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Case Report

Pneumocystis Pneumonia in a Thoroughbred Racehorse

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ABSTRACT

Pneumocystis pneumonia is an opportunistic respiratory infection that occurs in immunocompromised animals. In horses, pneumocystic pneumonia is observed mostly in foals and often progresses rapidly. Here, we report pneumocystic pneumonia in a Thoroughbred racehorse. A 3-year-old Thoroughbred racehorse colt had marked respiratory symptoms for 3 weeks and was unresponsive to antibiotic treatment. At necropsy, firm, tan, patchy lesions were scattered diffusely in the lungs. Microscopically, alveolar septa thickened by proliferation of collagen fibers and infiltration of inflammatory cells were observed. In the alveolar spaces, many brown-black yeast-like organisms similar to cystic forms of *Pneumocystis carinii* were recognized by staining with Gomori's methenamine silver. Bronchoalveolar lavage fluid (BALF) obtained before necropsy included macrophages engulfing the fungus bodies. Amplified products were obtained from BALF and lung tissue samples by *Pneumocystis*-specific nested PCR. Phylogenetic analysis based on the 18S rRNA gene sequence revealed that the *P. carinii* organism from BALF was related to the *Pneumocystis* spp. detected in other animals and was especially close to *P. carinii* derived from ferrets. This is a rare case of pneumocystic pneumonia in a colt with chronic pulmonary lesions.

1. Introduction

Pneumocystis spp. is a fungus in the class Archiascomycetes, phylum Ascomycota, that can cause opportunistic lung infections in immunodeficient individuals. Human immunodeficiency virus infection in humans and porcine circovirus type 2 infection in piglets are known to compromise immune systems and increase patients' susceptibility to *Pneumocystis* spp. infection [1,2]. In horses, there have been several reports of pneumocystic pneumonia. Most of the cases have been observed in foals younger than 6 months old [3-9], but there has been one case reported in an adult horse [10]. This report describes the pathological and pathogenic findings in a Thoroughbred colt with pneumocystic pneumonia.

2. Case Report

A 3-year-old Thoroughbred colt racehorse was referred to the horse clinic with a history of elevated rectal temperature (39.0°C). The colt had been forced to continue with daily training up until it visited the clinic, although it had had a mild fever for a week. At the first medical examination, abnormal respiratory sounds and a high count of peripheral white blood cells (14,800/mm^3) were detected. Infection of the respiratory tract was suspected clinically, and antibiotic treatment with cephalothin sodium (10 g, intravenous [IV] every 8 hours) was initiated. Blood biochemical values, including serum globulin level (3.4 g/dL) were normal. Radiographic inspection of the chest revealed reticular and ground-glass opacities. Arterial

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blood gas measurements were within normal ranges (PaO_2, 91.1 mm Hg; $PaCO_2$ 41.2 mm Hg; pH 7.422). Endoscopy of the respiratory tract revealed no abnormalities. No respiratory pathogenic bacteria were isolated from bronchoalveolar lavage fluid (BALF), and the cell composition of the BALF was in the normal range. Additional administrations of minocycline hydrochloride (1 g, IV, every 12 hours) and fosfomycin sodium (10 g, IV, every 12 hours) were begun 4 days (minocycline) and 1 week (fosfomycin) after the initial treatment. Treatment was continued for 3 weeks, but because of the poor clinical prognosis and for economic reasons, the colt was euthanized and necropsy was performed.

At necropsy, the lungs were heavy and poorly aerated, with a diffuse scattering of tan, patchy lesions that were firm and in which the lobules were indistinct. The cut surfaces of the lesions were solid, with no exudation. The other organs had no specific lesions. The organs were fixed in 20% neutral buffered formalin, processed routinely, and embedded in paraffin. Tissue was sectioned at 3 μm thick and stained with hematoxylin-eosin, elastica van Gieson, and Gomori's methenamine silver. The lung tissue was only slightly aerated but retained some areas of normal consistency. In the alveolar spaces there were marked accumulations of plump alveolar macrophages; an eosinophilic exudate partially filled the spaces. The type II pneumocytes were very enlarged and had proliferated in the alveoli, the septae of which were thickened (Fig. 1) by the proliferation of collagen fibers and the infiltration of macrophages, lymphocytes, and neutrophils (Fig. 2). At the sites of lesions stained with Gomori's methenamine silver, many brown-black yeast-like organisms measuring 5-8 μm in diameter and resembling the cystic forms of *Pneumocystis carinii* were recognized on the pneumocytes and in the alveolar spaces (Fig. 3). The plump alveolar macrophages included several fungus bodies in their cytoplasm. Staining of BALF smear specimens with Gomori's methenamine silver revealed moderate numbers of macrophages engulfing the fungus bodies (Fig. 4). The other organs, including the lymphoid organs, were pathologically unremarkable.

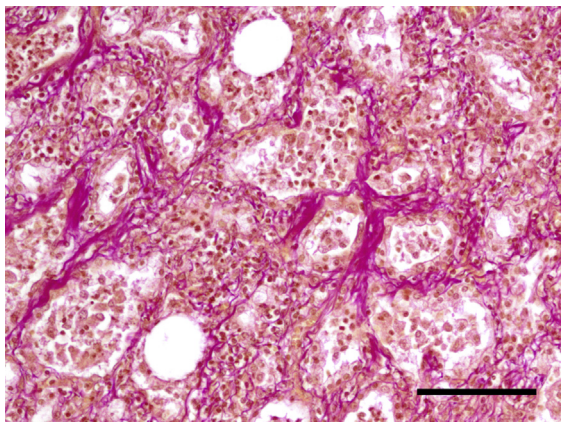

Fig. 2. Lung. Alveolar septae were markedly thickened by the proliferation of red-stained collagen fibers and infiltration of inflammatory cells. elastica van Gieson stain. Bar = 100 μm.

To detect *P. carinii* in BALF and lung tissue, we used a *Pneumocystis*-specific nested PCR targeting the 5S rRNA subunit. DNA was extracted from the specimens by using a commercially available blood and tissue kit (DNeasy; Qiagen GmbH, Hilden, Germany). We used the primers pAz102-H/pAz102E and pAz102-X/pAz102Y, described previously by Boondireke et al [11]. The PCR conditions were as follows: incubation at 94°C for 5 minutes; 40 cycles of 94°C for 30 seconds, 52°C for 60 seconds, and 72°C for 60 seconds; and a final extension step at 72°C for 5 minutes. By using PCR, we obtained *Pneumocystis*-specific products from both BALF and lung tissue samples.

To classify the taxonomic position of the *P. carinii* isolated from the colt, we performed a phylogenetic analysis based on the 18S rRNA gene sequence. To amplify DNA encoding eukaryotic species 18S rRNA, we performed PCR

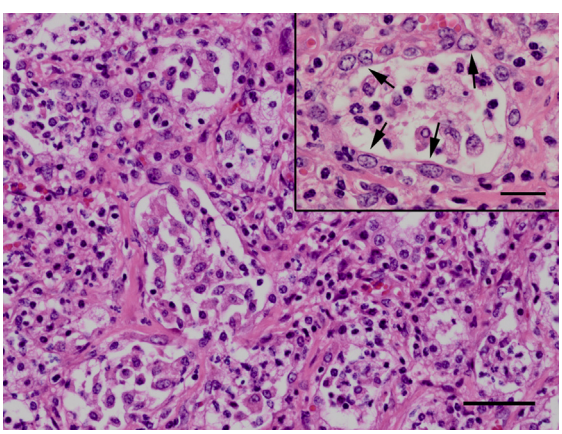

Fig. 1. Lung. There are marked accumulations of plump alveolar macrophages, and eosinophilic exudate partially fills the alveolar spaces. Hematoxylin-eosin stain. Bar = 50 μm. (Inset) Type II pneumocytes were markedly enlarged and proliferated in the alveoli (arrows), which had thickened septa. Hematoxylin-eosin stain. Bar = 20 μm.

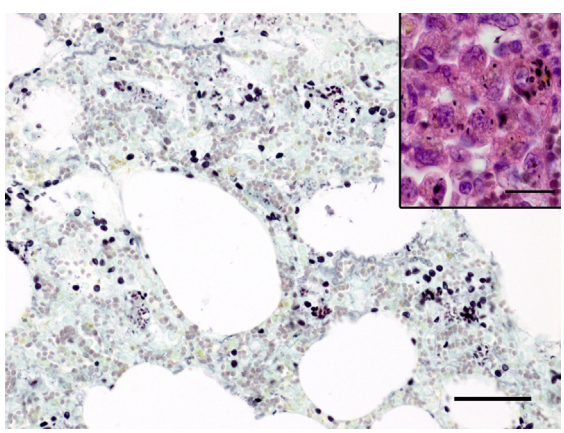

Fig. 3. Lung. Many yeast-like organisms similar to cystic forms of *Pneumocystis carinii* were recognized on the pneumocytes and in the alveolar spaces. Gomori's methenamine silver stain. Bar = 50 μm. (Inset) Plump alveolar macrophages included several fungus bodies in their cytoplasm. Triple staining with hematoxylin, eosin, and Gomori's methenamine silver. Bar = 25 μm.