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Case Report

Ruptured Mycotic Aortic Aneurysm in a Sooty Mangabey (*Cercocebus atys*)

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Mycotic aortic aneurysm is a local, irreversible dilatation of the aorta associated with destruction of the vessel wall by infection and is a grave clinical condition associated with high morbidity and mortality in humans. Rupture of aortic aneurysms can be spontaneous, idiopathic, or due to severe trauma, and the condition has been associated with bacterial and, rarely, fungal infections in humans and animals. Here, we describe a case of ruptured spontaneous aortic aneurysm associated with zygomycetic infection in a 21-y-old female sooty mangabey. The animal did not present with any significant clinical signs before being found dead. At necropsy, she was in good body condition, and the thoracic cavity had a large amount of clotted blood filling the left pleural space and surrounding the lung lobes. Near the aortic arch, the descending thoracic aorta was focally perforated (diameter, approximately 0.15 cm), and clotted blood adhered to the tunica adventitia. The aortic intima had multiple, firm, pale-yellow nodules (diameter, 0.25 to 0.5 cm). Histopathologically, these nodules consisted of severe multifocal pyogranulomatous inflammation intermixed with necrosis, fibrin, and broad, infrequently septate, thin-walled fungal hyphae. Immunohistochemistry revealed fungal hyphae characteristic of Mucormycetes (formerly Zygomycetes), and PCR analysis identified the organism as *Basidiobolus* spp. Dissemination of the fungus beyond the aorta was not noted. Spontaneous aortic aneurysms have been described in nonhuman primates, but this is the first reported case of a ruptured spontaneous aortic aneurysm associated with entomophthoromycetic infection in a sooty mangabey.

A mycotic aneurysm is a local, irreversible dilatation of an artery associated with destruction of the vessel wall by infection.[41] Mycotic aneurysms can result from infection of the arterial wall or can occur due to secondary infection of a previously existing aneurysm.[41] Despite the use of the term 'mycotic' to denote appearance of "fresh fungus vegetations,"[20,34] the majority of these aneurysms are caused by bacteria and only rarely by fungi.[13] Currently, this term is used broadly to describe any infected aneurysm regardless of its etiology.[28] Aneurysms can be classified as 'true' aneurysms, which involve all layers of the arterial wall, and 'false' aneurysms (hematomas), which form outside the arterial wall due to a leak in the artery.[38]

Mycotic aneurysms are rare in humans[10] and can occur in the cerebral or systemic circulation of patients with endocarditis, in whom septic emboli can occlude the vasa vasorum or the entire arterial lumen, damaging or destroying the muscle layer of the vessel.[1,35] The subsequent intraarterial pressure causes dilatation and aneurysm formation.[42] Although these aneurysms can vary in size or even disappear during treatment of the underlying etiology, they are always life-threatening, as they develop rapidly and may rupture days, weeks, months, or even years after successful therapy.[45,53] Mycotic aneurysms can be solitary or multiple and can occur anywhere in the body, including the left ventricle; coronary artery; abdominal aorta; and femoral, superior mesenteric, carotid, iliac, and brachial arteries.[9,27,30]

Rupture of an aortic aneurysm in humans can occur spontaneously due to trauma, increased diastolic pressure or anteroposterior diameter, or chronic obstructive pulmonary disease.[9] In animals, trauma is a more common than a spontaneous event.[38] Ruptured mycotic aortic aneurysms associated with bacterial infections have occurred in humans,[21,40] but those caused by fungi are rare.[46] A case of a horse with an aneurysm in the aortic arch associated with *Aspergillus* infection has been reported.[38] Spontaneous aortic aneurysms have been diagnosed in nonhuman primates including gorillas (*Gorilla* spp.); squirrel (*Saimiri* spp.), howler (*Allouatta* spp.), capuchin (*Cebus* spp.), patas (*Erythrocebus patas*), African green (*Chlorocebus aethiops*), and spider (*Atelles* spp.) monkeys; and pygmy chimpanzees (*Pan paniscus*).[5,7,8,24,25] Dissecting aneurysms have been seen in nonhuman primates used as experimental models of atherosclerosis.[24] Our report describes a unique case of a ruptured spontaneous aortic aneurysm associated with entomophthoromycosis in a sooty mangabey.

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Case Report

History. This 21-y-old female mangabey was born at the Field Station of the Yerkes National Primate Research Center and was

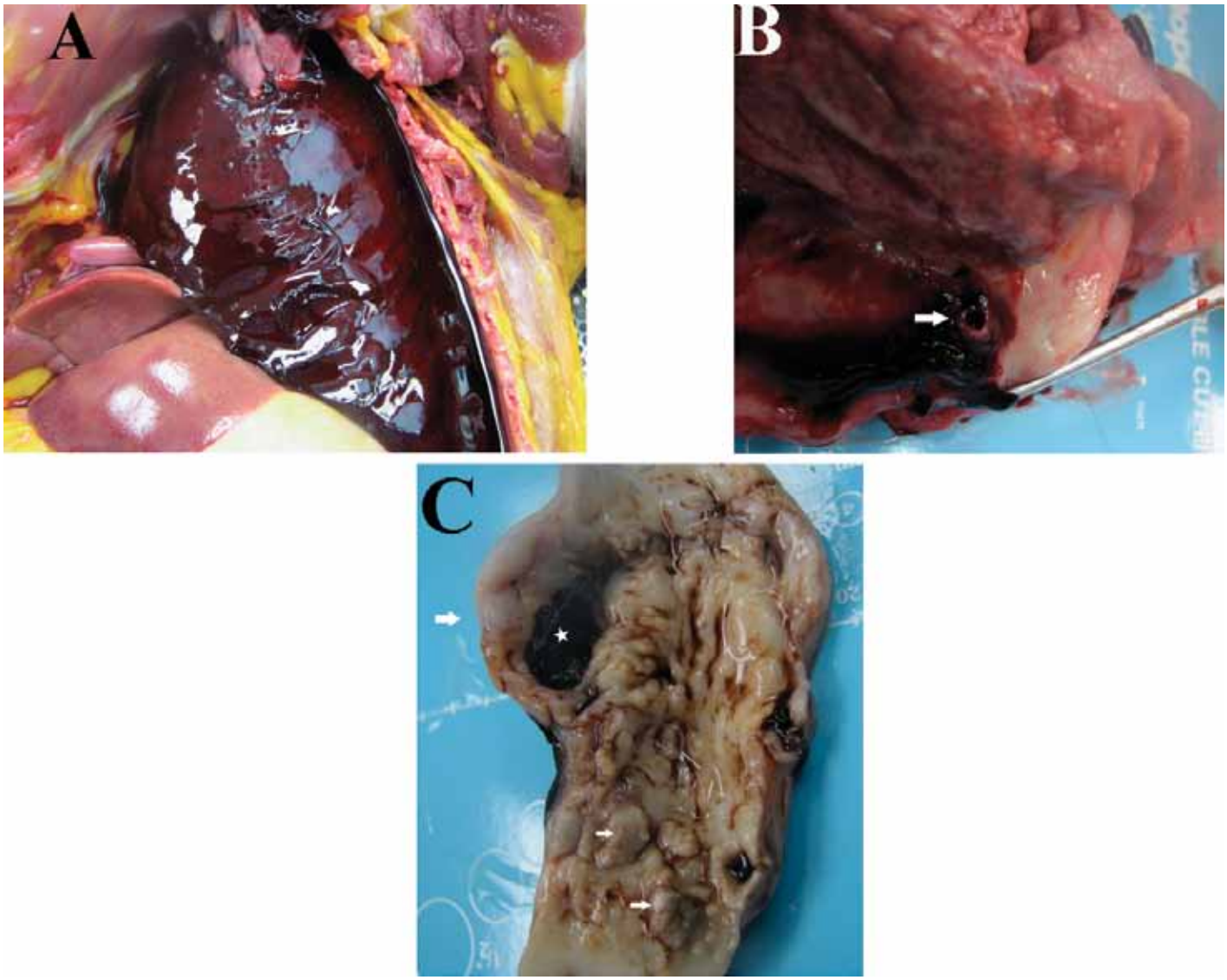

Figure 1. Gross findings. (A) Hemothorax, left side of the thoracic cavity. (B) Distended aortic arch and focal rupture site (arrow). (C) Formalin-fixed aorta, with the aneurysm (large arrow), rupture site covered by clotted blood (asterisk), and multiple intimal vegetative growths (small arrows).

housed in accordance with the *Guide for the Care and Use of Laboratory Animals*[18] and Animal Welfare Act Regulations.[2,3] She had been maintained in a social colony in an indoor–outdoor compound since birth and was fed a standard monkey chow diet (Purina LabDiet 5037, PMI, St Louis, MO) with daily approved enrichment. A physical examination and diabetes screening performed 3 mo earlier did not reveal any significant findings. No significant clinical signs were reported, and she had been observed eating and acting normally approximately 1 h prior to being found dead in the indoor housing area.

Pathology. At necropsy, she weighed 7.25 kg and was in good body condition. A large amount of clotted blood filled the left pleural space around the lung lobes (Figure 1 A). The wall of the thoracic aorta was moderately thickened and focally perforated (diameter, approximately 0.15 cm; Figure 1 B and C). The perforation had smooth edges and clotted blood adhered to the tunica intima and adventitia (Figure 1 B and C). The tunica intima had multiple, firm, pale yellow nodules (diameter, 0.25 to 0.5 cm; Figure 1 C). Tissue specimens collected at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. Histopathologic analysis confirmed thinning (aneurysm) and rupture of the aortic wall. The aortic wall had moderate multifocal intimal fibrosis intermixed with necrosis and infiltrates of degenerate and viable eosinophils and neutrophils (Figure 2 A through D). The aortic intimal nodules contained pyogranulomatous inflammation intermixed with abundant eosinophils, necrosis, fibrin (Figure 2 B and C) and broad, infrequently septate, thin-walled fungal hyphae, which occasionally showed focal bulbous dilatations and irregular branching (Figure 3 A and B). The inflammatory infiltrate often extended to the tunica media and occasionally to the tunica adventitia. No significant lesions or fungal hyphae were apparent in any other tissue.

Immunohistochemistry. Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4 µm. Immunohistochemical tests using a multistep

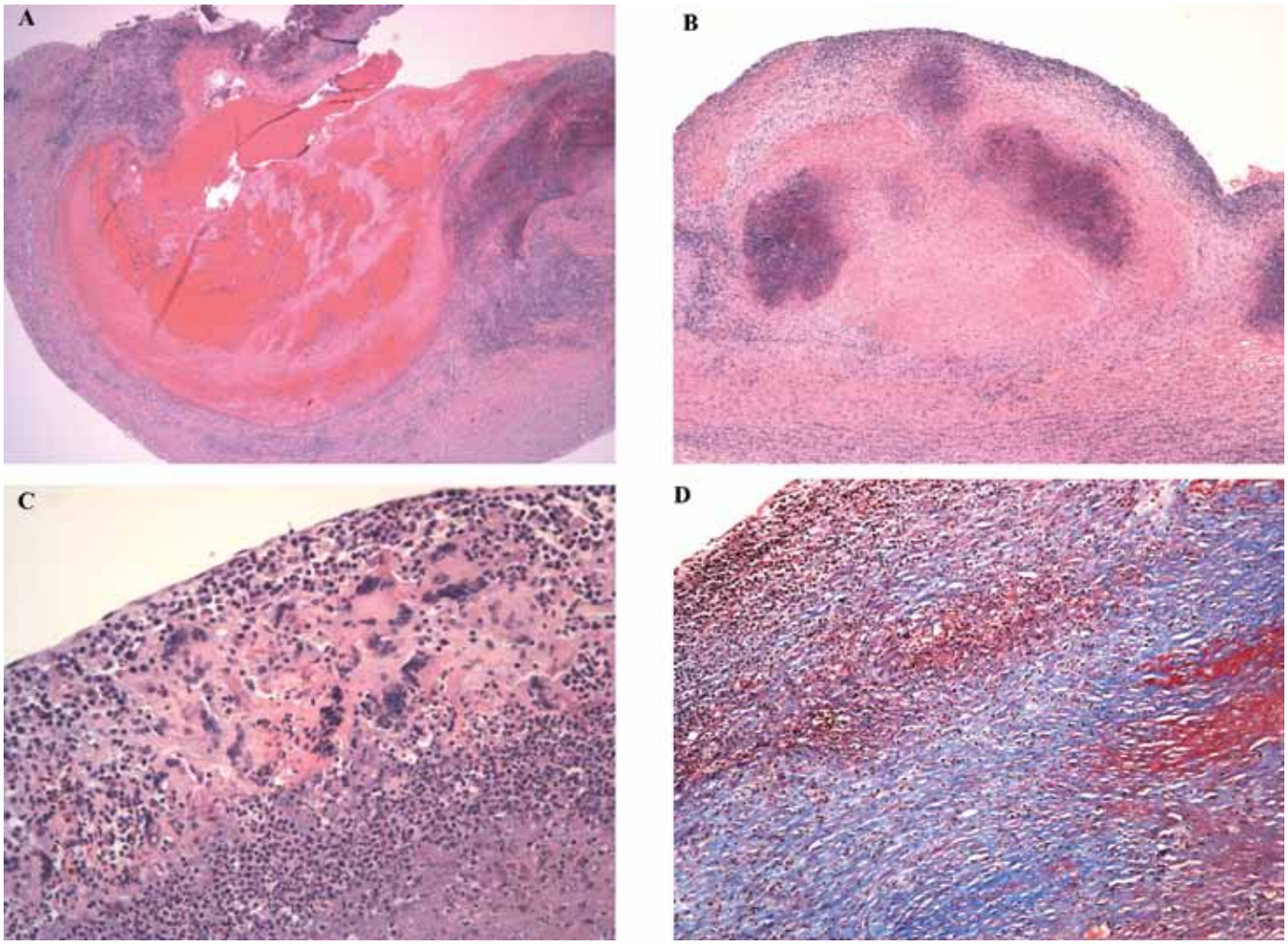

Figure 2. Histopathology. (A) Aortic aneurysm; magnification, ×20. (B) Section of an aortic intimal nodule or vegetative growth showing severe multifocal inflammation, necrosis, and fibrin thrombi. Hematoxylin and eosin stain; magnification, ×40. (C) Pyogranulomatous inflammation with necrosis in the aortic intima. Hematoxylin and eosin stain; magnification, ×200. (D) Increased amount of collagen with necrosis and inflammation in the aortic wall. Masson trichrome stain; magnification, ×100.

immunoalkaline phosphatase technique[43] were performed on sections of aorta. Primary antibodies included mouse anti*Rhizomucor* (cat no. M3565, Dako, Carpinteria, CA) and anti*Aspergillus* spp. (cat no. M3564, Dako) antibodies. Appropriate positive and negative controls were run in parallel. The *Rhizomucor* antibody was raised against *Rhizomucor* and is known to cross react with other mucormycetes including Mucoraceae (such as *Mucor*) and Entomophthoraceae (such as *Basidiobolus*). The immunohistochemistry for mucormycetes demonstrated irregular, wide, nonseptate fungal hyphae (Figure 3 C) in areas of intense eosinophilic inflammation in the aortic intima. Immunohistochemistry was negative for *Aspergillus* spp.

PCR. Formalin-fixed paraffin-embedded tissue was processed to produce genomic DNA as previously described.[29] Primers ITS3 and IT4, which amplify the ITS2 region of the 18S rDNA of most fungal species, were used for amplification. A single 423-bp product was amplified and sequenced in both directions by using the amplification primers. A BLAST search of the Genbank database revealed 96% homology to multiple isolates of the genus *Basidiobolus* in the order Entomophthorales. There were no acceptable matches to other species, confirming *Basidiobolus* as the likely causative agent.

Other findings. No significant pathogen was isolated from the blood, heart, liver, or colon contents. The serum was negative for antibodies to SIV and simian T-lymphotropic virus.

Discussion

An aneurysm is a localized abnormal dilatation or outpouching of a thinned and weakened portion of a vessel.[38] This abnormality usually affects large elastic arteries and therefore can be fatal when it ruptures.[38] In humans, aortic aneurysms are most commonly a complication of atherosclerosis and have been reported in association with uncontrolled diabetes, high blood pressure, vasculitis, cystic medial necrosis, infections, and physical injuries.[23,41,48] Causes of aneurysms in animals include copper deficiency in pigs,[44] *Spirocerca lupi* infection in dogs,[6,19] and *Strongylus vulgaris* infection in horses.[47] Aortic rupture with dissecting aortic aneurysm has been studied in cattle due to similarities with

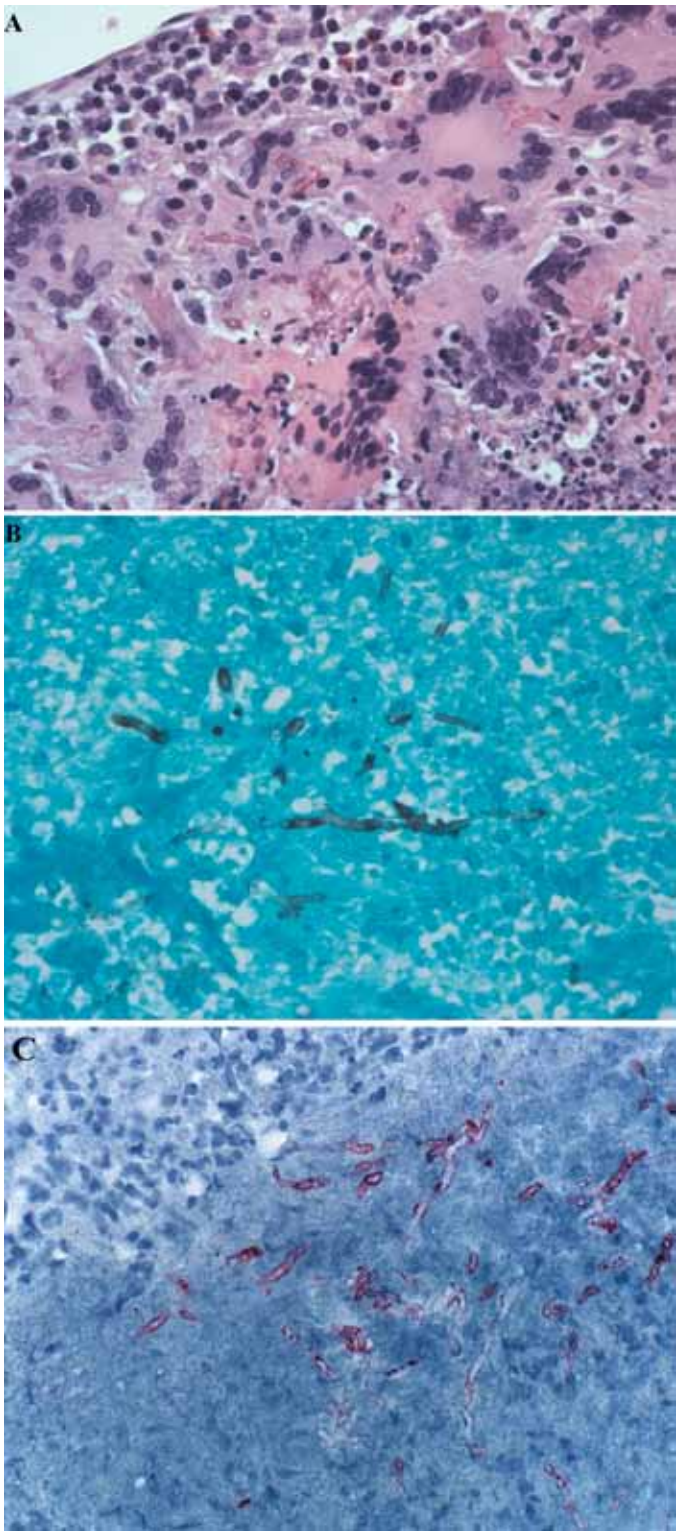

Figure 3. Intralesional fungal hyphae in an aortic intimal nodule. (A) Hematoxylin and eosin stain. (B) Gomori methenamine silver stain. (C) Immunohistochemistry for zygomycetes. Magnification, ×400.

Marfan syndrome in humans[36] and also occurs in ostriches, pigs, rats, and turkeys.[14,22,31] Sudden rupture of the ascending aorta associated with marked exertion and excitement (due to breeding and racing) in horses has been associated with rapid death due to cardiac tamponade.[38] The aortic aneurysm and rupture in the mangabey reported here was spontaneous and associated with severe infection with an entomophthoralean fungus.

Ruptured mycotic aortic aneurysms associated with bacterial infections like *Staphylococcus aureus*, *Streptococcus* spp., *Salmonella*, and *Treponema pallidum* have been occasionally documented in humans,[17,21,40] but those caused by fungi are rare and are often associated with poor prognosis.[26,46] Aneurysm in the aortic arch associated with *Aspergillus* infection was reported in a horse,[33] and fungal infections of the guttural pouch have been associated with ulceration of the internal carotid or maxillary artery resulting in their rupture and fatal hemorrhage in horses.[38] Spontaneous aortic aneurysms in nonhuman primates have been described to occur with dissecting aneurysms in monkeys used as experimental models of atherosclerosis,[5,7,8,24,25] and disseminated zygomycosis in a cynomolgus macaque has been described.[50] The current report describes a unique case of rupture of a spontaneous aortic aneurysm associated with entomophthoromycosis in a sooty mangabey.

Mucormycosis is the preferred descriptive term for angiotropic infection caused by a member of the subphylum Mucoromycotina or the subphylum Entomophthoromycotina (formerly Zygomycetes).[15] Histopathology typically reveals angioinvasion with associated necrosis.[15] Currently approximately 665 species of zygomycetes have been described, but reports of infections of immunocompetent humans or animals with these primitive, rapid growing, terrestrial, widespread, and largely saprophytic fungi are generally rare.[15] Medically important orders and genera include Mucorales, causing subcutaneous and systemic zygomycosis (Mucormycosis) and including Rhizopus, Lichtheimia (Absidia), Rhizomucor, Mucor, Cunninghamella, Saksenaea, Apophysomyces, Cokeromyces, and Mortierella; and Entomophthorales, causing subcutaneous zygomycosis (Entomophthoromycosis) and including *Conidiobolus* and *Basidiobolus*.[15]

In the current study, immunohistochemistry detected a mucormycetic infection in the aorta and PCR further identified the fungus as *Basidiobolus* spp. *Basidiobolus* is a true pathogen, causing infections in immunocompetent and immunocompromised human and animal hosts.[37] This organism occurs in decaying vegetation and soil and as a saprobe in the intestinal contents of reptiles like lizards (chameleons), amphibians (toads), and mammals (bats).[12,32] The fungus is believed to enter the skin of a host after an insect bite, scratch, or minor cut.[49,51] With the exception of *Basidiobolus ranarum*, fungi in the Entomophthorales order, compared with those in Mucorales, generally do not invade vascular tissue.[37] The character of *Basidiobolus* infection can range from acute infiltrates of eosinophils, lymphocytes, and plasma cells to granulomas with a predominance of eosinophils.[52]

Although healthy subjects can be affected, most human patients with mucormycosis have immunosuppression, secondary to diabetes, malignancy, or solid-organ or bone marrow transplantation, leading to disseminated infection associated with a very high mortality rate.[16,39] Other contributing risk factors were considered based on human literature, including atherosclerosis, uncontrolled diabetes, high blood pressure, vasculitis, cystic medial necrosis, infections, and physical injuries.[23,41,48] In this case,

the mangabey had no known history of diabetes, hypertension, or increased cholesterol levels. Although this animal had sustained fight wounds in the past, evidence of recent fighting or any trauma-associated lesion was not detected at necropsy. Clinical signs had not been reported prior to death of the animal, and complete gross and histopathologic analysis did not reveal significant predisposing disease, including atherosclerosis or neoplasia. In addition, the 2 common routes for contracting *Basidiobolus* infection, the respiratory and gastrointestinal tracts, did not exhibit any significant gross or histologic lesions.

In conclusion, the aneurysm in the thoracic aorta of this sooty mangabey was a true aneurysm, involving all the layers of the aortic wall. In the absence of any significant clinical signs and based on the gross and histologic lesions, the cause of death of this animal was acute rupture of an aortic aneurysm associated with a fungal infection. The mucormycotic infection of the aorta was severe and multifocal, but infection of other organs was not noted. Although systemic mucormycosis has occurred occasionally in the sooty mangabey colony at our institution,[4] the current presentation is unique, and to our knowledge, is the first report of ruptured spontaneous aortic aneurysm associated with entomophthoromycetic infection in a sooty mangabey.

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