Journal of Zoo and Wildlife Medicine 40(2): 365–368, 2009

COCCIDIOIDOMYCOSIS IN A BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Abstract: A 5-yr-old female black rhinoceros (*Diceros bicornis*) was euthanized 11 mo after arrival at the Milwaukee County Zoo (Milwaukee, Wisconsin, USA) from Glen Rose, Texas (USA) for a severe progressive rear leg lameness of 6-mo duration. Gross necropsy revealed complete rupture of the capital ligament of the left femur with synovitis and osteomyelitis. Multifocal lymphadenopathy with chronic suppurative lymphadenitis of the tracheobronchial, left supramammary, and iliac lymph nodes was present. Granulomatous pneumonia with a focal abscess was also noted. Histologically, fungal elements were seen in the lung, lymph nodes, and synovium, and *Coccidioides immitis* was isolated on fungal culture. *Coccidioides immitis* is not endemic to Wisconsin; therefore, the animal had to have been infected, although asymptomatic, at the time of arrival at the Milwaukee County Zoo. Whether the disease was active at the time of arrival or whether it was quiescent and then became active with the stress of shipment or injury is unknown.

Key words: Black rhinoceros, *Coccidioides immitis*, coccidioidomycosis, *Diceros bicornis*, fungal disease, valley fever.

BRIEF COMMUNICATION

In September 2005, a 4-yr-old captive-born female black rhinoceros (*Diceros bicornis*) arrived at the Milwaukee County Zoo (Milwaukee, Wisconsin, USA) from Glen Rose, Texas (USA). The rhinoceros had been born, mother-reared, and housed at a facility in Glen Rose until shipment to Milwaukee. During a 30-day quarantine period, repeated fecal samples were negative for endoparasites and enteric pathogens, and complete blood count (CBC) and serum chemistry values were within normal limits.

On 6 February 2006 (day 0), an acute onset of a moderate to severe (grade III/V) weight-bearing lameness of the left rear limb was noted. Trauma was suspected because of the suddenness and severity of the lameness. Visual examination did not reveal any wounds or swelling of the leg, and palpation of the foot and lower leg using behavioral training did not reveal any areas of sensitivity. Over the next 2 mo, the lameness continued unchanged despite stall rest and oral treatment with anti-inflammatory medication (Banamine®, Schering-Plough Animal Health Corp., Union, New Jersey 07083, USA) and antibiotics (Tucoprim®, Pfizer Inc., New York, New York 10017, USA), but the animal's appetite remained good and it continued to respond to routine behavioral training sessions. Thermography performed on day 47 revealed focal areas of increased temperature consistent with inflammation in the area of the left stifle, groin, and hip region. Complete blood cell count and serum chemistry results remained normal until a sample collected at day 64 showed an elevated total protein (8.9 mg/dl; International Species Inventory System [ISIS]: 7.9 ± 0.8). The globulins, although within normal limits (ISIS: 5.4 ± 1.1), had increased from 4.8 mg/dl while in quarantine to 6.0 mg/dl. Serologic testing on serum collected 85 day after the onset of lameness was negative for antibodies to *Blastomyces*, *Aspergillus*, *Histoplasma*, and *Coccidioides* by semiquantitative radial immunodiffusion assay (RID) (ID Fungal Antibody System, Immuno-Mycologics, Inc., Norman, Oklahoma 73071, USA, www.immy.com).

On day 108, the rhinoceros was immobilized with etorphine (1.8 mg intramuscularly [i.m.]; ZooPharm, Laramie, Wyoming 82070, USA) and detomidine (12.5 mg i.m.; Pfizer, Inc., New York, New York 10017, USA) for further examination, palpation, and radiographs of the affected leg. Radiographs of the lower left rear limb and stifle did not reveal any abnormalities, and palpation and manipulation of the leg and hip did not reveal any dislocation or fracture. Blood collected for CBC and serum chemistry revealed a white blood cell count of 10.2×10^3 µl (ISIS: $8.07 \pm 2.01 \times 10^3$/µl), with 8.57×10^3/µl

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neutrophils (ISIS: 5.06 ± 1.706 × 10^3/μl). The total protein was 9.3 mg/dl, and the globulins were 6.3 mg/dl. Over the next 2 mo, the animal progressed to a non–weight-bearing lameness in which the left leg was held stiffly and would drag. Ultrasound of the pelvis and hip performed under operant conditioning at day 171 did not reveal any fluid pockets indicating abscessation, asymmetry, or discontinuity of the left hemipelvis, and the femur appeared intact. Because of the deterioration of the animal's physical condition; lack of improvement with rest, antibiotic, and anti-inflammatory therapy; and increasing evidence of poorly controlled pain, the animal was anesthetized and humanely euthanized at day 175. RID assays on serum collected the day of euthanasia tested negative for antibodies to blastomyces, *Aspergillus*, and histoplasma but positive for coccidioides.

Gross necropsy revealed complete rupture of the round ligament of the left femur, with marked synovitis, osteoarthritis, and osseous metaplasia of the left coxofemoral joint. The joint capsule was intact, and the hip was not displaced. The depth of the right and left acetabula was asymmetrical, with approximately 1 cm of the left rim worn away. A corresponding area of wear was present on the left femoral head, indicating that the femur would subluxate when the animal was weight-bearing. A mild, chronic, granulomatous pneumonia with focal abscessation of the right hilar lung field was present. The area was firm on cut surface, with evidence of exudation. Sections of the left lung parenchyma revealed multiple 3–6-mm firm nodules. Moderate lymphadenopathy of the left supramammary and iliac lymph nodes and focal, chronic, suppurative, lymphadenitis of the tracheobronchial lymph node were present. Multiple adrenal cortical adenomas were an incidental finding.

Histologic findings included moderate to severe, chronic, multifocal, suppurative, granulomatous, and pyogranulomatous lymphadenitis with multinucleated giant cells and intracytoplasmic fungal yeasts of the tracheobronchial, left iliac, and left supramammary lymph nodes; severe chronic granulomatous and pyogranulomatous synovitis of the left coxofemoral joint capsule with multinucleated giant cells containing yeasts; and severe, multifocal, chronic, suppurative, granulomatous, and pyogranulomatous pneumonia, again with intralesional yeasts (Fig. 1). All intralesional fungi had morphologic features consistent with *Coccidioides* spp. No acid-fast bacteria were seen. Fungal culture of both the lung and tracheobronchial lymph node grew *Coccidioides immitis*.

Coccidioidomycosis, also known as valley fever, is caused by the pathogenic dimorphic fungi *C. immitis* and *Coccidioides posadii*.[9] In the United States, the fungi is endemic to the lower Sonoran life zone, including west Texas.[9] It is present in the soil and can survive in sea water.[17] Soil disturbances such as earthquakes, windstorms, construction, and archeologic digs allow the infectious arthroconidia to become airborne, causing episodic increases in the number of cases.[9,11,14,15] The primary route of infection is by inhalation, and the disease is primarily a pulmonary disease. Roughly 60% of the cases in humans are subclinical. Two to three percent of the remaining symptomatic cases progress to disseminated disease, which may involve the skin and soft tissue, bones, joints, and meninges.[9,11] The disease has been reported in other terrestrial domestic mammals and wildlife,[1,2,4,10,13,19,20] marine mammals,[5,7,17] and reptiles.[21] Although considered noncontagious, there has been one report of transmission from a female macaque to its infant[3] as well as a report of a veterinarian contracting coccidioidomycosis during necropsy of an infected animal.[12] Clinical susceptibility, course of the disease, and the tendency for dissemination vary among species, and signs range in severity from entirely subclinical to progressive and fatal.[18] Pregnant women and immunocompromised individuals are at higher risk for developing disseminated disease.[9,11]

Subclinical or self-limited disease in humans is usually not treated. Patients with disseminated coccidioidomycosis who are at risk for complications because of immunosuppression require treatment that may include antifungal therapy, surgical debridement, or both.[6,9] Amphotericin B is often used in patients who are in respiratory failure or who display rapidly progressive infections. Itraconazole is typically used to treat chronic progressive pulmonary or disseminated disease, and the duration of therapy may be protracted. In some cases, lifetime treatment is needed to prevent relapse.[9] Prolonged itraconazole therapy has been used successfully in a domestic horse.[8]

In humans, serologic testing can be used to assess the response to treatment. Serum concentration of complement-fixation type antibodies (primarily immunoglobulin G) declines with successful treatment and increases with recrudescence or dissemination of disease.[16] The use of

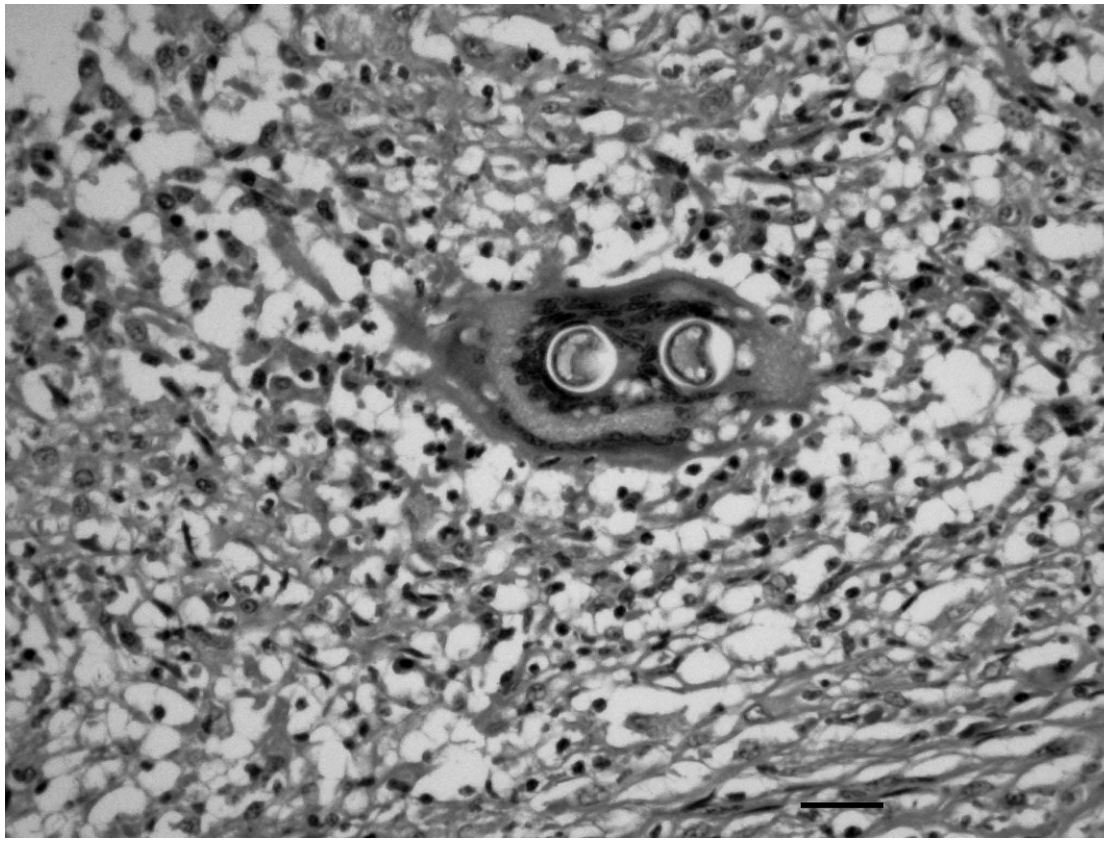

Figure 1. Foci of pyogranulomatous inflammation and chronic granulomas present in the lung of a black rhinoceros. Several foci have central multinucleated giant cells, occasionally containing phagocytosed *Coccidioides immitis* organisms. Hematoxylin and eosin, ×100. Bar = 80 μm.

this test as a prognosticator has not been well studied in other species.[18] In this rhinoceros, the initial negative result was followed in 91 day by a positive titer at the time of euthanasia. This conversion, in conjunction with increasing serum globulins, may indicate that the disease was inactive initially, then recrudesced and disseminated, primarily to the damaged tissues.

This is the first reported case of coccidioidomycosis in a rhinoceros. General susceptibility and the typical course of disease in this species are unknown. Another perissodactyl, the Przewalski's horse (*Equus caballus przewalskii*), is reported to be susceptible to infection with *Coccidioides*.[20] Studies have shown that captive black rhinoceros may have diminished T-cell reactivity, perhaps increasing their propensity for certain diseases, including fungal disease.[23] However, black rhinoceros have been housed for many years in endemic areas, yet the disease has not previously been recognized, indicating that this species may not be inherently more susceptible.

Coccidioides spp. are not endemic to Wisconsin; therefore, this animal must have acquired the disease while in Texas. Glen Rose, Texas, is just outside of what is considered to be the endemic area for *Coccidioides* spp.[11,22] The only confirmed case of coccidioidomycosis at the Glen Rose facility was a goat born and raised on site that was euthanized in 2006 and found to have concurrent thymoma and coccidioidomycosis (H. Haefele, pers. comm.). While the endemic area may have spread to include the Glen Rose region, it is possible that strong winds or some other cause of disturbance of contaminated soil may have introduced infectious spores into the area.

Whether the animal arrived in Milwaukee with active lesions or whether the disease was quiescent but became active and disseminated from the stress of shipment or from trauma to the leg and subsequent pain is unclear. This case serves as a reminder that when contemplating differential diagnoses for ill animals, the region of the

country in which the animal previously lived and the endemic diseases specific to that region must be considered. It should be emphasized that proper precautions should be taken to minimize the generation and inhalation of aerosols when performing necropsies of animals suspected of having coccidioidomycosis or with pathologic lesions consistent with coccidioidomycosis.

Acknowledgments: The authors are deeply grateful to and thank the dedicated and compassionate Milwaukee County Zoo keeper staff for their care of this animal. In addition, the authors appreciate the information provided by Dr. Holly Haefele regarding the history of coccidioidomycosis at the Glen Rose facility. The authors also thank all the large animal specialists who helped with this case, especially colleagues Dr. Kevin Nelson and Dr. Phil Burns.

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Received for publication 12 May 2008