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DISSEMINATED PROTOTHECOSIS IN A RUWENZORI LONG-HAIRED FRUIT BAT (ROUSETTUS LANOSUS)

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Abstract: An adult male Ruwenzori long-haired fruit bat (Rousettus lanosus) presented for lethargy and unthriftiness. Physical examination revealed cranial alopecia, mandibular ulceration, and dehydration. Supportive care and antibiotic therapy were initiated. The bat was found dead 3 days after presentation. Necropsy revealed alopecia on the head and body, exposed dried bone on the rostral tip of the mandible, and excessive clear pleural fluid. Lungs were congested and contained miliary white foci disseminated randomly throughout the parenchyma. Subcutaneous, intra-thoracic, and intra-abdominal adipose depots were minimal. Histologic examination of skin and lung revealed the presence of algal-like organisms morphologically consistent with Prototheca spp. Polymerase chain reaction amplification revealed >99% sequence identity match with Prototheca zopfii. Protothecosis has been previously reported in a single bat, Lyle's flying fox (Pteropus lylei), in Switzerland, but definitive protothecal speciation was not possible.

Key words: Disseminated, protothecosis, Prototheca zopfii, Rousettus lanosus, Ruwenzori long-haired fruit bat.

BRIEF COMMUNICATION

An approximately 9-yr-old captive-bred male Ruwenzori long-haired fruit bat (Rousettus lanosus) weighing 114 g presented with a history of lethargy and unthriftiness. Physical examination revealed general unthriftiness, cranial alopecia, mandibular ulceration, and 5–8% dehydration. The bat was bright, alert, and responsive at the time of examination. The bat was initially treated with subcutaneous saline (0.9% sodium chloride, Abbott Laboratories, Abbott Park, Illinois 60064, USA) and enrofloxacin (11.3 mg/ml suspension, Taylors Pharmacy, Winter Park, Florida 32789, USA; 5 mg/kg po) then isolated for supportive care and continued symptomatic treatment. No diagnostics were performed. The bat was found dead 3 days after presentation. Gross necropsy revealed areas of alopecia and thinning of the pelage on the head and body, exposed desiccated bone at the rostral tip of the mandible, a notable volume of clear pleural fluid, congested lungs with miliary white foci distributed randomly throughout the parenchyma, a partially discolored black liver, and no appreciable adipose stores. Representative samples from all organs and gross lesions were collected and fixed in 10% neutral buffered formalin and routinely embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

Aerobic culture of pulmonary tissue revealed rare growth of two enteric lactose-fermenting gram-negative rod strains and few Staphylococcus aureus. Histologic examination identified large irregular spherical organisms having a large, basophilic central nucleus surrounded by a distinct outer capsule in the heart, lung, spleen, testicle, skin, jejunum, pancreas, and peripancreatic lymph nodes (Fig. 1). Many of the larger organisms contained endospores. The wall of the organisms stained strongly positive by Periodic Acid-Schiff (PAS) methods (Fig. 2). Disseminated protothecosis was suspected on the basis of the morphological appearance and staining characteristics of the organisms. Hepatic hemochromatosis, a common disease in captive bats of various species, was an incidental finding upon histopathology.¹

DNA was extracted from the formalin-fixed paraffin-embedded sample using a commercial kit (Qiagen DNeasy, Qiagen, Inc., Valencia, California 91355, USA) according to manufacturer’s instructions. A portion of the 28S rDNA was amplified by polymerase chain reaction (PCR) using universal fungal primers U1 and U2 as previously described.⁹ Nucleotide sequencing of amplicons was carried out by a commercial company (GENEWIZ, Inc., South Plainfield, New Jersey 07080, USA). Sequences were confirmed by sequencing both strands. Forward and reverse sequences were aligned by a sequence

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alignment program and each sequence was compared by use of the GenBank nucleotide sequence database for similarity with sequences in an online genomic database (Nucleotide Basic Local Alignment Search Tool, www.ncbi.nlm.nih.gov/blast/blast, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland 20892, USA). Based on the nucleotide sequences, *Prototheca zopfii* was identified. The 28S rDNA (large subunit) sequence from this sample most closely matched that of *P. zopfii* (99% sequence identity [223/224 bp] with *P. zopfii* strain ATCC MYA-4771, GenBank acc. # JQ070131). The next closest match for the 28S amplicon was *P. zopfii* strain ATCC MYA-4770 with 98% sequence identity.

**DISCUSSION**

Protothecosis is an uncommon disease reported in numerous species including humans (*Homo sapiens*), domestic cattle (*Bos taurus*), dogs (*Canis lupus familiaris*), cats (*Felis catus*), roe deer (*Capreolus capreolus*), a beaver (*Castor canadensis*), a Cape hyrax (*Procavia capensis*), a flying fox (*Pteropus lylei*), and Atlantic salmon (*Salmo salar*).2,3,5,6,10–12 The causative agent is an achlorophyllic, aerobic, unicellular alga of the genus *Prototheca*. Members of this genus are saprophytic, ubiquitous in the environment, and have a worldwide distribution.11,12 *Prototheca* organisms are often found in tree slime flux, soil, sewage, feces, and water. Despite this common association with organic material and waste, disease remains rare in both people and animals.8,11,12 Two species of *Prototheca* have been reported to cause disease in humans and mammalian animal species: *Prototheca wickerhamii* and *Prototheca zopfii*.11,12 A third species, *Prototheca salminis*, has been reported in salmon.3 Various manifestations of disease in humans and animals caused by *Prototheca* include cutaneous, subcutaneous, and systemic infections and mastitis. Limited reports of protothecal infections in nondomestic mammalian species vary in presentation from cutaneous granulomatous lesions in beaver and deer to disseminated granulomatous lesions in the gastrointestinal mucosa and kidneys in a hyrax.2,6,7,10,14 A single previous report of protothecosis in a bat identified lesions in the lymphatic system, spleen, central nervous system (CNS), heart, muscle, and kidneys. These lesions resulted in a severe granulomatous lymphadenitis and splenitis and a widespread granulomatous meningoencephalitis. When comparing the described case with the prior report, comparable gross lesions include varying granular nodules or foci and/or abnormal coloration in various organs. Histological similarities included morphologically consistent spherical organisms in affected tissues that stained strongly PAS-positive.5

In domestic species, cutaneous disease and mastitis are seen most commonly in cats and cattle, respectively, whereas canine protothecosis...
is usually a widely disseminated fatal disease. *P. zopfii* is the causative organism most often associated with both protothecal mastitis in cattle and disseminated canine protothecosis.12 Speciation of infective protothecal organisms in reported nondomestic species appears to vary or was not performed. The disseminated presentation in canids appears most similar to findings in the described case, with affected organs typically including large intestine, eyes, CNS, kidneys, liver, skeletal muscle, heart, lymph nodes, thyroid, and pancreas.11

Little is known about the pathogenesis of *Prototheca* infections. It is theorized that cutaneous infection results from introduction of contaminated environmental material via traumatic wounds in humans and cats. In canine cases with disseminated disease, the colon is presumed the primary site of infection after ingestion of contaminated material. Dissemination occurs via lymphatic or hematogenous routes. Immune suppression has been considered a predisposing factor in both canine and human protothecal infections, but definitive evidence of this is lacking.11,12 The pathogenesis in the described case is unknown, though similar theories could be ascribed.

Clinical signs commonly associated with canine disseminated protothecosis include hemorrhagic diarrhea and weight loss. Evidence of chronic colitis is often present for months prior to clinical signs suggestive of disseminated disease. Signs of ocular and CNS infection include blindness and seizures. Ataxia may be seen in some cases. Other nonspecific signs such as fever, lymphadenopathy, and dehydration may present. Less commonly, dogs may present with firm nodular skin lesions.3,11,12 One presentation in dogs that may warrant higher suspicion of protothecosis is an individual with hemorrhagic enteritis concurrent with acute blindness.13 Macroscopic lesions on postmortem examination may include granulomatous nodular lesions in affected tissues.11,12 It is undetermined if the gross cutaneous lesions seen in the described case were primary sequelae of the protothecal organisms or secondary to trauma or other opportunistic infection(s). However, it is presumed the protothecal infection contributed to these lesions as organisms were identified in submitted samples.

Differentials in canine cases, particularly cutaneous infections, include * Blastomyces* sp., *Cryptococcus* sp., *Candida* sp., *Histoplasma capsulatum*, *Coccidioides immitis*, *Geotrichum candidum*, and *Pneumocystis* sp., as well as protozoan-induced *Caryospora* sp. Other differentials for canine protothecal colitis include parasitic and viral etiologies such as parvovirus and coronavirus.14

Diagnosis of protothecosis can be made using molecular techniques such as PCR, cytologic or histopathologic examination, or culture of representative tissues or fluids. Indirect immunofluorescence microscopy or use of special staining techniques on histologic samples employing Gomori methenamine silver (GMS), PAS, or Giemsa stains can aid in diagnosis.8,11,12 Of note is that on histopathologic examination protothecal organisms elicit minimal inflammatory response, which is considered characteristic of protothecosis (Fig. 1).4,7 Complete blood count and serum chemistry changes are typically nonspecific.8

Treatment for protothecosis is not well established. Infections demonstrate minimal to no response to empirical antibiotic therapy, and potential worsening of signs after immunosuppressive treatment.8,12 Localized human cases have been successfully treated with surgical excision in combination with various therapeutic agents, including amphotericin B (AMB), itraconazole, fluconazole, amikacin, and tetracycline, though there is little consistency in response to a particular antimicrobial.12 A combination of intravenous AMB, oral itraconazole, and AMB enemas has been reported in treating canine protothecosis.13 As of 2007, intravenous AMB was thought to be the most effective drug to treat protothecosis, though no drug or combination of drugs demonstrate convincing efficacy in treating disseminated or CNS canine infections.12

Protothecosis has been previously reported in a single bat, Lyle’s flying fox (*Pteropus lylei*), in Switzerland, but definitive speciation was not possible, though organisms histologically resembled *P. wickerhamii*.5 Protothecosis remains a relatively obscure disease in exotic, nondomestic, and wildlife species, and as such may not be considered in a differential diagnosis. In cases where clinical signs and gross lesions at necropsy are consistent with a fungal or algal infection, *Prototheca* infection should be a consideration regardless of species.

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LITERATURE CITED


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