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A RETROSPECTIVE STUDY OF 11 CASES OF LUNGWORM (*DIDELPHOSTRONGYLUS HAYESI*) INFECTION IN OPOSSUMS (*DIDELPHIS VIRGINIANA*)

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Abstract: A juvenile, female North American opossum (*Didelphis virginiana*) died of verminous pneumonia caused by *Didelphostrongylus haysei* despite aggressive treatment with oral fenbendazole, corticosteroids, and antibiotics. This prompted a retrospective study of lungworm infection in opossums, during which 19 additional necropsy reports from opossums were reviewed. Including the subject of this report, a total of 11 (55%) of these cases included a diagnosis of lungworm infection. This diagnosis was considered to have contributed to death in eight out of the 11 cases (73%). Histologically, 10 of the 11 (91%) opossums had granulomatous bronchopneumonia with small to moderate numbers of adult nematodes in the airways and parenchyma. Four of the 11 (36%) opossums had free larvae within the parenchyma or terminal airways. Inflammation was usually associated with larvae, degenerating parasites, and nonintact adult nematodes. Superimposed bacterial pneumonia was evident in three animals, and sections of lung examined from all the opossums were characterized by moderate to severe smooth-muscle hyperplasia in airways, including terminal respiratory bronchioles and alveolar ducts. Nine animals had prominent medial smooth-muscle hyperplasia in small- and medium-sized arterioles. Lesions in other organs, particularly in liver, heart, and gastrointestinal tract, were frequently identified. Three animals had concomitant septicemia or bacterial bronchopneumonia (or both), which contributed to the cause of death. Seven animals had gastric nematodosis (*Physaloptera* sp.), although three of them had been treated with a 14-day course of fenbendazole.

Key words: Opossum, *Didelphis virginiana*, lungworm, *Didelphostrongylus hayesi*, verminous pneumonia, marsupial.

CASE REPORT

A 5-mo-old, female North American opossum (*Didelphis virginiana*) was presented to the Veterinary Medical Teaching Hospital (VMTH), University of California (Davis, California 95616, USA), in November 1993, with the main complaint of dyspnea of 2-day duration. The wild-caught animal (Yolo County, California, USA) was orphaned 1 mo earlier and was currently under the care of a licensed wildlife rehabilitator. This animal had been housed in an outdoor enclosure comprising bare ground covered by straw bedding, with six other opossums of similar age but unknown relatedness. Two of these animals had died recently after bouts of dyspnea.

On presentation, this opossum was alert and able to ambulate normally but was dyspneic. The animal was emaciated (870 g), with flea dirt present on its haircoat. The animal was estimated by skin turgor to be at least 5% dehydrated. Oral mucous membranes were pale. The dyspnea, characterized by a respiratory rate of 60 breaths/min and increased expiratory effort, worsened when the animal was stressed. In addition to physical examination, diagnostic evaluation included whole-body radiographs, blood collection for a complete blood count and serum chemistry analysis, and fecal collection for flotation and direct smear.

Radiographic findings included increased pulmonary infiltrates and an associated peribronchial pattern. Complete blood count and serum chemistry analysis revealed a microcytic (mean corpuscular volume [MCV] = 63.8 fL, 63.8 μm^3), hypochromic (mean corpuscular hemoglobin concentration [MCHC] = 316 g/L, 31.6 g/dl; mean corpuscular hemoglobin [MCH] = 20.2 pg; Hb = 62 g/L, 6.2 g/dl), and regenerative anemia (RBC = 3.07 $\times(10^{12}$/L, 3.07 $\times(10^{6}/\mu l$; hematocrit [HCT] = 0.196, 19.6%; reticulocytes = 0.0188, 18.8%; nucleated RBCs = 162/100 WBC), hyperfibrinogenemia (9 g/L, 900 mg/dl), hypergammaglobulinemia (0.045 g/L, 4.5 g/dl), and hypoalbuminemia (10.6 g/L, 1.6 g/dl).[11,24,26] Circulating eosinophils were not observed in this patient. An elevated alanine aminotransferase (2.10 μkat/L, 126 IU/L), arginine aspartase (6.10 μkat/L, 366 IU/L), and total

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bilirubin (29.1 μmol/L, 1.7 mg/dl) were also suspected after comparison of these values with published reference values and in-house laboratory values from other apparently healthy opossums.[24] Centrifugal fecal flotation using zinc sulfate revealed numerous (>10/lower power field [LPF]) coiled metastrongylid larvae 220–240 μm long by 12.5 μm wide. They had a long, filariform esophagus approximately half their total length and an attenuated, lancet-shaped tail. No dorsal spine was observed. The radiographic findings and metastrongylid larvae identified from fecal flotation supported a diagnosis of verminous pneumonia.

Treatment of the lungworm infection consisted of oral fenbendazole (Panacur Suspension, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey 08876, USA) at 50 mg/kg/day and dexamethasone sodium phosphate (Azium, Schering-Plough Animal Health, Union, New Jersey 07083-1982, USA) at 0.25 mg/kg i.m. every 12 hr. Fenbendazole was chosen on the basis of its use in treating lungworm infection in domestic animals; the dose was extrapolated from domestic cats. Dexamethasone was used to decrease the suspected inflammatory reaction to dying larvae and, therefore, to decrease the severity of clinical signs. Enrofloxacin (Baytril, Bayer Corporation, Shawnee Mission, Kansas 66201, USA) at 5 mg/kg i.m. every 12 hr was used to prevent or treat secondary bacterial infections. Aminophylline (Aminophylline, American Regent Laboratories, Inc., Shirley, New York 11967, USA) at 10 mg/kg i.m. was used during periods of severe respiratory distress through bronchodilation. The opossum was maintained in an oxygenated chamber (40–50%) during hospitalization. Despite therapy the opossum died 3 days later.

At necropsy, representative tissues from all organs were placed in 10% formalin, and lung tissue was collected for bacterial culture and identification. The significant postmortem findings were limited to the lungs and the liver. Grossly, the lungs were dark red, wet, and slightly firm, with numerous pale, tan, 3- to 6-mm nodules, often coalescing, scattered throughout the parenchyma. Numerous black-and-white, thread-like worms, 1–2 cm long, were present in the trachea and on the cut surface of the lung. The liver was friable and pale tan with an enhanced reticular pattern.

Histologically, the pulmonary lesions were characterized by multifocal to diffuse areas of granulomatous bronchopneumonia with scattered focal areas of necrosis. Some of the airways contained cross sections of nematode parasites associated with small amounts of cellular debris and mucus, whereas other parasites were free within the parenchyma. Generally, there were few to no inflammatory cells associated with the adult nematodes. Small numbers of larvae were seen within the parenchyma, surrounded by granulomatous inflammation characterized by large numbers of macrophages and giant cells with smaller numbers of lymphocytes. Occasionally, the alveoli were lined by type 2 pneumocytes, and there was prominent smooth-muscle hyperplasia of the respiratory bronchioles and alveolar ducts. *Salmonella typhimurium* was cultured from the lung. Additionally, there was severe hepatic lipidosis.

Feces were submitted to the VMTH Diagnostic Parasitology Laboratory for zinc sulfate 33% centrifugal fecal flotation. Numerous (>10/LPF) coiled larvae were recovered. The larvae were approximately 240 μm long by 12.5 μm wide. A 3-cm^3 section of lung was submitted to recover adult nematodes. Gravid, adult, female nematodes were approximately 2.5 cm long by 845 μm wide. Adult male nematodes were approximately 1.0 cm long. The buccal cavity was reduced and surrounded by six lips. The cuticle was inflated and almost twice the width of the nematode's body. The male nematode had a weakly developed bursa supported by bursal rays. The bursal lobes were not well demarcated. The males had short, stout spicules and a complex gubernaculum. The dorsal bursal ray of the male was rounded by two long branches. The lateral and ventral rays of the bursa were each joined to common stalks. On the basis of these morphologic features, the nematodes were further identified as *Didelphostrongylus hayesi*.[15]

Between January 1993 and January 1994, 20 additional North American opossums were necropsied by the VMTH Pathology Service. Eleven (55%) of these cases included a diagnosis of *D. hayesi* infection. These opossums had been brought to the VMTH by a licensed wildlife rehabilitator. Seven of the opossums affected were females, three were males, and for one the sex was unrecorded. Six of these were juveniles, three were adults, and two were of unknown age. Six opossums were dead or died shortly after arrival at the VMTH. Four opossums were presented with the main complaint of dyspnea, one was presented with seizures, and one was presented with severe bite wounds on the head and the neck. Before being presented, six of the 11 (55%) opossums had been treated for lungworm infections with 50 mg/kg fenbendazole p.o. from 3 to 14 days. Three of these treated animals were dyspneic when presented, and three were found dead at the wildlife rehabilitation center without premonitory clinical signs.

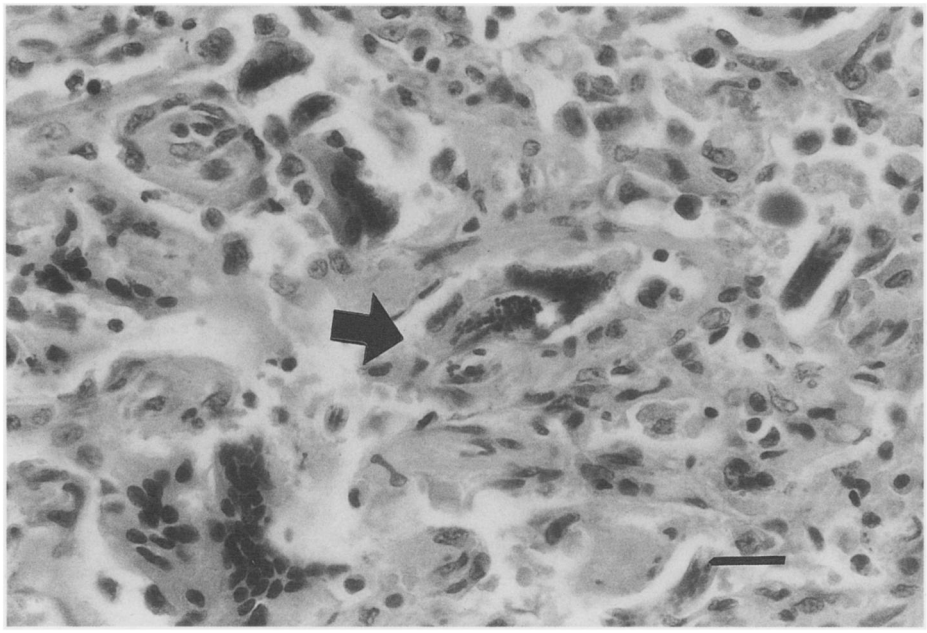

Figure 1. Photomicrograph of lung from an opossum with verminous pneumonia and severe granulomatous inflammation surrounding metastrongyle larvae (arrow). H&E. Bar = 25 μm.

In the 11 opossums with lungworms, the infection ranged from mild to severe, depending on the numbers of larvae detected in the feces, and it was considered the cause of death or contributed to the cause of death in eight of the 11 cases (73%). Striking gross pulmonary lesions were seen in six of the 11 (55%) opossums examined. They were characterized by firm, dark red, wet lungs with discrete single to coalescing 2- to 6-mm nodules scattered throughout the parenchyma. In the other five opossums examined, the gross lesions were less severe. The lungs were dark red, wet, and heavy (3/5) or congested, firm, and shrunken (2/5).

Although several pathologists performed the necropsies, histologic sections were reevaluated by a single pathologist. Microscopically, there was great variation in the severity of the pulmonary lesions and parasite burden. Pulmonary lesions were graded mild, moderate, or severe on the basis of the extent of the histologic lesions. Ten of the 11 (91%) opossums had few to moderate numbers of adult nematodes in the airways and pulmonary parenchyma. The parasites in the parenchyma were generally associated with mucus and cellular debris. Four of the 11 (36%) opossums had free larvae within the parenchyma or terminal airways. Larvae were associated with moderate to severe granulomatous inflammation characterized predominately by macrophages with some multinucleated giant cells and smaller numbers of lymphocytes (Fig. 1). There was generally very little inflammation associated with the adult nematodes, although in two of the treated animals there was mild to moderate inflammation associated with degenerating parasites. The sections examined from all the opossums were characterized by moderate to severe smooth-muscle hyperplasia in terminal respiratory bronchial and alveolar ducts, and three animals also had prominent medial smooth-muscle hyperplasia in small- and medium-sized arterioles (Fig. 2). The most severely affected lungs had extensive areas of alveolar collapse (atelectasis) and mild, type 2 pneumocyte hyperplasia.

Only one of the animals diagnosed with lungworms antemortem and treated with fenbendazole at 50 mg/kg/day for a period of 14 days lacked evidence of pulmonary inflammation or nematodes. Pulmonary arterial medial and bronchiolar smooth-muscle hyperplasia was present, consistent with previous lungworm infection. This animal did, however, have widespread eosinophilic inflammation within multiple organs, which is suggestive of systemic parasitism. Only one larva was found in a section of lung from the second animal treated for 14 days. This animal had concurrent bacterial bronchopneumonia. The third animal treated with fenbendazole for 14 days had moderate to severe pneumonia associated, in part, with degenerating nematodes. Severe hepatic lesions consistent with right-sided heart failure also were present and were

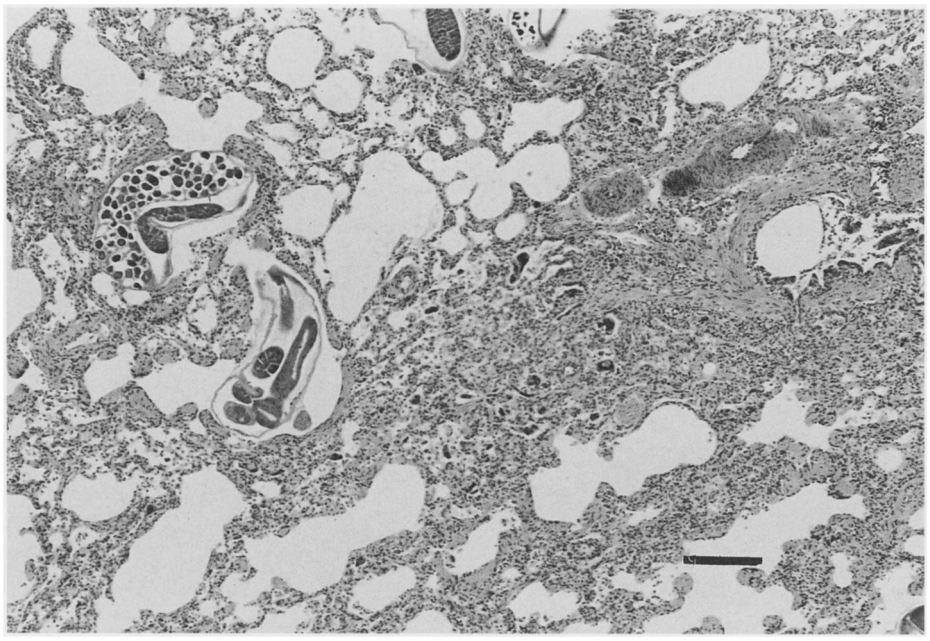

Figure 2. Photomicrograph of lung from an opossum with lungworm infection caused by *D. hayesi*. Note the adults without host response and the arteriolar and bronchiolar smooth-muscle hypertrophy. H&E. Bar = 200 μm.

possibly caused by pulmonary hypertension from the parasitic pneumonia.

In addition to parasitic pulmonary lesions, many of the opossums had concomitant disease, diagnosed by histologic examination. One animal had bacterial pneumonia. Two were septicemic, with multiple lesions including broncho- and interstitial pneumonia, hepatitis, myocarditis, vegetative valvular endocarditis, splenitis, glomerulointerstitial nephritis, and lymphadenitis. *Salmonella typhimurium* was isolated from the lungs and livers of two of these animals and the intestinal tract of a third animal without histologic evidence of septicemia. Pleural and peritoneal fluids were noted in 45 and 36% of the cases, respectively. Ten of the 11 (91%) animals had one or more histologic lesions in the liver, including vacuolar hepatopathy or hepatic lipidosis (n = 4), centrilobular hepatic necrosis (n = 2), centrilobular passive congestion (n = 2), and periportal hepatitis (n = 3). Six of the 11 (55%) animals had one or more cardiac lesions, including endocarditis (n = 2), myocarditis (n = 3), thrombus (n = 2), cardiomyopathy (n = 1), right-sided heart failure (n = 2), and supraventricular septal defect (n = 1). Two of the 11 (18%) opossums had numerous puncture wounds and cellulitis. Seven animals (64%) had gastric nematodosis (*Physaloptera* sp.), although three of them had been treated with a 14-day course of fenbendazole. One animal also had an oxyurid infection (*Cruzia* sp.) at the time of death, despite recent fenbendazole treatment.

DISCUSSION

The pulmonary lesions in opossums in this report were characterized by a mild to severe, granulomatous bronchopneumonia, with variable numbers of adult nematodes and larvae. There was generally little to no inflammation associated with the adult nematodes either within the airways or in the parenchyma, although occasional degenerating adult parasites in the treated animals were surrounded by a mild to moderate inflammatory response. Lack of cellular response to adult metastrongyles found in bronchioles has been reported in other metastrongylid infections.[22] The most severe inflammation was associated with free larvae within the parenchyma. Developing larvae are more metabolically active and are known to secrete immunogenic substances.[9]

The nematodes were likely responsible for the dyspnea. Although adult nematodes in the airways contributed little to the inflammatory process, they were associated with abundant mucus and likely caused obstruction and subsequent atelectasis. Granulomatous inflammation around the larvae caused significant loss of pulmonary parenchyma. The lesions observed in opossums in this report were similar to those reported previously in *D. hay-*

esi infections in opossums from the southeastern United States, and these cases demonstrate the range of severity of the lesions.[3,16]

Another characteristic feature of the pulmonary lesions was the prominent smooth-muscle hyperplasia in the small airways and arterioles. This lesion has been associated with parasitic pneumonia in pigs and cats as a result of infection with *Metastrongylus* spp. and *Aelurostrongylus abstrusus*, respectively. But the pathologic mechanism is as yet unknown.[6] *Salmonella typhimurium* cultured from the lungs was considered secondary to the verminous pneumonia because the wild opossum is a natural reservoir for *Salmonella* spp., with systemic spread occurring in moribund animals.[8,10,17,23] The hepatic vacuolar change likely represented lipidosis secondary to debilitation and mobilization of body fat reserves.

The Virginia opossum occurs naturally from New Hampshire to Colorado and from southern Ontario to Costa Rica.[14] The species was first introduced into California in 1890 and has since spread from southwestern British Columbia to San Diego, California.[14] Although *D. hayesi* has been reported in opossums from the southeastern United States, until recently this parasite has not been reported in California.[1,3,13,15,16] The cause of this unusually high prevalence of verminous pneumonia in opossums that were presented to the VMTH between January 1993 and January 1994 has not been determined.

A study conducted using opossums from the Opossum Care Program in Davis, California, revealed *D. hayesi* infections in 13 out of 20 (65%) resident opossums and 10 out of 13 (77%) newly arriving opossums on the basis of fecal examination for larvae. In that study the authors concluded that infection is common in opossums in northern California, that infections were most likely acquired in the wild rather than at the rehabilitation center, and that oral fenbendazole (50 mg/kg/day × 14 days) eliminated larval shedding in the feces of 11 out of 15 (73%) opossums.[1] On the basis of the findings in this report, however, fenbendazole at 50 mg/kg/day administered for 14 consecutive days did not ensure the survival of or eliminate gastric nematodes (*Physaloptera* sp.) in three animals.

The American opossum is held in collections of zoological parks and is used as a laboratory animal.[7,20] To meet these needs, opossums are occasionally wild-caught.[25] Therefore, zoo personnel, biologists, veterinarians, and others working with these animals should be aware of the prevalence of helminth parasites as well as the spectrum of diseases (including verminous pneumonia) common to opossums in the wild.[2,4,5,12,18,19,21] This report describes clinical and pathologic changes associated with *D. hayesi* infection in American opossums.

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