Journal of Zoo and Wildlife Medicine 26(3): 403–408, 1995

PREVALENCE, ACQUISITION, AND TREATMENT OF *DIDELPHOSTRONGYLUS HAYESI* (NEMATODA: METASTRONGYLOIDEA) INFECTION IN OPOSSUMS (*DIDELPHIS VIRGINIANA*)

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Abstract: Several cases of didelphostrongylosis (lungworm infection) were diagnosed in opossums (*Didelphis virginiana*) at a wildlife rehabilitation center in California. A study was initiated to determine the source and distribution of infection in resident and newly arriving opossums and the efficacy of fenbendazole in treating the infection. Fecal samples were collected before treatment and 1, 2, 3, 4, and 8 wk after the start of a 14-day course of fenbendazole. The Baermann procedure was performed for the detection of lungworm larvae. Lungworm infection was diagnosed in 13 (65%) of 20 resident opossums and 10 (77%) of 13 newly arrived opossums, for an overall pretreatment prevalence of 70%. Four uninfected animals housed with infected animals did not become infected. These observations and others suggested that most infections were acquired in the wild rather than in the rehabilitation center. Fourteen days of oral fenbendazole treatment at 50 mg/kg/day eliminated larval shedding in the feces of 11 (73%) of 15 infected animals from which individually identifiable fecal samples had been collected. Information from this study should be of use to those responsible for the care of captive marsupials.

Key words: Opossum, *Didelphis virginiana,* lungworm, *Didelphostrongylus hayesi,* wildlife rehabilitation.

INTRODUCTION

Several genera of lungworms have been reported from marsupials in Australia,[9] where marsupials are common. In the USA, natural infection of *Didelphis virginiana,* the Virginia opossum, with *Didelphostrongylus hayesi* was first reported in Georgia.[5] Recently, several cases of clinical didelphostrongylosis were diagnosed at the Veterinary Medical Teaching Hospital at the University of California–Davis. Affected animals were from a local opossum care program (OCP). These cases provided the impetus for studying lungworm infection in a large group of captive marsupials. A study was initiated to determine the prevalence of infection in resident and newly arriving opossums, the likely source of infection for the opossums, and the efficacy of fenbendazole in treating the infection.

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MATERIALS AND METHODS

Thirty four live opossums were admitted to an OCP in a residential section of Davis, California, between June 1992 and January 1994. Of the animals included in this study, 15 were males and 19 were females (Table 1). At the time of admission, nine were infants (<3 mo), 16 were juveniles (3–8 mo), and nine were adults (>8 mo). Age estimates were based on morphologic characteristics. Opossums were considered resident if they had been in the OCP longer than 1 mo before the start of the study; otherwise, they were classified as new arrivals. Thirty-three of the animals had been collected in Yolo, Solano, or Sacramento counties in northern California. Opossum habitat in these counties consists primarily of riparian zones, irrigated farmland, and residential areas. One additional animal was from a residential section of Los Angeles County. Opossums were collected when observed to be injured (six), weak (three), or orphaned (15). The remainder (10) were collected from residential areas for reloca-

Table 1. Sex, age at admission, length of time in captivity, and age at the start of the study, for opossums admitted to a wildlife rehabilitation center.

Group no.	Opossum no.	Sex	Age at admission (mo)	Time in captivity (mo)	Age at start (mo)
1	1	M	1	4	5
	2	F	1	4	5
	9	M	5	1.5	6.5
	10	F	3.5	1.5	5
	11	F	3	3.5	6.5
	12	F	4.5	3	7.5
	13	M	2	4	6
	14	F	1	4	5
	15	F	3	3.5	6.5
	16	F	4	2.5	6.5
	19	M	4.5	3	7.5
	26	M	5	1.5	6.5
2	3	F	12+	0	12+
	6	M	9	0	9
	7	M	3	7	10
	8	F	2	8	10
	17	M	2	17	19
	18	F	12+	7	19+
	20	F	2	7	9
	21	M	2	17	19
	22	F	24+	0	24+
	23	M	7	1	8
	24	F	3.5	0	3.5
	25	F	5	0	5
	30	F	2.5	6	8.5
	31	F	7	0	7
	32	M	3	2.5	5.5
	33	M	11	0	11
	34	F	12	0	12
	35	F	5	0	5
	36	F	12	0	12
	37	M	12+	0	12+
	38	M	8	0	8
	39	M	10	0	10

tion to more rural surroundings and appeared healthy. Opossums that were in the OCP at the time the study was initiated and those arriving thereafter were included in the study and were considered representative of the population at large.

Upon admission to the OCP and during the course of the study, opossums were housed either singly or communally. Housing consisted of either wood frame enclosures or standard stainless steel dual cages. Seven wooden enclosures were used, each 1.8 m high and varying in floor space from 1.8 to 4.3 m^2. Siding consisted of hardware cloth screening with 1.3- × 1.3-cm and 1.3- × 2.5-cm openings. Roofs were constructed of plywood and sealed to prevent leakage. Plastic sheeting was attached to the eaves and pulled down during rainy weather. Flooring consisted of bare ground with 5–8 cm of straw covering. Enclosures were raked daily to remove feces, wet straw, and spilled food. Plywood nest boxes with straw bedding were provided for sleeping quarters, and climbing branches were available for exercise. Two sets of stainless steel cages were located outdoors and one was located in a garage. The outdoor steel cages were lined with dry newspapers and straw, and cardboard boxes containing dry straw were provided. Indoor steel cages were lined with newspapers only, and sleeping boxes containing clean toweling were offered.

Dry cat or dog chow was provided ad libitum to all animals. This diet was supplemented with a variety of cooked meats, fruits, and vegetables to an approximate proportion of 10% of the total diet. Water was always available, and water bowls were cleaned daily. Animals were examined weekly for fleas and sprayed with pyrethrin flea spray as needed. Disturbances were kept to a minimum.

Opossums were assigned to one of two groups based on whether the identity of animals producing individually collected fecal samples was known. Group 1 included 12 opossums housed communally in two subgroups of six opossums each. A similar number of fecal samples were collected without knowledge of which animals produced specific samples. Animals in Group 1 were separated for collection of fecal samples at 8 wk. Group 2 included 22 animals housed either singly (10) or communally (12). The identity of animals in Group 2 producing collected fecal samples was known and recorded. Approximately 5–10 g of feces were collected before anthelmintic treatment and at 1, 2, 3, 4, and 8 wk after start of treatment. Feces were refrigerated and

examined within 1 wk of collection. A Baermann procedure for the detection of lungworm larvae was performed, using the entire collected sample and allowing for overnight soaking in tap water.[8] Sediment aspirated from the bottom of the collection vessel was placed on a glass slide with a 22-mm^2 coverslip. The area under the entire coverslip was examined at 40× magnification for the presence of lungworm larvae. Larval burden was estimated for the entire area under the coverslip as + = 1–100 larvae, ++ = 101–300 larvae, +++ = 301–600 larvae, and ++++ = >600 larvae.

Following the pretreatment fecal examination, all opossums in group 1 and all infected opossums in Group 2 were treated orally with 50 mg/kg fenbendazole (Safe-Guard, Hoechst-Roussel Pharmaceuticals, Somerville, New Jersey 08876, USA) s.i.d. for 14 days. Weights of animals in group 1 were estimated visually. Animals in group 2 were weighed. Animals from group 1 were weighed later, and comparison of actual and estimated weights revealed a high level of accuracy for previous visual estimations. Dosages of fenbendazole were increased in proportion to weight gain as necessary. To prevent potentially deleterious inflammation associated with the death of lungworms, all animals receiving fenbendazole were also treated orally for 14 days with aspirin (Bayer Children's Aspirin, Bayer Co., New York, New York 10016, USA) at 20 mg for animals <2.5 kg and 40 mg for those >2.5 kg. Seven days after start of fenbendazole treatment, nine opossums in group 2 were also treated orally with trimethoprim-sulfa (Sulfatrim Pediatric Suspension, Barre-National, Baltimore, Maryland 21244, USA) at 0.62 ml/kg/day for 14 days to guard against possible secondary bacterial infections following the death of lungworms.

RESULTS

Results of the Baermann fecal examinations are presented in Table 2. Of the 11 pretreatment fecal samples collected from animals in group 1, seven (64%) contained lungworm larvae. At 1, 2, 3, and 4 wk after initiation of treatment, 2/6 (33%), 2/12 (17%), 4/11 (36%), and 2/12 (17%) samples, respectively, contained lungworm larvae. However, at 8 wk, fecal samples from 11 animals remaining in this group were free of lungworm larvae. One animal (no. 10) in group 1 died acutely before completion of the study.

Of the 22 opossums in group 2, 16 pretreatment samples (73%) contained lungworm larvae. Thereafter, the percentage of animals shedding larvae declined until at 8 wk after initiation of treatment only 4/19 (21%) of the animals remaining in group 2 were still shedding lungworm larvae. Before completion of the study, one of these animals (no. 20) was released and two animals (nos. 21, 23) died of verminous pneumonia, which was confirmed at necropsy. Of the 15 infected opossums from group 2 that completed the study, lungworm larvae were eventually cleared from the feces of 11 (73%) of these animals.

DISCUSSION

In the USA, natural infection of the Virginia opossum with *Didelphostrongylus hayesi* was first reported in Georgia[4–6] and later in Louisiana[1] and Tennessee.[3] The development of *D. hayesi* is indirect. In an experimental completion of the life cycle, first-stage larvae released in the feces were infective to the terrestrial snails *Mesodon perigraptus* and *Triodopsis albolabris*. Third-stage larvae recovered at least 3 wk later from the snails were fed to parasite-free opossums. The prepatent period to first-stage larval shedding was 22 days.[5] It was important in the present study to confirm that *D. hayesi* was not directly transmitted, because direct transmission within the OCP facility could compromise efforts at rehabilitation.

Findings in the present study suggest that lungworm infections in these opossums were primarily acquired in the wild rather than in the OCP. Eight of the opossums in group

Table 2. Fecal larval lungworm burden[a] from opossums following 14 days of oral fenbendazole treatment (50 mg/kg/day).

Group no.	Opossum no.	Pretreatment	Weeks after start of treatment				
			1	2	3	4	8
1	all[b]	7/11 (64%)	2/6 (33%)	2/12 (17%)	4/11 (36%)	2/12 (17%)	0/11 (0%)
2	3	−	++	+	+	−	−
	6	+	+	−	−	−	−
	7	+	−	+	+	−	+
	8	−	−	−	−	−	−
	17	+	−	−	−	+++	−
	18	+	ND	−	−	−	−
	20	−	Released				
	21	+	ND	+	Died		
	22	+++	++++	++	+	+	++
	23	+	ND	+	−	+	Died
	24	−	ND	−	−	−	−
	25	++++	++	+	+	+	+
	30	−	−	−	ND	−	−
	31	+++	+	+	+	+	+
	32	+	+	+	−	+	−
	33	+++	+++	+	+	−	−
	34	++	+	+	−	−	−
	35	−	ND	−	−	−	−
	36	++	+	−	−	−	−
	37	++	+	+	−	−	−
	38	+	+	+	−	−	−
	39	+++	++	−	−	−	−
No. infected/no. examined		16/22	12/16	12/21	6/19	6/20	4/19
% infected		73%	75%	57%	32%	30%	21%

[a] − = negative; + = 1–100 larvae; ++ = 101–300 larvae; +++ = 301–600 larvae; ++++ = >600 larvae. ND = not determined; R = released; D = died.

[b] Group 1 included animal nos. 1, 2, 9–16, 19, 26. Results for this group are number of fecal samples positive for lungworm larvae/number examined (% positive).

1 were at least 3 mo of age when admitted to the OCP, and four were <3 mo of age. Three months is the approximate age at which juvenile opossums become independent.[7] The maximum number of fecal samples from this group that contained lungworm larvae at any one time during the study was seven. The four animals <3 mo of age at admission probably never became infected. Of the infected animals in group 2, only two (nos. 17, 21) appeared to be <3 mo of age at admission to the OCP. These two were brothers and had been in the OCP 17 months before start of the study. They may have become infected while in the OCP; unlike other animals, both were let out into the yard area three to four times each week and were frequently observed eating snails. We were unable to recover lungworm larvae from a small number of snails collected at the OCP facility (data not shown). However the possibility remains that infection might have been infrequently acquired in the OCP through ingestion of infected snails.

Four animals (nos. 8, 24, 30, 35) that were uninfected at the pretreatment fecal examination did not appear to become infected during the course of the study, although all were housed at some time with infected animals. Also, one opossum (no. 3) tested 3 days after arrival was apparently uninfected initially but subsequently excreted lung-

worm larvae and so was treated. The initial fecal exam for this animal probably was performed during the prepatent period of the parasite. In one animal, larval shedding had ceased by 4 wk after start of treatment, but larvae were subsequently observed at the 8-wk fecal exam. This animal was housed with two uninfected animals throughout the course of the study, so the positive sample at 8 wk was likely due to resumption of egg production, a phenomenon previously reported with benzimidazole usage.[2] Therefore, reinfection did not occur in any of the opossums, even though until the fourth week of the study most of these animals were housed with at least one animal that was shedding larvae. Had reinfection occurred, it would have been revealed at the 8-wk exam.

Several patterns of larval shedding followed fenbendazole treatment. For animals in group 1, there was an initial decrease in larval shedding, probably because of temporary sterilization of female worms,[2] followed by a transient resumption of larval release at 3–4 wk. However, by 8 wk no larvae were detected in the 11 remaining opossums.

In group 2, most infections were no longer evident 2–4 wk following start of treatment. In a few animals (e.g., nos. 7, 17), larval shedding was eliminated but resumed at a later sampling date. Larvae were eventually eliminated from opossum no. 17 but not from opossum no. 7. This situation illustrates the importance of follow-up examinations 6–8 wk following treatment with benzimidazoles.[2] In addition to animal no. 7, in three additional animals (nos. 22, 25, and 31) infections remained evident 8 wk after start of treatment, although the amount of larval shedding from these animals was greatly reduced. Fenbendazole given orally at 50 mg/kg/day for 14 days was 73% effective in eliminating larval shedding for 14 animals of group 2. Because necropsies were not performed, we cannot be positive that all infections were cleared. An improvement in efficacy might be realized by extending treatment to 21 or 28 days. Alternatively, a higher dose (e.g., 100 mg/kg) given for 14 days may be more effective. However, because three animals died following treatment at 50 mg/kg/day, presumably as a result of lungworm death, higher dosages of fenbendazole should be used with caution.

Although no infected animals were left untreated as controls, this omission in no way compromised the value of the study. This was a field study that arose from what was perceived as an acute need and was not primarily a drug trial. Because of recent mortality and because we had not at the time ruled out direct transmission of infection it was deemed imprudent to withhold treatment from animals known to be infected. However, given the low likelihood of transmission within the OCP and the length of time some infected animals had remained at the OCP, it is highly unlikely that elimination of larval shedding was due to anything other than the fenbendazole treatment.

The results of this study indicate that a high percentage of feral opossums may be infected with *D. hayesi,* that most infections were acquired in the wild, that direct transmission of the parasite in the OCP was uncommon if it occurred at all, and that fenbendazole given orally at 50 mg/kg/day for 14 days eliminated larval shedding in the feces of most animals. Future studies should seek to refine the fenbendazole treatment regimen and/or evaluate additional anthelmintics.

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Received for publication 1 September 1994