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Infection of a Three-Toed Sloth (*Bradypus variegatus*) by a *Pneumocystis*-like Organism in Panama

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Since its first description over 75 yr ago, *Pneumocystis carinii* has been a parasite of uncertain taxonomy. It was originally considered to be a trypanosome (Chagas, 1909, Mem. Inst. Oswaldo Cruz Rio de J. 1: 159–218) and later proposed to be a yeast (Csillag, 1957, Acta Microbiol. Acad. Sci. Hung. 4: 1–8; Vávra and Kucera, 1970, J. Protozool. 17: 463–483). Most recently

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FIGURE 1. Multiple 1–2-cm irregular nodules are visible through pleura on cut surface of the lungs of a three-toed sloth from Panama infected with a *Pneumocystis*-like organism. These nodules extend deep into lung parenchyma.

Matsumoto and Yoshida (1984, J. Protozool. 31: 420–428) have studied this organism ultrastructurally and proposed a scheme for the life cycle. It is classified presently as a protozoan belonging to the class Sporozoa, subclass Coccidia (Beaver et al., 1984, Clinical Parasitology, Lea and Febiger, Philadelphia, Pennsylvania, pp. 167–170). Recently, *Pneumocystis carinii* has received much attention as the cause of pneumonia in patients with acquired immunodeficiency syndrome (Jaffe et al., 1983, Ann. Intern. Med. 99: 145–151) and it has been identified also as the cause of pneumonia in monkeys with a naturally occurring immune deficiency syndrome (Letvin et al., 1983, Proc. Natl. Acad. Sci. USA 80: 2718–2722). Experimentally induced immunosuppression in a variety of laboratory animals including rats (Chandler et al., 1980, Handbook: Animal Models of Human Disease, Arm. Forc. Inst. Pathol., Washington, D.C., Model No. 181), rabbits (Yoshida et al., 1981, Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A 250: 206–212), and non-human primates (Long et al., 1975, J. Am. Vet. Med. Assoc. 167: 651–654) has been used to study pneumocystosis. *Pneumocystis carinii* is apparently present as a latent infection in many species. It was demonstrated in the lungs of 23 zoo animals in the Netherlands that died from various causes over an 11-yr period (Poelma, 1975, Z. Parasitenkd. 46: 61–68). In that study, *P. carinii* was identified in the lung of a three-toed sloth (*Bradypus tridactylus*) that died of unknown causes. The animal had been imported from Surinam 4 mo prior to death.

An adult 3.1-kg female three-toed sloth was captured manually from the Panamanian jungle approximately 6 km north of the village of Escobal and 1 km west of Gatun Lake as part of an epidemiologic survey to determine the mammalian reservoirs of *Leishmania* spp. in Central America. Twenty-four hr after capture, the sloth exhibited tachycardia (160 beats per min), dyspnea with moist rales, and appeared to be in extreme respiratory distress. Rectal temperature was 32.3 C and no evidence of abdominal pain was noted on palpation. Due to the poor prognosis for survival upon return to the jungle, the sloth was immobilized with ketamine hydrochloride (20 mg/kg) and xylazine hydrochloride (2 mg/kg) by intramuscular injection in the quadriceps muscles and killed with a lethal injection of sodium pentobarbital via cardiac puncture. At necropsy impression smears were made of lung, liver, and spleen and stained with Gram stain. Samples of lung were taken

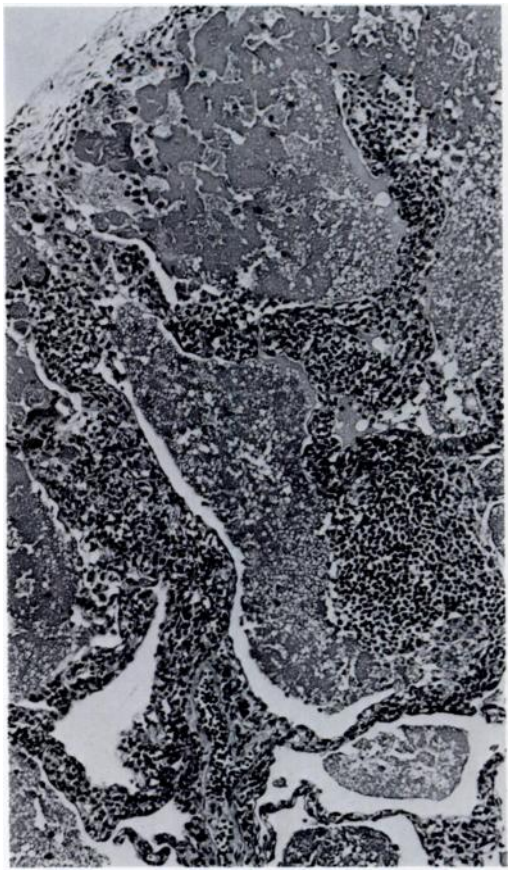

FIGURE 2. Subpleural area of lung of a three-toed sloth from Panama showing alveolar spaces distended with frothy proteinaceous exudate. Alveolar septae are thickened by a cellular infiltrate of lymphocytes and macrophages. H&E, ×100.

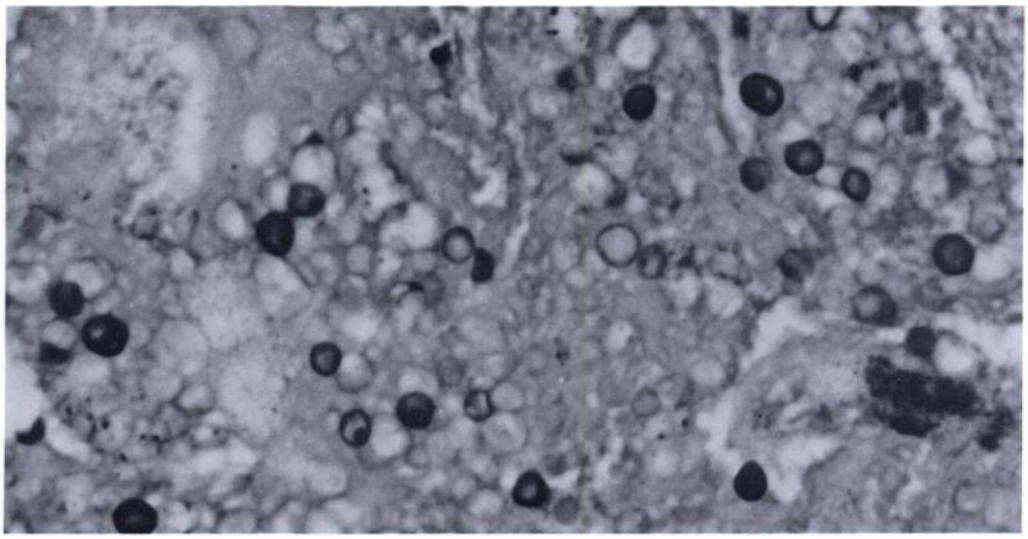

FIGURE 3. Deeply stained round to oval *Pneumocystis*-like organisms in alveolar exudate of a three-toed sloth from Panama. GMS, ×1,000.

for bacterial culture. All major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E), Gomori's methenamine silver (GMS), and Brown and Brenn tissue Gram stain for light microscopy.

Gross lesions were limited to the lungs which had a mottled appearance and numerous 1–2-cm irregular, raised, yellow-tan nodules under the pleural surface. On cut surface these nodules were demarcated poorly, often coalescing and extending throughout the lung to occupy approximately three-quarters of the lung parenchyma (Fig. 1).

Gram stain of lung impression smears revealed a few Gram-negative organisms. No organisms were seen in smears of the spleen or liver. *Enterobacter* spp. and *Acinetobacter anitratus* were cultured from the cut surface of the lung.

Histologic examination of the lung revealed an interstitial pneumonia involving approximately 75% of the lung parenchyma. Pulmonary bronchioles and alveolar lumina were distended with a frothy proteinaceous exudate that contained a few lymphocytes and macrophages while the interstitium of the adjacent alveolar septae was distended with a similar inflammatory cell infiltrate (Fig. 2). Gomori methenamine-silver stain demonstrated multiple round to oval *Pneumocystis*-like organisms in the alveolar exudate that varied from 3–7 μm in diameter (Fig. 3). Histologic examination of the liver, kidneys, adrenal glands, various sections of the gastrointestinal tract, cervical lymph-nodes, salivary glands, cerebellum and cerebral cortex revealed essentially normal tissue.

Although *Pneumocystis carinii* has been demonstrated in the lung of a sloth in a zoo by Poelma (1975, op. cit.), our case is unique in that a significant pneumocystis pneumonia occurred in a sloth in the wild. The microscopic appearance of the pulmonary lesion and the morphology of the organisms are typical of the descriptions reported in other host species by Long (1975, op. cit.) and Chandler et al. (1980, op. cit.). In man and animals, pneumocystis pneumonia is associated routinely

with immunodeficiency. In the case reported here, the immunocompetence of the sloth was not known. The microbiologic findings of *Enterobacter* spp. and *Acinetobacter anitratus* were considered to be findings of opportunistic invading organisms. There was no gross or microscopic evidence of other significant concurrent disease.

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Sporulated Coccidian Oocysts Resembling *Goussia* Labbe, 1896 in the Viscera of Nile Crocodiles

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Three dead adult (3–4 yr of age) Nile crocodiles (*Crocodylus niloticus* Laurenti, 1768) from a crocodile farm in Zimbabwe were submitted for evaluation. They had been dead 1–2 hr prior to necropsy. No gross lesions were seen at necropsy. Available for histology were pieces of liver, spleen and lung fixed in neutral-buffered 10% formalin. Tissues were processed routinely (i.e., dehydrated in ethanol series, embedded in paraffin, sectioned at 5 μm). For electron microscopy formalin-fixed tissues were post-fixed in Dalton's osmium-dichromate solution (Dalton, 1955, Anat. Rec. 121: 128), embedded in Epon, and cut at 8 nm. Sections were stained with lead citrate and uranyl acetate.

Histologically the red pulp of the spleen was infiltrated by leucocytes, primarily lymphocytes, but the white pulp was still easily recognizable. There were many sporulated oocysts of a coccidian in the red pulp area and impinging on and within the periphery of the lymphoid sheaths. A mild hemosiderosis was present in the red pulp. Numerous oocysts were found within the interstitium of the lung. It was difficult to determine the exact location of the oocysts. It was determined that they were within macrophages or endothelial cells. Some oocysts were extracellular. Diffuse hydropic changes were present in hepatocytes. Oocysts were numerous in sinusoids and often were found in cells with flattened nuclei. These parasites were probably in Kupfer cells, but may have been in circulating macrophages. In general there was no inflammatory response to the parasites in any organ.

Oocysts were found singly or in clusters. The only stage of parasite found was sporulating or sporulated oocysts (Fig. 1). The oocyst wall was thin (less than 0.5 μm) and was folded or collapsed during sectioning and oocysts were spherical and approximately 20 μm in diameter. Four sporocysts were present within each oocyst. These were ovoidal and measured approximately 15 × 6 μm when mature. Each sporocyst contained two elongate sporozoites with their sides abutting one another. Sporozoites measured 12 × 2.5 μm and each contained a small basophilic nucleus and large eosinophilic globules (when stained with hematoxylin and eo-