Abstract

Disseminated coccidioidomycosis was determined to be the cause of impaired ambulation in a 5-year, 10-month-old male cynomolgus monkey (Macaca fascicularis). The primary infection was considered to be from aerosol exposure. The diagnosis was based on clinical signs, gross lesions, histopathology, and periodic acid-Schiff special stains on collected tissues. Significant light microscopic findings included pyogranulomatous pneumonia, thoracolumbar vertebral osteomyelitis, and tracheobronchial lymphadenitis with fungal spherules. Infection with Coccidioides sp. was based on the presence of light microscopic examination of tissues and the presence of characteristic features of fungal spherules, consistent with those described previously for Coccidioides spp. To our knowledge, this is the first report of naturally acquired disseminated coccidioidomycosis in a presumably immunocompetent colony cynomolgus monkey (Macaca fascicularis).

Keywords: Coccidioides sp; Coccidioidomycosis; Cynomolgus monkey; Lung; Macaca fascicularis; Vertebrae

Introduction

Coccidioides spp. is a pathogenic fungi that resides in the soil in semi-arid regions in parts of the southwestern United States, Mexico, and Central and South America. Natural infection occurs in wild rodents, and high concentrations of the fungal organisms are often found in and around rodent burrows. Vegetative growth can occur after periods of rain, with dispersion of the organisms in dry windy climate conditions [1]. The route of infection is typically respiratory, and infection has been reported following experimental aerosol exposure, with as few as 50 arthrospores [2]. Animal to animal transmission has been reported in rodents, following ingestion of infected tissue, but is not considered a common occurrence [3].

In primates, clinical signs of coccidioidomycosis include weight loss, skin lesions, and those associated with respiratory disease, such as nasal discharge, cough, and dyspnea [1,4]. Pulmonary lesions have been reported to result in consolidation, collapse, pleural adhesions and variably sized cavitations [1,5]. Infection in the vertebral column has been reported to cause lameness, reluctance to move and paralysis [1,4]. Radiographic evidence of osteolytic lesions of the ulna has also been reported in one Japanese macaque [4]. In affected vertebral column, lesions can result in lysis of bone. Paravertebral masses, with invasion into the vertebral column and spinal cord, have also been reported to lameness, and/or paralysis [1]. Disseminated infection can involve multiple organs, including regional lymph nodes, liver, spleen, kidneys or esophagus [1,5]. The inflammatory response can vary from neutrophilic to granulomatous, and vary from miliary to massive. Microscopic lesions that are pyogranulomatous, typically have characteristic peripheral multinucleated giant cells, often with spherules present that are thick-walled, approximately 10-60 μm in diameter, and filled with 1-4 μm endospores [1].

Differential diagnosis in primates should typically include tuberculosis, neoplasia and other disseminated mycotic infections. Serologic testing for specific antibody, tube precipitin test, or skin testing for coccidioidin may help detect infection, yet skin testing is not considered specific. Etiologic diagnosis is often made from the characteristic appearance of Coccidioides spp. organisms (and the use of special stains such as periodic acid Schiff) in cytology specimens, histology sections of affected tissue, or by culture.

Case Report

In this case report, a 5-year, 10-month-old male cynomolgus monkey (Macaca fascicularis) housed at Covance Research Products (CRP) facility in Alice, TX, USA, was presented for physical examination due to locomotion difficulty and urinary bladder incontinence. This animal was previously tested three times for Herpes B virus, simian immunodeficiency virus, simian T-lymphotropic virus type 1, and was determined to be negative in all cases. Tests for Shigella, Salmonella, and Campylobacter were also evaluated two times previously, and were also negative. Hematology and clinical chemistry parameters were not available based on a retrospective search of this animal’s record. During physical examination, the left leg was paretic, with no movement of the left foot, and the right leg had significant weakness with a limited range of motion. There was no apparent perception of deep pain in the toes, or in stifle of the affected left foot.

The animal was euthanized, and a necropsy was performed based on the declining clinical condition. There was no presence of Mycobacterium sp. organisms noted in collected tissues, using an acid-fast bacillus (AFB) stain. Light microscopic evidence of pneumonia in the lung was evident, and was characterized by multifocal to coalescing pyogranulomas, often containing fungal organisms. Similar inflammation was also observed in the thoracolumbar vertebrae, associated with some localized loss of bone, and in the tracheobronchial lymph, where the inflammation obscured preexisting...
noidal architecture (Figures 1-3). Numerous spherules consistent with *Coccidioides* sp. were observed within pyogranulomatous areas in the lung, vertebrae and tracheobronchial lymph node. A diagnosis of disseminated infection with *Coccidioides* sp. was made, based on the microscopic examination of tissues and the characteristic features of fungal spherules, consistent with those described for *Coccidioides* sp. Chronic lymphoplasmacytic inflammation was considered an incidental finding in the urinary bladder, as evidenced by no fungal organisms evident, and no other microscopic findings in the urinary bladder, suggestive of a specific cause for the inflammation. However, we could not rule out the possibility that a similar infectious process was in the urinary bladder, yet not in the sections examined.

**Discussion**

This case report represents disseminated coccidioidomycosis in a cynomolgus monkey (*Macaca fascicularis*). A review of this animal documented historical records indicated that this animal had been part of previous pharmacokinetic evaluations, yet there was no history of illness or inclusion in any immune modulating toxicity studies. Although we were unable to show conclusively, this animal presumably was not immune compromised. The primary infection was considered most likely respiratory. The disseminated nature of this infection, specifically, the osteomyelitis involving the vertebrae, was presumably secondary to hematogenous spread from the lung, and accounted for the clinical musculoskeletal symptomatology, similar to other previous reports [1,4].

There are relatively few reports of coccidioidomycosis in primates. These reports are limited to a ring-tailed lemur, baboons, rhesus monkeys and gorillas. In most cases, the animals were either housed in endemic areas or they originated from endemic areas [1,2]. One chronic progressive pulmonary form of coccidioidomycosis was described in a young adult male rhesus monkey [6]. Similar to this case report, the animal originated from Texas, which is a known endemic area. Also similar to this case report, there were numerous spherules present, characteristic of *Coccidioides* sp. Serum from 191 primates housed at the California Primate Center were assayed for the presence of coccidioidin [3]. These animals were reportedly housed outdoors from October 1977 to October 1978. Coccidioidomycosis was described in two of the animals; a rhesus monkey (*Macaca mulatta*) with a disseminated infection, and bonnet macaque (*Macaca radiate*) with a pulmonary infection. Two additional monkeys were also found to have coccidioidal antibody detected, but no clinical evidence of disease. This information, as in our case report, provides additional evidence that the incidence of exposure, and/or subclinical coccidioidomycosis in colony primates, may be greater than previously known based on the usual methods of testing.

The morphology of the organisms observed in infected tissues in this animal was consistent with either *C. immitis* or *C. posadasii*, both of which are closely related, morphologically identical and require genetic analysis and growth rate characteristics, in the presence of high salt concentrations (*C. posadasii* grows more slowly) to further differentiate [7,8]. The distribution of *C. immitis* is reportedly limited to California’s San Joaquin Valley region, whereas *C. posadasii* is reportedly found in the southwest United States, Mexico and South America [7,8].

Cynomolgus monkeys are commonly used in pre- and post-clinical studies designed to evaluate drug safety. Infections by various naturally occurring organisms may be observed, albeit uncommonly, during these studies and may complicate interpretation and relatedness to study specific conditions, especially in animals exposed to potentially immunomodulating agents. Only one confirmed case of active infection of *Coccidioides* sp. was found from a historical pathology records search of Covance Research Product animals housed in Alice, TX, USA, dating back to 2007. The origin of most of these animals was from China, with a few from Mauritius. To our knowledge, this is the first reported case of naturally acquired coccidioidomycosis in a cynomolgus monkey. The findings presented in this case report show...
that coccidioidomycosis may occur in presumably immunocompetent colony cynomolgus monkeys (Macaca fascicularis).

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References