelium is markedly hyperplastic. (HE, 400X).

cytoplasmic projections covering the capillaries (type I pneumocytes). The inflammatory component is represented by moderate, multifocal to diffuse interstitial infiltration of lymphocytes, plasma cells and heterophilic granulocytes. Multifocal heterophilic granulocytes and lymphocytes also infiltrate the respiratory epithelium. The lymphoid aggregates (equivalent to the mammalian bronchiolar-associated tissue) are activated. Abundant homogenous eosinophilic fluid (mucus) is filling the faveolar space, admixed with numerous heterophilic granulocytes, erythrocytes and cell debris.

Contributor's Morphologic Diagnosis:
Lung: Proliferative pneumonia, severe, diffuse with type II pneumocyte hyperplasia and mucus accumulation in the faveolar space.

Contributor's Comment: The *Morelia viridis* nidovirus belongs to the subfamily of the Torovirinae (Family Coronaviridae), which have so far mainly been associated with enteric diseases (humans, horses, cattle, swine).[4,11,13,16] However, recent studies on toroviruses have shown that they can be both entero- and pneumotropic.[14] In the last two years snake nidoviruses have been identified as the causative agent of a severe respiratory disease in ball pythons, green tree pythons[3] and Indian pythons.[2,8,9,12] Nidoviruses were also identified in the lungs of cattle and wild shingleback lizards with pneumonia, though their direct association with disease has so far not been examined.[9,14]



Lung, green tree python. Histological appearance of a healthy snake lung (HE, 400X). (Photo courtesy of: Institute of Veterinary Pathology Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich, Fax number +41 44 635 89 34, www.vetpathology.uzh.ch)

In the python lung, nidovirus infection is associated with a distinct proliferative activity and a degree of apoptotic cell death of both type I and type II pneumocytes, leading to an increased epithelial turnover and hyperplasia.[3]

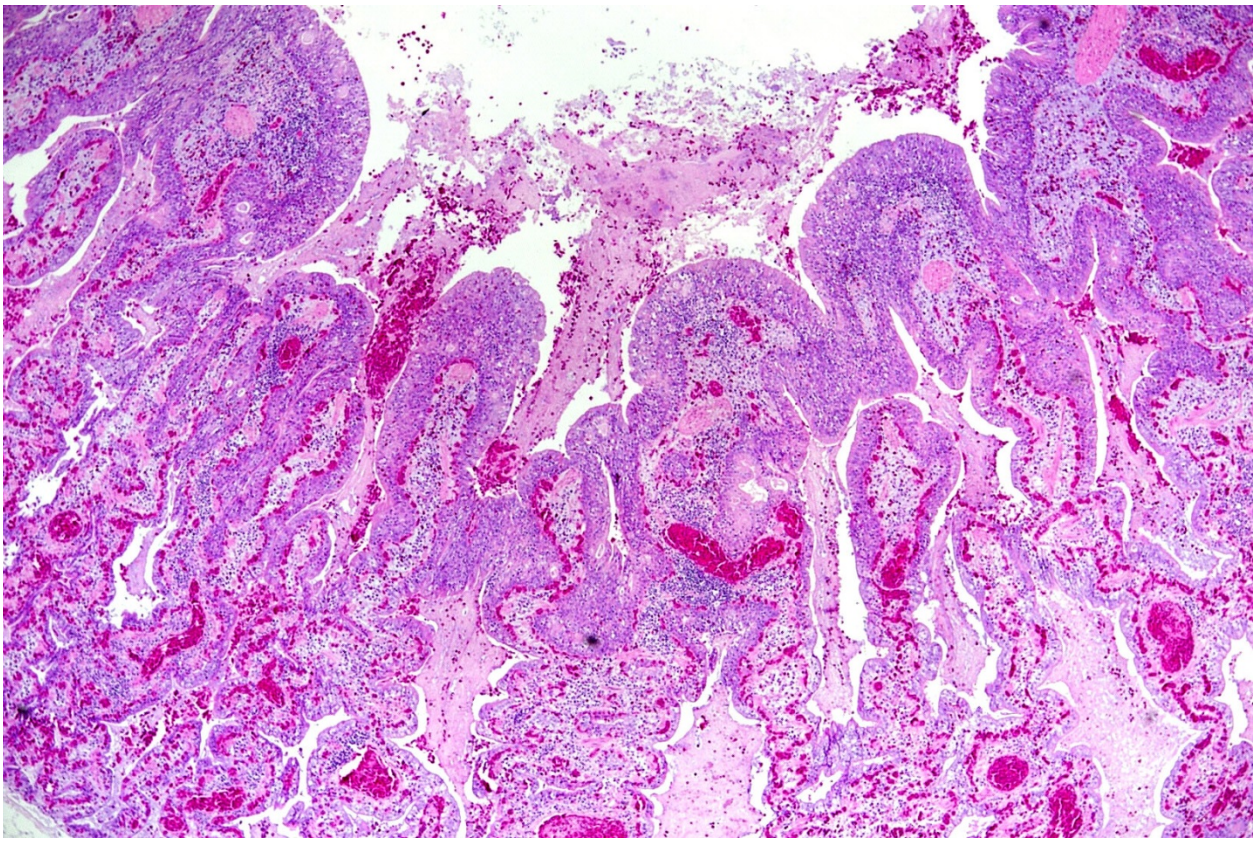
In the faveolar space of the healthy snake lung, thin cytoplasmic extensions of the type I pneumocytes cover the capillary walls, forming the gas-blood barrier.[10] This case nicely demonstrates a marked replacement of type I pneumocytes by type II pneumocytes in the faveolar space, known as an unspecific regenerative response of snakes to infectious agents, similar to response to lung injury in mammals.[5] In the healthy reptilian lung, lamellated bodies containing surfactant can be demonstrated through electron microscopy in type II pneumocytes. Following the nidoviral infection, "transformed" type II pneumocytes containing intracytoplasmic serous/mucous granules are noted ultrastructurally in the faveolar space.[3] This epithelial cell type has been previously described in various reptile species and is likely to explain the pathognomonic excess in mucus production in nidoviral pneumonia[7,10] and indicate an decrease in surfactant production.[3] In the present case viral protein could be detected in the epithelium of the entire respiratory tract (nasal cavity, larynx, trachea, lung and air sacs) and indicates a respiratory transmission (droplet) as the most likely infection route. Nidoviruses cause sudden outbreaks with high mortality in breeding collections, symptoms prior to deceasing/death are rare.

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing, diffuse, mild with marked respiratory and type II pneumocyte hyperplasia and faveolar edema, Green tree python (*Morelia viridis*), reptile.

Conference Comment: Nidoviruses are a diverse group, affecting humans and a growing population of veterinary species including: snakes, wild shingleback lizards, cattle, and nematodes. Nidoviruses that affect animal species are from the *Coronaviridae* family and *Torovirinae* subfamily. Specifically, the *Torovirus* genus affects mammals and the little known *Bafinivirus* genus affects ray-finned fish. There have been disagreements regarding the classification of reptile nidoviruses which have not been formally classified to date.[1] Regardless of the phylogenetic differences, toroviruses all share similar tissue tropism for the gastrointestinal and respiratory tracts.

Snake-associated nidoviruses were first identified in ball pythons (*Python regius*) and Indian rock pythons (*P. molurus*) in 2014[2,12,15] and green tree pythons (*Morelia M. viridis*) in 2017.[3] Gross findings in these snakes include: stomatitis, sinusitis, pharyngitis, tracheitis, esophagitis, and proliferative pneumonia with abundant mucus secretion.

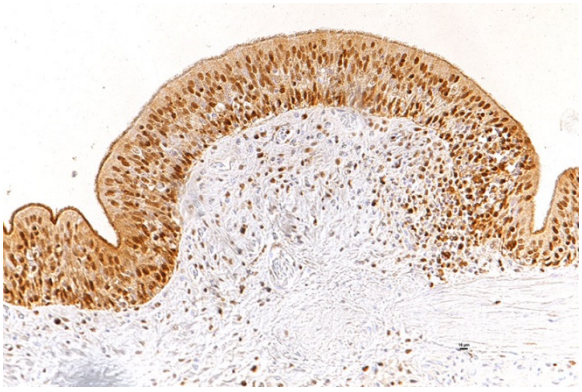
More and more over the past several years, nidovirus infection has been associated with fatal respiratory disease in several species of python, but Koch's postulates had not



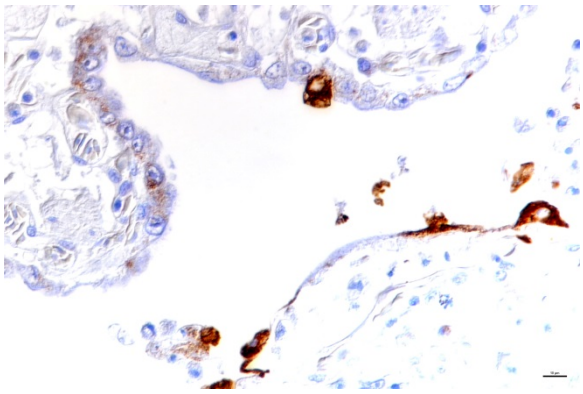
Lung, green tree python. Marked thickening of the entire epithelium (hyperplasia) with a moderate mixed-cellular interstitial inflammation and accumulation of mucus, cell debris and heterophils in the lung lumen (HE, 400X) (Photo courtesy of: Institute of Veterinary Pathology Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich, Fax number +41 44 635 89 34, www.vetpathology.uzh.ch)

definitively proven that it was the cause. In a recent study,[6] it was demonstrated through experimental infection of three ball pythons (*Python regius*) that nidovirus infection results in mucinous chronic inflammation and proliferative interstitial pneumonia. Possible transmission routes were suggested by identifying infectious virus in oral secretions and fecal material. Additionally, it was suggested that choanal and oral swabs were the best sample locations for antemortem diagnosis.

Conference participants thought there was enough necrosis within the submitted sections to include it as a modifier in the morphologic diagnosis. Morphologically, the participants suggested that the material present within the faveolar spaces suggested proteinaceous edema fluid rather than mucus. The location descriptor of bronchopneumonia as used in the JPC morphologic is based on the presence of hyperplastic and inflammatory changes in the respiratory epithelium at the tips of the faveolar septa as well as the type II pneumoncyte hyperplasia deep in the faveolae.



Lung, green tree python. Evidence of proliferative activity in all epithelial cell layers of the multilayered respiratory epithelium (PCNA, 200X). (Photo courtesy of: Institute of Veterinary Pathology Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich, Fax number +41 44 635 89 34, www.vetpathology.uzh.ch)



Lung, green tree python. Multifocal infected type I and type II pneumocytes (Immunohistochemistry for nidoviral-antigen, 400X). (Photo courtesy of: Institute of Veterinary Pathology Vetsuisse-Faculty (University of Zurich), Winterthurerstrasse 268, CH-8057 Zurich, Fax number +41 44 635 89 34, www.vetpathology.uzh.ch)

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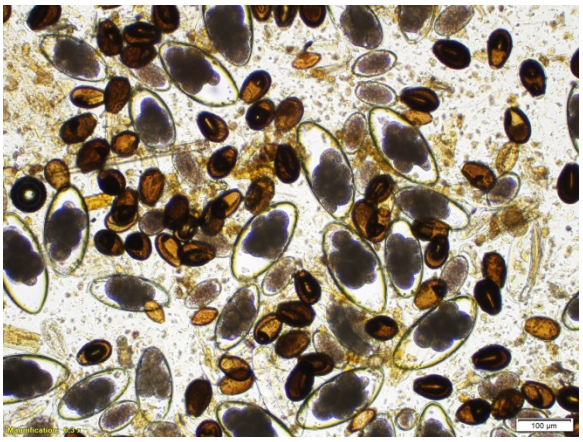
CASE II: 1704 0830 (JPC 4101141).

Signalment: 1-year-old, female, Suri alpaca, *Vicugna pacos*, alpaca.



Fecal flotation, alpaca. Higher magnification of an Eimeria macusaniensis oocyst. The oocyst is pyriform shaped with a distinct brown-colored wall and has a micropyle and micropylar cap. (Scale=20µm) (Photo courtesy of: Dr. Y. Nagamori. Oklahoma Animal Disease Diagnostic Laboratory)

History: The alpaca was recently found depressed with decreased appetite. She had free access to a small Bermuda grass pasture but no known exposure to any toxic plants. She had no history of any medical treatment. Upon presentation, she was weak, dyspneic, recumbent and unable to stand. Physical examination showed dilated pupils with absence of pupillary light reflexes. Euthanasia followed by necropsy examination was elected by the owner with the aim of identifying any potential herd health problems.

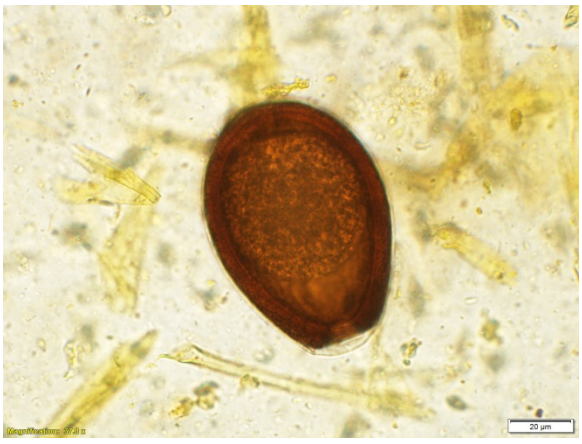
Gross Pathology: The wall of the ileum was mildly and subjectively thickened, but the mucosa was grossly unremarkable. In multiple segments of the spiral colon, the mucosa was uniformly reddened and mildly thickened with multifocal dark red, ulcerative foci overlain by a moderate amount of fibrin. The liver was diffusely pale with an accentuated lobular pattern and greasy texture on cut surfaces (hepatic lipidosis) with multiple petechial and occasional ecchymotic hemorrhages scattered throughout the hepatic parenchyma. In the heart, multifocal petechial hemorrhages were present in the endocardium and myocardium of the left ventricle.

Laboratory results: An iStat Chem 8 biochemical analysis performed upon presentation revealed mild acidemia (AnGap 36 mEq/L; reference interval: 14-21 mEq/L), mild hypoglycemia (89 mg/dL; reference interval: 102-149 mg/dL) and azotemia (creatinine of 6.3 mg/dL; reference interval: 1-2.4 mg/dL).

Fecal flotation revealed numerous *Eimeria macusaniensis* oocysts, *Nematodirus* sp. eggs, and moderate numbers of Trichostrongyle-type of eggs, *Capillaria* sp. eggs and *Trichuris* sp. eggs. *Eimeria macusaniensis* oocysts were also observed in the direct fecal smears.

Aerobic bacterial culture recovered very large numbers of *Escherichia coli* and the anaerobic culture recovered very large numbers of *Clostridium perfringens* from the ulcerated spiral colon. The bacterial culture for *Salmonella* spp. was negative.

Microscopic Description: Small intestine: The lamina propria is moderately expanded by inflammatory infiltrates, predominantly lymphocytes, plasma cells and eosinophils, as well as large numbers of intra-epithelial, intracytoplasmic protozoan schizonts,



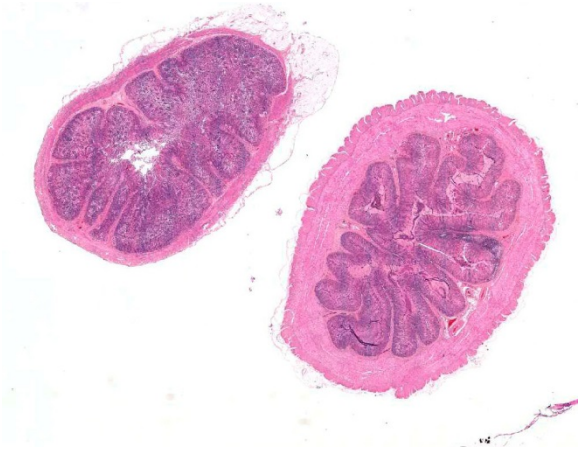
Fecal flotation, alpaca. Numerous parasitic eggs of different types are observed, including Eimeria macusaniensis oocysts. (Scale=100µm) (Photo courtesy of: Dr. Y. Nagamori. Oklahoma Animal Disease Diagnostic Laboratory)

gamonts, and oocysts. Most of these protozoa are located in the deep mucosa. The protozoa are present in the cytoplasm of cryptal epithelial cells, compressing the nuclei of the host cells. Schizonts are 70-100 µm in diameter, round to elliptical, and are surrounded by a large, clear parasitophorous vacuole. Within schizonts, numerous small, basophilic merozoites and occasional blastophores are discernible. The microgamonts are 120-200 µm in diameter and contain multiple highly condensed nuclei arranged in irregular bands, anastomosing trabeculae or circular patterns with prominent blastophores formation. Fully-developed microgamonts contain numerous linear or needle-shaped microgametes. In comparison, the macrogamonts are relatively small (50-90 µm in diameter) and are surrounded by a prominent parasitophorous vacuole. The developing macrogametes exhibit multiple, large, variably-sized, characteristic wall-forming bodies at the periphery of the cytoplasm. A few non-sporulated oocysts, approximately 80 x 55 µm, are seen in the epithelial cells or free in the intestinal lumen. The oocysts are pyriform with thick (8-10 µm) walls and have a visible micropyle and micropylar cap. The mucosa associated lymphoid tissues (Peyer's patches) are prominent and hypercellular.

Spiral colon: Multiple mucosal erosions are accompanied by acute necrosis of surface epithelial cells characterized by shrunken, pyknotic nuclei and hypereosinophilic cytoplasm. The erosive foci are overlain by karyorrhectic cellular debris intermingled with degenerate neutrophils, fibrin, mucus, erythrocytes and bacterial colonies. There are mildly increased numbers of lymphocytes, plasma cells and eosinophils present in the lamina propria of the affected segments.

Contributor's Morphologic Diagnosis:

Ileum: Moderate, subacute, diffuse lymphocytic and eosinophilic ileitis with numerous intralesional protozoan schizonts, gamonts and oocysts (morphologically consistent with *Eimeria macusaniensis*).



Ileum, colon: There are no obvious abnormalities on subgross examination. (HE, 4X)

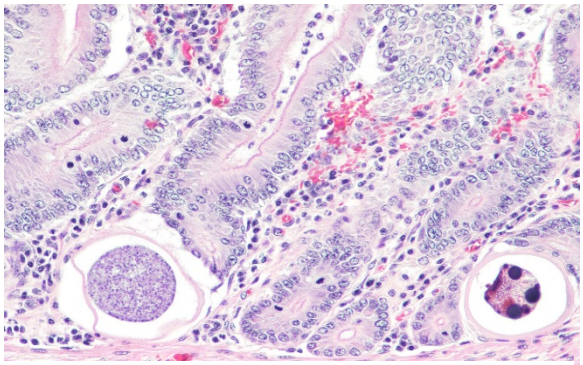
Spiral colon: Moderate, acute, multifocal erosive colitis, with intralesional bacterial colonies.

Contributor's Comment: This is a presumed case of acute sepsis secondary to erosive colitis with coexistence of intestinal *Eimeria macusaniensis*. *E. macusaniensis* has been recognized as a significant intestinal pathogen in alpacas and llamas.[1,2,4,5,6] Along with the other 5 species of *Eimeria* known to infect camelids (*E. lamae*, *E. alpacae*, *E. punoensis*, *E. peruviana*, and *E. ivitaensis*), coccidiosis in alpacas was generally considered subclinical and only occasionally caused disease in young animals.[5] However, recent reports suggest *E. macusaniensis* infection in both adults and juveniles can cause varying degrees of clinical disease and death, either by sole infection, co-infection with other *Eimeria* spp., or complicated by secondary bacterial infections.2-4 It appears *E. macusaniensis* infected animals may be predisposed to other diseases.[1] A relationship between enterotoxemia caused by *Clostridium perfringens* and *E. macusaniensis* infection in alpacas has been

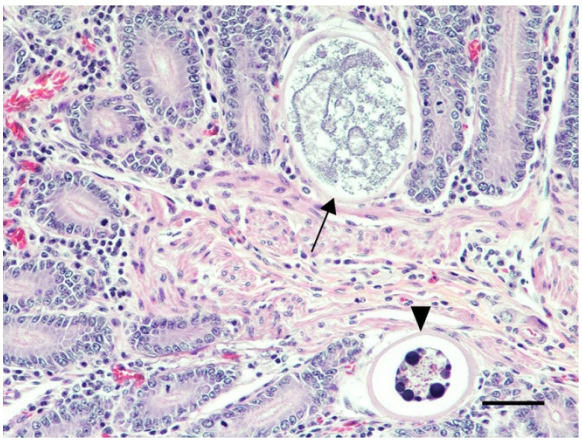
proposed and described.[4,6] In the present case, the erosive colitis, potentially caused by *C. perfringens* overgrowth, may have been predisposed by the heavy burden of *E. macusaniensis*.

Although the small intestine was grossly unremarkable except for subjective, mild thickening of mucosa of the ileum, large numbers of asexual and sexual stages of *E. macusaniensis* were revealed histologically. *E. macusaniensis* is recognized by the distinct, relatively large oocysts with average sizes of 75.5 µm by 54.9 µm during endogenous stages in tissue sections[7] and 106.6 µm by 80.5 µm in the feces.6 The oocysts are pyriform shape, with brown, 9-10 µm thick walls, a micropyle and a micropylar cap.[7,8] Other endogenous stages of *E. macusaniensis* (schizont, macrogamont and microgamont) are reported to be relatively indistinguishable from other *Eimeria* spp. in camelids.[5,7]

Antemortem diagnosis of *E. macusaniensis* can be challenging due to several factors, including nonspecific clinical signs, relatively long pre-patent period (≧30 days), and variable numbers of oocysts shed in the feces of affected animals. In addition, the oocysts of *E. macusaniensis* have a relatively high specific gravity, which makes it possible for them to be missed by routine fecal flotation methods and requires specific techniques (e.g. saturated sugar solution or flotation solutions with higher specific gravity) to achieve a better detection rate.[2,4] Therefore, the prevalence of *E. macusaniensis* may sometimes be underestimated.



Ileum, alpaca. Schizont (left) containing numerous merozoites is surrounded by a prominent parasitophorous vacuole. The developing macrogamont (right) shows multiple distinct wall-forming bodies at the periphery of cytoplasm. (HE, 200X) (Photo courtesy of: Oklahoma State University, Department of Veterinary Pathobiology, College of Veterinary Medicine, 250 McElroy Hall, Stillwater, OK 74078, https://cvhs.okstate.edu/Veterinary_Pathobiology)



Ileum, alpaca. The developing microgamont (arrow) contains multiple irregularly arranged, highly condensed nuclei with blastophore formation. Note the macrogamont (arrowhead) is relatively small and encompassed by a prominent parasitophorous vacuole. (HE, 200X) (Photo courtesy of: Oklahoma State University, Department of Veterinary Pathobiology, College of Veterinary Medicine250 McElroy Hall, Stillwater, OK 74078, https://cvhs.okstate.edu/Veterinary_Pathobiology)

In a report of 34 alpacas and 15 llamas,[2] the most commonly reported clinical signs of animals infected by *E. macusaniensis* include lethargy, anorexia and weakness with few cases presented with diarrhea and colic. It is also important to note that there is no significant age or sex predisposition, although juveniles and females at breeding ages comprise a considerable subset among the infected population, which is hypothesized to be associated with the depressed immune status of the animals.[2]

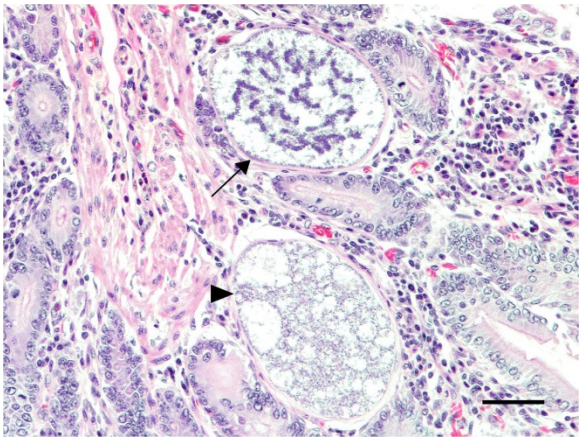
JPC Diagnosis:

1. Ileum: Intraepithelial apicomplexan schizonts, gamonts, and oocysts with minimal lymphocytic inflammation, Suri alpaca (*Vicugna pacos*), alpaca.
2. Spiral colon: No abnormal findings.

Conference Comment: There are innumerable species of the genus *Eimeria* which demonstrats a direct coccidian life cycle characterized by both sexual and asexual reproduction. The Eimerian life history is prototypical and may be used as a basis for understanding the life cycle of all other coccidians. Rare exceptions include coccidians that utilize blood-feeding arthropods as hosts (*Hepatozoon* or *Schllackia*) where the oocysts develop in the arthropod and the mammalian host is infected by ingesting the infected arthropod.[1]

The life cycle begins when an infective sporulated oocyst is ingested by an appropriate host. There the sporozoites emerge, and schizogony, or asexual replication, begins. Free sporozoites enter cells of the lamina propria or enterocytes within the duodenum, form round trophozoites, and divide to become first-generation schizonts (same as meronts). It is worth noting that all intracellular forms of *Eimeria* (trophozoite, schizont, etc.) are all located within a parasitophorous vacuole which is best seen on electron microscopy. The first-generation schizont subsequently forms first-generation merozoites that burst out of the infected cell and infect adjacent cells to become second-generation schizonts. Schizogony continues in a cyclical fashion for two to three generations and then final merozoites (telomerozoites) infect a final naïve host cell and develop into either a female macrogamont or male microgamont. At this point sexual replication, or gametogony, can occur. When gamonts mature, they are called gametes. The female macrogamete is unicellular and fills the entire parasitized cell, whereas the male microgamont undergoes multiple divisions and eventually contains biflagellated microgaametes which exit the microgametocyte to fertilize the macrogamete and form zygotes. Oocysts develop which are leased by rupture of the host cell and passed out with the feces to undergo sporulation, be ingested, and start its life cycle all over again.[1]

Generally, *Eimeria* sp. can be identified based on host specificity and oocyst form (see chart below), and identified postmortem using direct smears of intestinal contents or H&E stained histologic sections. Wright's or Giemsa stain can be useful in identifying sporozoites. Simply identifying coccidian oocysts in animal feces is not enough to implicate disease since healthy animals may have large numbers. The history and clinical signs must fit with a diagnosis of coccidiosis: bloody diarrhea, weight loss, and ill thrift.[1,9]



Ileum, alpaca. The developing microgamont (arrow) exhibits irregular bands and anastomosing trabeculae. A fully-grown microgamont (arrowhead) contains numerous microgametes. (HE, 200X) (Photo courtesy of: Oklahoma State University, Department of Veterinary Medicine250 McElroy Hall, Stillwater, OK 74078, https://cvhs.okstate.edu/Veterinary_Pathobiology)

Of interest, recent paleoparasitological investigation into the pre-incan to pre-hispanic contact period in northern Chile identified *Eimeria macusaniensis* as well as three other parasite eggs (*Enterobius vermicularis, Trichostrongylus* sp.*,* and *Trichuris* sp.*)* within human coproliths (fossilized feces).[3] The presence of *E. macusaniensis* in human fecal matter is related to the use of llamas as food, transportation, and sacrificial offerings, and underscores the importance of llamas and alpacas to societal evolution.

Table 1: *Eimeria* sp. by host and organs affected[9]

Animal	**Coccidia**	**Organ affected/Clinical signs**
Birds		
Chickens	*E. acervulina*	Duodenum/enteritis
	E. necatrix/maxima	Jejunum/enteritis
	E. brunette	Ileum/enteritis
	E. tenella	Ceca/typhylitis
Turkey	*E. meleagridis*	Cecum
	E. adenoeides	Cecum, ileum
	E. meleagrimitis	Upper intestine
	E. gallopavonis	Ileum, large intestine
Geese & ducks	*E. truncata*	Kidney/anorexia, depression
	E. anseris/nocens	Intestine
Sandhill/whooping cranes	*E. reichenowi*	Disseminated
	E. psittaculae	Intestine
Parrots		
Cattle	*E. bovis/zuernii*	Cecum and colon/diarrhea
	E. alabamensis	Small intestine
Sheep	*E. ahsata/christenseni*	SI
	E. brakuensis	SI
	E. crandallis	SI
	E. ovinoidalis	Cecum, colon
Goats	*E. christenseni*	SI
	E. arloingi	SI
	E. hirici	SI
	E. ninakohlyak-imovea	LI
Equine	*E. leukarti*	SI
	Klossiella equi	
Swine	*E. debliecki*	SI (in 1-3 week old piglets)
Canine	*I. canis*	Ileum, colon occasionally
Feline	*I. felis*	SI, colon occasionally

Mice	*Klossiella muris* *E. falciformis* *E. vermiformis* *E. papillata* *E. ferrisi*	kidney Colon Intestine Intestine Intestine
Rabbit	*E. stiedae* *E. intestinalis* *E. flavescens*	Bile ducts Ileum & cecum Ileum & cecum
Ferret	*E. furonis*	SI

The sections made available to participants did not contain eosinophils in the small intestine, or bacterial colonies and ulceration within the large intestine. Attendees vigorously debated the severity of the parasitism, inflammation, and crypt hyperplasia within the small intestine and concluded that it would not be severe enough to induce ulcer formation and secondary bacterial infections further down the alimentary tract. Therefore, an additional pathogen is suspected.

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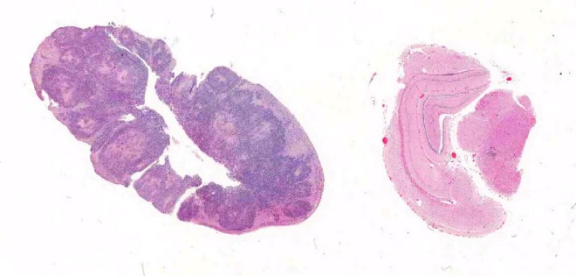
CASE III: H15-0067L (JPC 4067142).

Signalment: Unknown age, male, Bilby, *Macrotis lagotis*, marsupial.



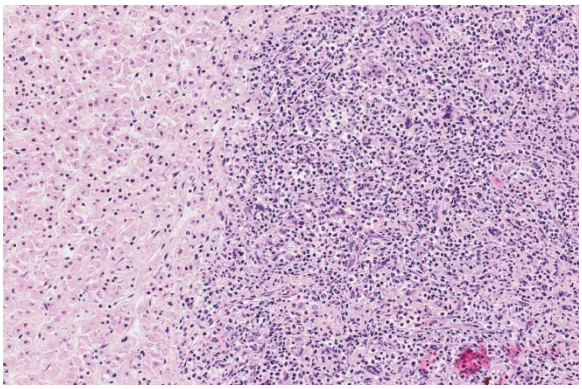
Adrenal gland left (right); cross section through diencephalon (right), bilby: The normal architecture of the adrenal gland is effaced by a densely cellular inflammatory infiltrate as well as multiple foci of inflammation in the cerebrum and thalamus. (HE 5X)

History: A captive young adult male bilby (*Macrotis lagotis*) was euthanized at a wildlife park following the onset of severe lethargy and was subsequently presented to the anatomic pathology service at Murdoch University Veterinary Hospital for necropsy examination. Approximately two months prior, the bilby was captured for an annual health check. He was healthy and weighed 2098 grams. On that day he was transferred to a neighboring enclosure which contained 10 other bilbies, including one young male and nine adult and sub-adult females. Approximately seven weeks later, the bilby became lethargic and was recaptured. He weighed 1630 grams. He was isolated and euthanized three days later following no improvement. A zoo keeper was bitten during capture.

Gross Pathology: On gross examination, the bilby was emaciated with a focal circular, ulcerated skin lesion on the dorsal tail base. Dozens of variably sized, poorly demarcated, red to tan nodules were present throughout the lungs and kidneys. The adrenal glands were markedly enlarged, pale tan and soft with loss of corticomedullary distinction.

Laboratory results: Samples of aseptically collected lung and spleen were stored at 4^0C prior to submission for fungal culture. Tissue cultures isolated *Sporothrix schenckii* demonstrating thermal dimorphism. At 26^0C the isolates were expanding, crumbly and grey in color and at 36^0C an atypical yeast-like organism was identified. Microscopically, organisms were characterized by clavate conidia arranged predominantly terminally on erect conidophores attached by fine denticles. Dematiaceous sessile conidia were also observed. Subsequent sequencing of the internal transcribed spacer (ITS) regions confirmed the isolate as *Sporothrix schenckii sensu lato*.

Microscopic Description: Adrenal glands: Diffusely effacing the normal medullary architecture and extensively infiltrating and effacing over 90% of the cortex, are large numbers of degenerate neutrophils and epithelioid macrophages admixed with moderate numbers of multinucleate giant cells, and large amounts of eosinophilic and karyorrhectic debris (necrosis). Admixed are frequent pleomorphic 2-15 μm diameter yeast. These organisms are predominately intracellular within the cytoplasm of macrophages and multinucleate giant cells,



Adrenal cortex, bilby. The adrenal cortex (left) is replaced by a dense pyogranulomatous infiltrate composed of numerous macrophages, viable and degenerate neutrophils, and scattered multinucleated giant cell macrophages (HE, 80X)

but are also present in fewer numbers extracellularly within areas of necrosis. Yeasts are round, oval or elongate (cigar-shaped) and larger yeast (10-15 μm diameter) are round to oval with rare narrow-based budding. Yeasts are sometimes surrounded by a clear thin halo and/or a slightly refractile clear cell wall, and stain intensely positive with periodic acid-Schiff (PAS). The smaller forms (2-9 μm diameter) are elongate, cigar-shaped to oval and stain intensely positive with PAS and are also Gram positive.

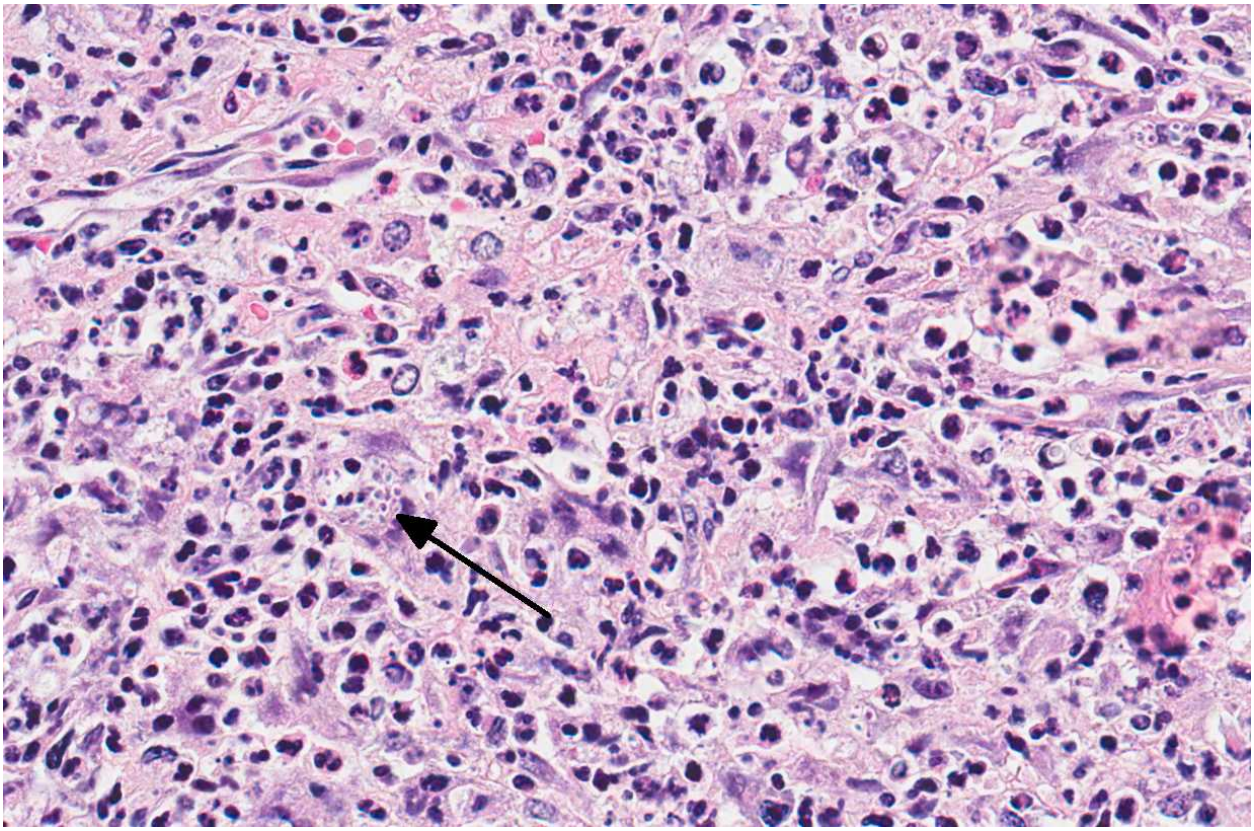
Brain: In multiple regions of brain, including the cerebrum, hippocampus and midbrain, the neuropil and leptomeninges are disrupted by multifocal to coalescing, randomly distributed foci of pyogranulomatous inflammation as previously described. Low numbers of the previously described fungal yeast are present scattered throughout the inflammatory foci. Mildly increased numbers of glial cells are present immediately surrounding these inflammatory foci.

Similar inflammatory foci are also present within the kidneys, lungs, testes, lymph nodes, heart, liver, spleen, salivary glands and tail wound.

Contributor's Morphologic Diagnosis:

Adrenal glands: Severe, regionally extensive, subacute, pyogranulomatous and necrotizing adrenalitis with numerous intrahistiocytic and extracellular fungal yeast.

Brain: Moderate, multifocal to coalescing, random, subacute, pyogranulomatous meningoencephalitis with occasional intrahistiocytic and extracellular fungal yeast and mild gliosis.

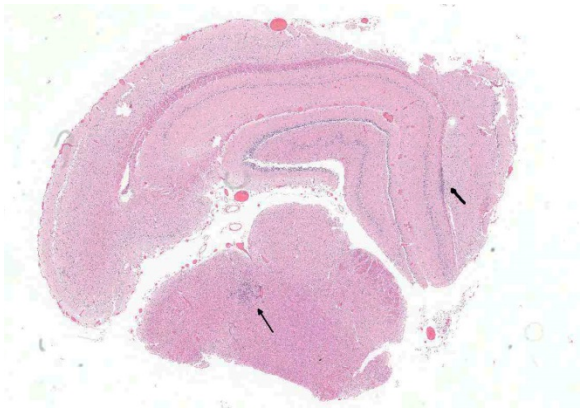


Adrenal cortex, bilby. Macrophages and multinuclearted giant cell macrophages cotnin one or more intracytoplasmic 3-5um, round to ellipsoid yeasts. (HE, 400X)

Contributor's Comment: The histological and fungal culture findings are consistent with disseminated sporotrichosis, an uncommon fungal disease of humans and animals which, outside of Brazil, is usually caused by the fungus *Sporothrix schenkii*. Gene sequencing of isolates from Brazil have shown the most common species as *S. brasiliensis*, with *S. globosa, S. albicans*, *S. luriei* and *S. Mexicana* also isolated.[1,5] *Sporothrix* spp. are thermally dimorphic, cosmopolitan fungi found in soil, hay and decaying plant matter.

Based on the size and pleomorphic nature of the yeast, *Sporothrix schenckii* was strongly suspected following initial microscopic evaluation; however, other yeast that can appear morphologically similar include *Cryptococcus* spp. and *Blastomyces dermatitidis* (see table below for summary of common fungal yeast pathogens).

Given the presence of the organism within the chronic skin wound on the tail base, the most likely route of infection in this case was considered to be either wound inoculation or direct inoculation from a penetrating wound, with subsequent dissemination via hematogenous or lymphatic spread to numerous internal organs.



Diencephalon, bilby. There are two foci of inflammation in the diencephalon – one in the internal capsule and one in the thalamus (arrows). (HE, 10X)

Although multiple cases of fungal disease have been described in a variety of native Australian marsupials and monotremes, to our knowledge this is the first reported case of sporotrichosis in a native Australian marsupial. *Trichophyton* spp. and *Microsporum* spp. have been described as the cause of dermatophytosis in both native Australian macropods and koalas. *Mucor amphibiorum* causes significant morbidity and mortality in the platypus, with severe ulcerative dermatitis, occasional spread to underlying muscle and dissemination to other organs. *Candida* spp. have been reported to cause gastrointestinal infections in macropods and koalas and *Cryptococcus gatii* and *C. neoformans var grubii* have been associated with cryptococcal meningitis, pulmonary and disseminated cryptococcosis in a number of macropod species, koalas and a single dusky antechinus (*Antechinus swainsonii*).

In the veterinary literature, sporotrichosis has been reported in a variety of animals. Cats are the most commonly affected domestic species; however, cases in dogs, horses, cattle, pigs, fish, rodents and armadillos have also been reported.[6,12] In cats, sporotrichosis typically presents as ulcerated, nodular cutaneous lesions, most commonly on the head and ears. Respiratory mucosal involvement is common; ascending lymphangitis and dissemination are reported less frequently.[8,13] Disseminated disease is rare in all species, with human cases occurring most often in immuno-compromised patients. In cats, however, it usually occurs in the absence of immunosuppression.[8,11,13] Feline sporotrichosis is an important source of zoonotic sporotrichosis in humans, with cases in Brazil associated with bites and scratches from infected domestic cats (Felis catus). *S. schenkii* has been isolated from the

nail beds of healthy cats.[4] Human infections resulting from bites or scratches from rodents, armadillos, and a single opossum have also been reported.[3,12] In this case, zoonotic infection did not occur following the bilby bite.

Table 1. Summary of common fungal yeast pathogens.[10]

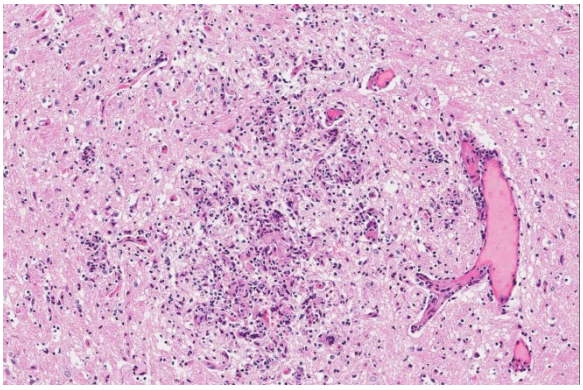
Features	Sporothrix spp	Cryptococcus neoformans	Blastomyces dermatidis	Coccidioides immitis	Histoplasma capsulatum	Histoplasma farciminosum
Histopathology						
Tissue morphology Budding	Pleomorphic cigar shaped yeast cells Narrow base	Oval yeast cells with prominent thick capsule Narrow base	Thick walled yeast cells Broad base & unipolar	Spherules containing endospores	Oval yeast cells within macrophages Narrow base	Oval yeast cells within macrophages Narrow base
Size (diameter)	3-6 µm	4-8 µm	8-10 µm	Spherules 10-80 µm, endospores 2-5 µm	2-5 µm	2-5 µm
Staining	PAS, silver stains, small forms: Gram positive	PAS, silver stains, capsule: mucicarmine	PAS, silver stains	PAS, silver stains	PAS, silver stains	PAS, silver stains
Epidemiology & clinical disease						
Disease Species most affected Environmental habitat Geographical distribution Site of lesions Route of infection	Sporotrichosis Horses, cats, dogs & humans Decaying vegetation, soil, moss Worldwide, mainly tropical and subtropical Lymphocutaneous, occasional dissemination Traumatic implantation	Cryptococcosis Cats horses and humans Soil enriched with bird faeces & Australian red gum trees Worldwide Nasal cavity, lungs, brain, eye, skin Inhalation	Blastomycosis Dogs and humans Acidic soil rich in organic matter North America, India and the Middle east Lungs with metastasis to skin and other tissues Usually inhalation	Coccidiomycosis Dogs, horses and humans Arid or semi-arid soil in low-lying areas Southwestern USA, Central and South America Lungs with metastasis to bones Usually inhalation	Histoplasmosis Dogs, cats and humans Soil enriched with bird and bat faeces Endemic to Mississippi and Ohio river valleys with sporadic cases in other countries Lungs with occasional dissemination Inhalation	Epizootic lymphangitis Equidae Soil Africa, the Middle East and Asia Skin, lymphatic vessels and nodes Traumatic implantation
Mycological characteristics						
Fungal culture	Dimorphic 25ºC mould colonies white to black/brown, wrinkled and leathery; conidia pear shaped arranges in rosettes on conidiophores 35-37ºC yeast colonies cream to tan	37ºC mucoid colonies with capsules, urease activity; brown colonies on birdseed agar	Dimorphic 25-35ºC mould colonies white and cottony producing pear shaped conidia borne on conidiophores or hyphae 37ºC yeast colonies cream to tan, wrinkled and waxy	25-30ºC mould colonies shiny moist and grey to white and cottony; septate hyphae with barrel shaped arthrospores separated by empty cells	Dimorphic 25-30ºC white to buff colonies with cottony aerial hyphae; septate hyphae bear small conidia and sunflower-like macroconidia 37ºC yeast colonies cream to tan, round and mucoid	Dimorphic similar to H. capsulatum

JPC Diagnosis:

1. Adrenal gland: Adrenalitis, pyogranulomatous, multifocal to coalescing, severe with numerous intracellular and extracellular yeasts, Bilby (*Macrotis lagotis*), marsupial.
2. Brain, diencephalon: Encephalitis, pyogranulomatous, multifocal, moderate with rare intracellular yeasts.

Conference Comment: Sporotrichosis is a subacute to chronic disease of animals and humans caused by fungi of the *Sporothrix* spp. of which *Sporothrix schenckii* is the most common. *S. schenckii* is a dimorphic fungus, meaning it is in its mycelial form at room temperature and when at 37 ^{0}C (body temperature) it develops into the yeast form. Microscopically, the yeast form is oval to cigar-shaped with a thin refractile cell wall, 2-6 um in diameter by 2-10 um in length, exhibit narrow based budding, and may intracellular (within multinucleated giant cells or macrophages) or extracellular. Like many other types of fungi, *Sporothrix* elicits a diffuse or nodular pyogranulomatous response. When cutaneous, the epidermis is often hyperplastic or ulcerated and often accompanied by fibrosis. In some cases, yeasts are surrounded by Splendore-Hoeppli material or asteroid bodies, but this is not pathognomonic for sporotrichosis and can be seen when there is antigen-antibody complex formation surrounding other organisms or foreign objects.[2,9]

Sporotrichosis has been reported in cats, dogs, horses, mules, donkeys, cattle, goats, swine, camels, and humans. As discussed in this case, sporotrichosis carries a risk of zoonotic transmission, and the most common route is from cats to humans.[2] Disease is more common in cats, horses, and dogs living in temperate and tropical zones; particularly in intact male outdoor cats and hunting dogs. Infection is acquired via puncture wounds,



Diencephalon, bilby. Within the thalamus, there is a focus of pyogranulomatous inflammation with marked perivascular inflammation and edema. (HE, 200X)

and pulmonary infection occurs rarely via inhalation of spores. Sporothrix spores are quite hardy and can survive for months or years in soil, vegetation, and wood. The human form of disease is termed "rose handler's disease" which illustrates that point.[2] Clinically, there are three forms of sporotrichosis: (1) Primary cutaneous form; (2) Cutaneous-lymphatic form; and (3) Extracutaneous or disseminated form.[9]

The primary cutaneous form is most likely a result of strong host immunity and results from puncture wounds with the organism confined to the point of entry. This form tends to be chronic and clinically appears as multiple scattered raised, alopecic, ulcerated or crusted nodules along the head or distal extremities. Infected cats can spread the organism around their own epidermis through autoinoculation by grooming.[9]

The cutaneous-lymphatic form is the most common form in horses and humans and involves the skin and subcutis with spread through associated lymphatics. Affected lymphatic vessels become thickened and rope-like, regional lymph nodes are enlarged, and often nodules break open resulting in oozing sores. In this form, lesions are often along the proximal forelimbs, chest, and thighs.[9]

Finally, the extracutaneous or disseminated form occurs most frequently in cats and may involve a single extracutaneous tissue or multiple organs. This form develops often as a sequela to cutaneous-lymphatic infection or following inhalation of spores. In affected cats, no immunosuppressive factors have been identified and it is unclear what causes *Sporothrix* to disseminate. Clinically, affected cats are febrile, depressed, and anorexic.[9]

Microscopically, the cigar-shaped yeast is characteristic, and can be readily identified using standard fungal stains (GMS or PAS) or Gram stain (gram-positive),[2] but there are times when the cigar-shaped form is not present. In those cases, common differentials would include *Cyptococcus neoformans* or *Histoplasma capsulatum*. However, *Cryptococcus neoformans* can be much larger (up to 20um) with a very thick 2um capsule that stains with mucicarmine, and *Histoplasma capsulatum* is always intracellular.[9]

Contributing Institution:
http://www.murdoch.edu.au/Services/Veterinary-Hospital/About-us/Services/Pathology-and-Clinical-Pathology/

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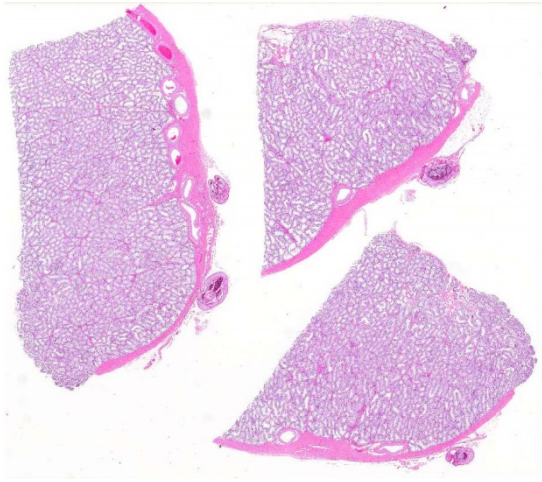
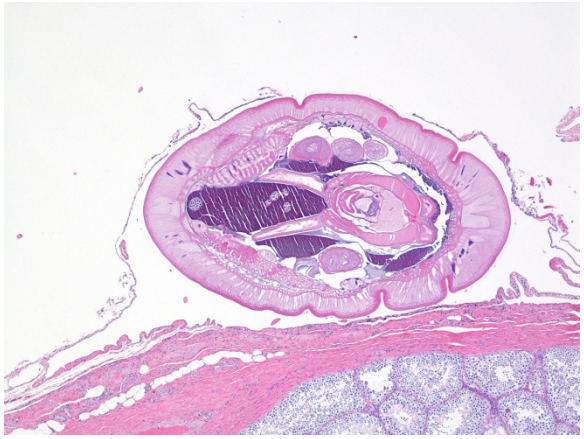
CASE IV: WSC 2017-2018 Case I (JPC 4100648).

Signalment: Adult, male, African green monkey, *Chlorocebus sabaeus*, primate.

History: This monkey was in a study to determine the efficacy of a therapeutic drug for treating pneumonic plague. All of the monkeys in this study were exposed to a lethal dose of aerosolized *Yersinia pestis*. Once an individual animal began displaying clinical signs, twice daily treatments were initiated with either a placebo (control group) or the therapeutic drug (experimental group). This monkey was in the experimental group and it developed clinical signs necessitating euthanasia on Day 15 after bacterial challenge. Note: All of the control animals died on Day 4 or Day 5.



Testis, African Green monkey. Within the fused vaginal tunics, there are multiple cross sections of larval acanthocephalan parasites. (HE, 5X)



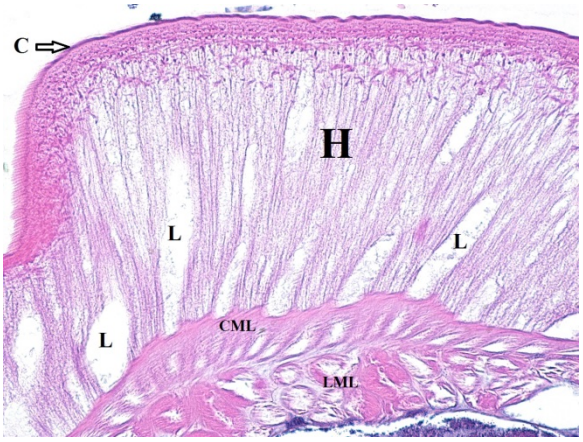
Testis, African Green monkey. Section of testis with an approximately 2 mm diameter metazoan parasite encysted in the tunica vaginalis. (HE, 40X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort D Detrick, MD http://www.usamriid.army.mil/)

This monkey was part of a research project conducted under an Institutional Animal Care and Use Committee (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: There were multiple fibrous adhesions between the left testis and the inner lining of the scrotum. In the areas of adhesion, numerous oval white nodules, measuring 1-2 X 2-3 mm, were located in the connective tissues over the tunica albuginea of this testis and within the surrounding scrotum. There were several fibrous adhesions between the right middle lung lobe

and the mediastinum and between the right inferior lung lobe and the thoracic wall. An approximately 1 cm diameter firm nodule was palpated within the middle of the right inferior lung lobe.

Laboratory results: None provided.



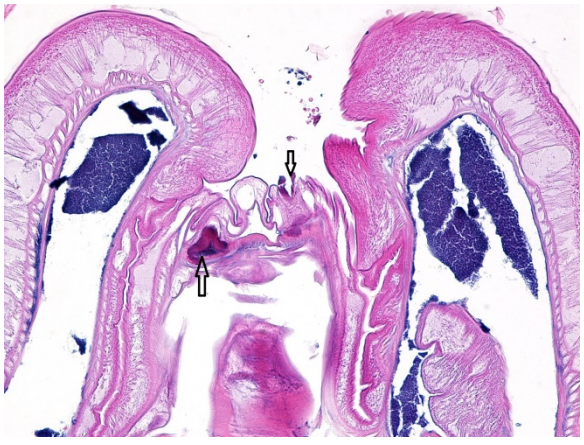
Testis, African Green monkey. High magnification of the wall of the parasite showing it has an outer cuticle (C), a thick hypodermis (H) containing lacunae (L), a circular muscle layer (CML), and a longitudinal muscle layer (LML). (HE, 400X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/)

Microscopic Description: Testis (3 sections): Adjacent to the tunica albuginea, within the tunica vaginalis, there are four sections of a metazoan parasite measuring 1.5–2.5 mm in greatest diameter. The parasites have a cuticle, a wide hypodermis (100-200 µm thick) containing multiple lacunae, and two layers of muscle (circular and longitudinal) that border a pseudocoelom. In one section of the parasite, there is a retracted proboscis with spines. In other sections, the pseudocoelom contains oval muscular structures, measuring <100 X <200 µm, which have peripheral muscles and a central canal; these are consistent with lemnisci. Neither a digestive tract nor mature eggs are evident. Multifocally, the tunica vaginalis is fused with the tunica albuginea which results in the parasites being encysted. In other tissue sections (not present on the scanned slide), the parasites reside in pseudocysts present in the left epididymis and the wall of the scrotum.

Contributor's Morphologic Diagnosis: Testis/tunica vaginalis; multiple encysted larval acanthocephalan parasites

Contributor's Comment: The anatomic features of the parasites in this monkey that are characteristic of the phylum Acanthocephala include: (1) a cuticle; (2) a thick hypodermis; (3) a pseudocoelom; (4) the lack of a digestive tract; (5) a proboscis with spines; and (6) the presence of lemnisci (pleural of lemniscus).[4,5] No other types of parasites have lemnisci.[5]

As adults, Acanthocephelans are parasites of the intestinal tract of vertebrate hosts and different species have been reported in all classes of vertebrates (i.e. fish, amphibians, reptiles, birds, and mammals).[4] The adult worm everts it spiny proboscis and this is used for attachment to the host's intestinal wall. This proboscis is responsible for the common names for Acanthocephalans:

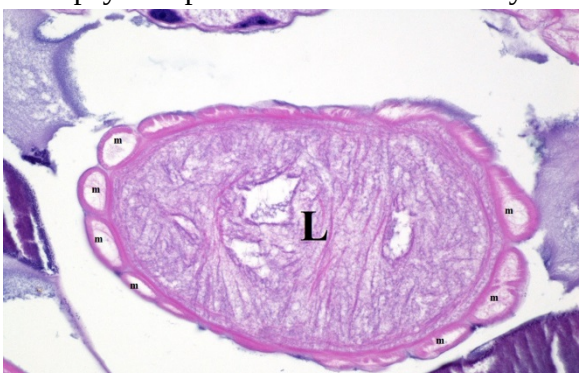


Testis, African Green monkey. Section of the parasite with a retracted proboscis that has spines (arrows). (HE, 200X)(Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/)

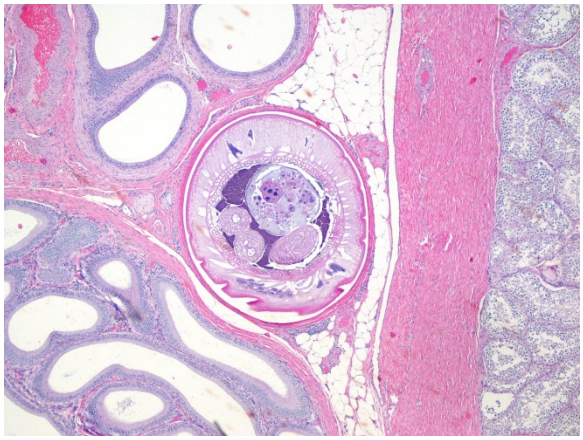
"thorny-headed" or "spiny-headed" worms.[4,5] Lacking a digestive tract, the worms absorb nutrients through their body wall and the channels (lacunae) in the hypodermis probably aid in transporting absorbed nutrients.[4]

Adult female worms produce eggs, each containing a larva called an acanthor,[2,4] which are passed in the feces of the host. The larvated eggs are ingested by an invertebrate intermediate host (usually an arthropod) in which the parasites develop and encyst at a stage called a cystacanth.[2,4] Usually, the life cycle is completed when the definitive host ingests the infected intermediate host and the cystacanths are then released and develop into adults in the intestines of the definitive host.

However, cystacanths can occasionally use vertebrates as paratenic hosts.[2,4,5] This occurs when a cystacanth-infected intermediate host is ingested by a vertebrate that is not a final host species; the cystacanths then encyst in the tissues of the paratenic host without any further maturation. The parasites in this monkey are consistent with cystacanths and the monkey is a paratenic host. A full necropsy was performed on this monkey and



Testis, African Green monkey. High magnification of the parasite showing a lemniscus (L) within the pseudocoelom. Around its periphery are compressor muscles (m). (HE, 400X) (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/)



Testis, African Green monkey. Section of testis and epididymis (from a different histology slide). The parasite is contained within a pseudocyst within the epididymis. (HE, 40X) (HE, (Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/)

the only locations that these parasites were found were in the connective tissues around the left testis and epididymis. These parasites were deemed to be incidental findings of no clinical significance. The lung lesions noted grossly in this animal were areas of subacute to chronic inflammation attributable to the experimental bacterial challenge.

This African green monkey originated from a semi-feral colony on the island of St. Kitts in the Caribbean. The monkeys on St. Kitts are believed to have descended from escaped or intentionally-released "pets" on slave-trading ships in the 1600's.[1] They became established on the island (as well as the Caribbean islands of Nevis, Anguilla, Saint Maarten, and Barbados) and their population increased to a level so that their crop-raiding tendencies caused them to be described as a "pest species" on St. Kitts as early as 1700,[1] where they are still considered to be an invasive pest.

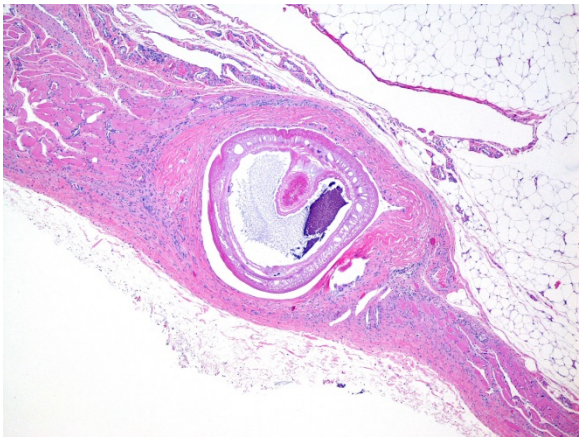
The species of Acanthocephala in this monkey was not determined. However, the

monkey most likely had plenty of opportunities to be exposed to potential intermediate hosts on St. Kitts before it was brought to the United States. Adult acanthocephalans in the genus *Prosthenorchis* have been reported in a variety of New World primates (NWPs) and cystacanths of these parasites have also been found encysted in the peritoneal membranes of NWPs.[2,6] However, it is unlikely that the parasites in the monkey of this report are a species of *Prosthenorchis* because NWPs do not occur on St. Kitts. It is interesting to note that feral pigs are present on St. Kitts; this suggests the possibility that the parasites in this monkey might be cystacanths of the "thorny-headed worm" of pigs: *Macracanthorhynchus hirudinaceus*.

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

JPC Diagnosis: Testis, tunica vaginalis: Multiple encysted acanthocephalans, African green monkey (*Chlorocebus sabaeus*), primate.

Conference Comment: Acanthocephalans were first described by Italian author Francesco Redi in 1684. The name "Acanthocephala" (which is derived from the Greek *akanthos*, thorn and *kephale*, head) was coined by scientist Joseph Koelreuter in 1771 but not formalized until 1809. Acanthocephalans are simple parasites that lack many internal organs for increased efficiency. For instance, they lack a mouth or gastrointestinal tract, and the adult stages of the parasite live in the intestines of their host where they uptake nutrients that have been digested by the host. Thorny-headed worms have marked variation in size from species to species depending on the host and can reach up to 65 centimeters in length



Testis, African Green monkey. Section of scrotum (from a different histology slide). The parasite is contained within a pseudocyst and there is disruption, mild mononuclear inflammation, and fibrosis of the tunica dartos (40X)(Photo courtesy of: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/)

(*Gigantorhynchus gigas*). In addition to their physical size, there is variation in the size of many of their adult cells. Polyploidy is common with some species having up to 343n. Finally, some species of thorny-headed worms have an interesting life cycle and have been referred to by some as "body snatchers". Generally, Acanthocephalans begin their life cycles within invertebrates, such as earthworms or crustaceans that live near water systems. When these invertebrates are infected, the thorny-headed worms cause the invertebrates to go towards the light (*i.e.* surface of the water or soil), where they will inevitably be eaten by a definitive host and allow for continuation of the life cycle.[3]

Contributing Institution:
US Army Medical Research Institute of Infectious Diseases
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