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PULMONARY LESIONS CAUSED BY THE LUNGWORM (*DIDELPHOSTRONGYLUS HAYESI*) IN THE OPOSSUM (*DIDELPHIS VIRGINIANA*) IN COLIMA, MEXICO

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Abstract: *Didelphostrongylus hayesi* is an important and prevalent pulmonary nematode in the opossum (*Didelphis virginiana*). An in-depth description of the pulmonary lesions caused by this nematode is lacking. The objective of this investigation was to make a detailed account of the gross, subgross, and microscopic changes that occur in the lungs of opossums naturally infected with *D. hayesi*. Forty-four opossums trapped in the state of Colima, Mexico, were euthanized by an overdose of barbiturates. Following a postmortem examination, the right lung was cut from the main bronchi and placed in a Petri dish containing a saline solution for the detection and identification of live parasites. The left lung was fixed and cut serially for subgross microscopic examination and sections of lung were cut and stained for histopathologic examination. The most remarkable gross change in parasitized lungs was a poorly collapsible pulmonary parenchyma and mild emphysema. The right lung tested positive for lungworms on gross examination in 20/44, and 11/44 (25%) of the left lungs showed tan nodules on the pleural surface. Microscopically, the bronchi of 20/44 animals harbored adult and larval stages of *D. hayesi* (left lung), the same 20 opossums from which nematodes were grossly evident at necropsy (right lung). Adults and larvae were present in bronchi, bronchioles, and alveoli mixed with desquamated cells and many eosinophils, and to a lesser extent neutrophils, alveolar macrophages, and giant cells. Bronchi and bronchioles exhibited goblet cell hyperplasia and metaplasia respectively, and infiltration of lymphoplasmacytic cells in the interstitium and lamina propria. The tan nodules consisted of focal alveolar endogenous lipidosis, which likely resulted from parasitic airway obstruction. The lungs of 3/20 parasitized opossums also showed alveolar bronchiolization (Lambertosis). The absence of *Eucoleus aerophilus* or bacterial pneumonia incriminates *D. hayesi* as the putative cause of pulmonary lesions in these opossums.

Key words: Alveolar metaplasia, *Didelphis virginiana*, *Didelphostrongylus hayesi*, lipid pneumonia, lungworms, opossum

INTRODUCTION

Didelphostrongylus hayesi, a nematode of the order Strongylida, superfamily Metastrongyloidea, is a lung parasite of the opossum (*Didelphis virginiana*) in North, Central and South America.[1,2,4,16,19] The prevalence of *D. hayesi* in the opossum population ranges from 48 to 79% and from 4.5 to 14% for the United States and Mexico respectively.[4,18,19,23] *D. hayesi* has an indirect life cycle requiring terrestrial snails such as *Mesodon perigraptus* or *Triodopsis albolabris* as an intermediate host.[25] Although it is well known that the opossum becomes infected after ingesting the intermediate host, the precise larval migratory pathway from the digestive tract to the lung is incompletely understood.[25] Once in the lung, third-stage larvae mature into the adult stage in the pulmonary airways, particularly intrapulmonary bronchi.[2,16] There have been some reports describing the pulmonary lesions associated with *D. hayesi* infection, but most of these reports were made from a casual lung sampling or with opossums having parasitic or bacterial comorbidities of the lung.[10,16,30] However, an in-depth description of the morphologic changes occurring in the lung of opossums naturally infected with only *D. hayesi* is lacking. The objective of this investigation is to make a detailed description of the gross, subgross, and microscopic changes that

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occur in the lungs of opossums naturally infected with *D. hayesi*.

MATERIALS AND METHODS

Forty-four opossums were trapped in the State of Colima, Mexico (19° 14′ 36″N; 103° 43′ 30″W), in accordance with the official norms for animal care (NOM-033-ZOO-1995), transportation of live animals (NOM-051-ZOO1995), and wildlife protection (NOM-059-SEMARNAT-2010) established by the government of Mexico. Opossums were trapped at night between 2300 and 0200 hours using Tomahawk live traps (12 × 24 × 42 inches) and canned sardines as bait. Those opossums that were found pregnant either visually or by palpation, lactating, or weighing less than 250 g were excluded from this study and immediately set free. After recording of the date and trap geographic location, live opossums were individually transported in cages to the Diagnostic Laboratory of the Faculty of Veterinary Medicine, University of Colima. Animals were euthanized with an intraperitoneal overdose of barbiturates (5 ml/1.0 kg body weight) (Pisabental® pentobarbital sodium 6.3%, Pisa, SAGARPA, Q-7833-215, Guadalajara, Jalisco, Mexico). The carcasses were weighed, measured from the nose to the tip of the tail, and necropsied following routine procedures. Weight and length values were expressed as mean ± SD. At necropsy, the lungs were removed in toto from the thoracic cavity, placed on a dissecting board, grossly examined, and photographed. The right lung was cut from the main bronchus and placed in a Petri dish containing 0.65% saline solution for 5 min. The fluid was examined with a dissecting microscope (Olympus SZ2-ILST®, Hicksville, NY 11801 USA) for the presence of live parasites, which were then removed using a dissecting probe and placed in a warm solution of 70% ethyl alcohol for further microscopic examination. For identification and preservation, nematodes were cleared in glycerin–lactophenol and their morphologic features recorded and photographed. Lastly, nematodes were submitted for archiving to the Helminth Repository of the National University of Mexico (UNAM; catalog # CNH-7547).

The left lung was detached by cutting the main bronchus and immersed in 10% buffered formalin for 48 hr. After fixation, transverse sections of the left lung were cut serially from cranial to caudal lobes, and each slide was examined for parasites. The subgross pulmonary changes were investigated using a dissecting microscope, and hereafter are referred to as subgross lesions. Finally, lung sections were processed, embedded in paraffin, cut at 6 μm, and stained with hematoxylin-eosin and periodic acid–Schiff following standard techniques.[27]

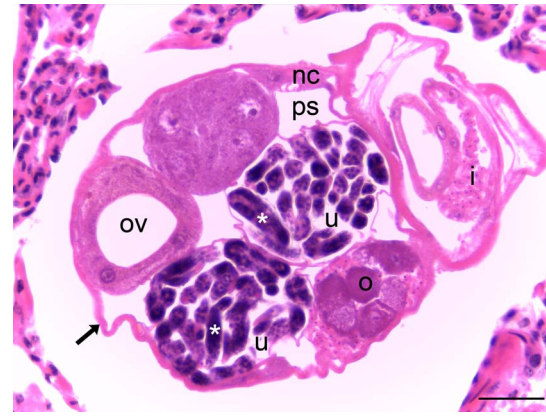

Figure 1. Cross section of a female *D. hayesi* showing a thin cuticle with no ornamentation (arrow), intestine (i), pseudocele (ps), nerve cord (nc), ovaries (o), oviduct (ov), and the uterus (u) with many parasitic larvae (asterisks). Hematoxylin-eosin stain. Bar 50 μm.

RESULTS

Opossums and lung parasites

From a total of 44 trapped opossums, 28 (64%) and 16 (36%) were male and female respectively. The average weight was 1.63 ± 0.44 kg (range 0.25–2.55 kg), and the average nose–tail length was 75.14 ± 10.23 cm (range 50–90 cm). The left lung of 20/44 captured opossums (45.45%) was positive for lungworms on necropsy examination; the frequency of lungworm infection was similar in male (13/28; 46.42%) and female (7/16; 43.75%) opossums.

The lungworms were identified as *D. hayesi*. Female *D. hayesi* had a mean length of 16.53 mm and an average width of 0.31 mm, and male nematodes were 7.20 ± 0.99 mm in length and 0.19 ± 0.05 mm in width. According to sex, 55.20% and 44.80% of the nematodes were female and male respectively. No other types of nematodes were detected in these lungs.

On tissue section, *D. hayesi* exhibited a smooth, thin, nonornamented cuticle (3 μm) with underlying hypodermis containing few nuclei and forming lateral cords. The body musculature was closely associated with the hypodermis and projected into the body cavity (pseudocoelom) in a platymyarian arrangement. The pseudocoelom of female nematodes enclosed the esophagus, intestine, ovaries, and oviduct (Fig. 1). Because *D.*

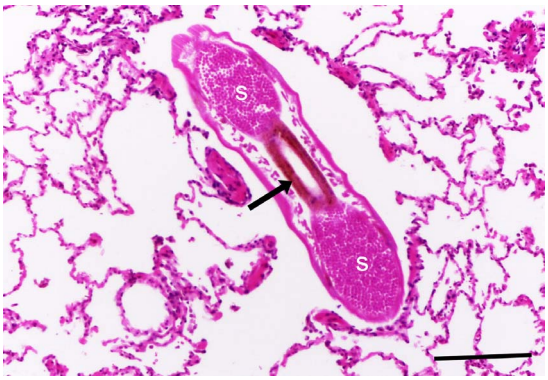

Figure 2. Longitudinal section of a male *D. hayesi* showing the intestine (arrow) and testis with sperm in the sperm duct (s). H&E stain. Bar 200 μm.

hayesi is ovoviviparous, the uterus contained many larvae (90 μm in length and 10 μm in width) (Fig. 1). The male pseudocoelom included testis and sperm ducts that were filled with sperm (Fig. 2). The oral aperture connected with a uniform muscular esophagus (20 μm in diameter). Contractile fibers were visible in the body musculature as well as in the esophagus, intestine, uterus, and oviduct.

Gross findings

The most apparent gross finding in parasitized opossums was the inadequate collapse of the pulmonary parenchyma after opening the thorax in 20/44 (45%). The margins of the pulmonary lobes had focal to coalescing areas of emphysema (Fig. 3a). There were also well-delineated, multifocal (0.5–3 cm), tan, raised plaques in the dorsocaudal pleura in 11/44 (25%) of the opossums (Fig. 3a, b). These nodules occurred individually or clustered in groups that typically followed the branching of intrapulmonary airways. There was subtle focal emphysema in the caudal lung lobes in 20/44 (45%) associated with serpiginous tracks containing adult *D. hayesi* (Fig. 3c). Formalin fixation notably enhanced the gross appearance of pulmonary lesions, particularly of the pleural tan nodules (Fig. 3d).

Subgross findings

On cut surface, the tan nodules were restricted to the subpleural region and did not extend deep into the pulmonary parenchyma (Fig. 3e). Under the dissecting microscope, parasitic tracks containing adult *D. hayesi* were further evident underneath the visceral pleura, particularly on the caudal lung lobes (Fig. 3c inset). Another remarkable change detected under the dissecting microscope consisted of small yellow plaques (0.3–0.8 mm) that had been missed by the naked eye on the pleural surface. Transverse sections of the fixed lung parenchyma also revealed that the walls of some bronchi were distended and showed uneven contour (Fig. 3f)

Histopathologic findings

Histopathologic examination of the left lungs revealed a wide variety of changes involving bronchi, bronchioles, and alveoli (Table 1). Microscopically, the intrapulmonary bronchi of 20/44 opossums contained adult and larval stages of *D. hayesi* (Figs. 1, 3g). These 20 animals were the same from which nematodes had been collected from the right lung at the time of necropsy. Adult nematodes and larvae were surrounded by mucus admixed with desquamated cells and inflammatory cells, largely eosinophils and to a lesser extent neutrophils and macrophages (Fig. 3h). The bronchial mucosa exhibited goblet cell hyperplasia, and the lamina propria contained conspicuous infiltrates of eosinophils and few lymphocytes and plasma cells (Fig. 3h). In the most severely parasitized lungs, the bronchi also exhibited distention of the wall and peribronchial fibrosis, changes that were microscopically consistent with bronchiectasis as grossly shown in Fig. 3f. Some bronchial glands were moderately enlarged. Hyperplasia of the bronchial-associated lymphoid tissue was a consistent microscopic finding in the lungs of all parasitized opossums (100%) (Fig. 3h).

The bronchiolar lumens of all (20/20) parasitized opossums also contained adult *D. hayesi,* and in some bronchioles, the entire lumen was occluded by nematodes surrounded by numerous eosinophils (Fig. 3i). The bronchiolar mucosa in many cases exhibited mild to moderate goblet cell metaplasia with eosinophilic and lymphoplasmacytic infiltrates in the lamina propria (Table 1). The peribronchiolar interstitial tissue was thickened because of collagen deposition, and in some cases, the peribronchiolar interstitium also showed densely packed aggregates of lymphocytes.

Some alveoli also contained adult *D. hayesi* but to a much lesser extent than bronchi and bronchioles (Fig. 3j). Alveoli often included clusters of parasitic larvae surrounded by many eosinophils and few macrophages and giant cells (Fig. 3k, l; Table 1). The alveolar interstitium had sporadic micronodules composed almost exclusively of lymphocytes.

The large and small tan nodules grossly and subgrossly observed in the lungs were microscopically composed of aggregates of macrophages positioned primarily in the terminal bronchiolar and proximal alveolar regions (Fig. 3m). These macrophages were notably vacuolated (lipid-laden) and were associated with minimal inflammatory changes in the surrounding tissue, a feature indicative of pulmonary alveolar lipidosis (Table 1).

The pulmonary vasculature in 10/20 parasitized opossums (50%) exhibited moderate to severe smooth muscle hypertrophy and perivascular fibrosis (Fig. 1n; Table 1). In the more severely affected cases, the arterial lumens were virtually occluded by these proliferative changes.

Finally, the lungs of 3 of 20 parasitized opossums (15%) showed conspicuous aggregates of bronchiolar cells lining the alveolar surface, a lesion that was interpreted as alveolar bronchiolization (Fig. 3o). None of the parasitized opossums showed mineralization in the lungs.

DISCUSSION

Morphologically, the lungworms recovered from the opossums captured in Colima, Mexico, were similar to those reported for *D. hayesi* in the United States, Canada, and South America.[2,18,25] The 45% prevalence of *D. hayesi* in opossums in Colima was lower than the 74% first reported in California,[19] but closer to the 55% subsequently reported in the same state.[16] It should be noted that these percentages were based on the microscopic detection of adult *D. hayesi* in the lung, and not on the detection of eggs or larvae in feces as commonly reported by other investigators.[4,23] Male and female opossums appeared to be equally affected, suggesting that there may not be sex predilection for *D. hayesi* infection as sporadically happens with some other parasites.[34]

The most noticeable change visible at the time of necropsy was the lack of pulmonary collapse following the opening of the thorax. This lack of pulmonary recoil could have resulted from two distinct mechanisms: first, from the airway obstruction caused by the presence of *D. hayesi* in the lumen of the airway, or second, from the interstitial thickening of the airways arising from inflammation and fibrosis.[6,17] Adult forms of *D. hayesi* were visible by the naked eye on the visceral pleura, but this was a subtle finding that could be missed by the untrained eye. For this reason, it is recommended to use a dissecting microscope for routine examination of opossum lungs. The emphysematous lesions at the margins of the pulmonary lobes were likely a consequence of parasitic airway obstruction, a change that is especially evident in the caudal lobes where nematodes were more abundant.[6,17,22]

As occurs in some, but not all, types of verminous pneumonia of domestic and wild animals,[17,22] the adult stage of *D. hayesi* populated both the bronchi and bronchioles and to a much lesser extent the alveoli. A similar distribution was reported in the lungs of foxes infected with *Crenosoma vulpis*.[21,22] It's hard to ascertain if the bronchial hyperplastic changes, particularly goblet cell hyperplasia, were the result of the direct contact between the mucosa and *D. hayesi*, or resulted indirectly from the release of inflammatory mediators in response to the antigens and secretory products secreted by the nematode.[24,31]

The presence of goblet cells in bronchioles was a real metaplastic lesion because the normal bronchioles of the opossum, like all domestic mammals, lack this mucus-producing cell in the bronchiolar mucosa.[8] Bronchiolar goblet cell metaplasia occurs in chronic bronchiolitis and may result in mucus plugs and small-airway obstruction.[6,17,32] Bronchiolitis obliterans, another common lesion that follows chronic bronchiolitis, was absent in the opossums harboring *D. hayesi*. Parasitized opossums also exhibited bronchial gland hyperplasia, which microscopically translated into a high glandular to wall thickness ratio, known as the Reid index.[17,22] Increased Reid index occurs during prolonged mucosal irritation such as allergy, inflammation, and parasitic bronchitis.[9] Further studies should quantify the severity of bronchial gland hyperplasia by calculating the Reid index in opossums infected with *D. hayesi*. The lungs that were harboring *D. hayesi* also showed bronchointerstitial fibrosis, which is a nonspecific lesion that reflects chronic inflammation in various forms of bronchitis, bronchiolitis, and pneumonia, whether instigated by allergy, infection, or parasitism.[6,13]

The infiltrates of eosinophils and alveolar macrophages with the occasional giant cell were classic for the alveolar inflammatory response provoked by parasitic larvae and parasitic cuticles.[15,16,24] Two other important microscopic lesions related to the presence of larvae and inflammatory cells in alveoli were hyperplasia of type II pneumocyte and alveolar bronchiolization. Type II hyperplasia is a common finding in alveolar inflammation when there is an underlying necrosis of type I pneumocytes, which subsequently, as part of the alveolar repair, are replaced by rapidly dividing type II cells.[6,9,17] The mecha-

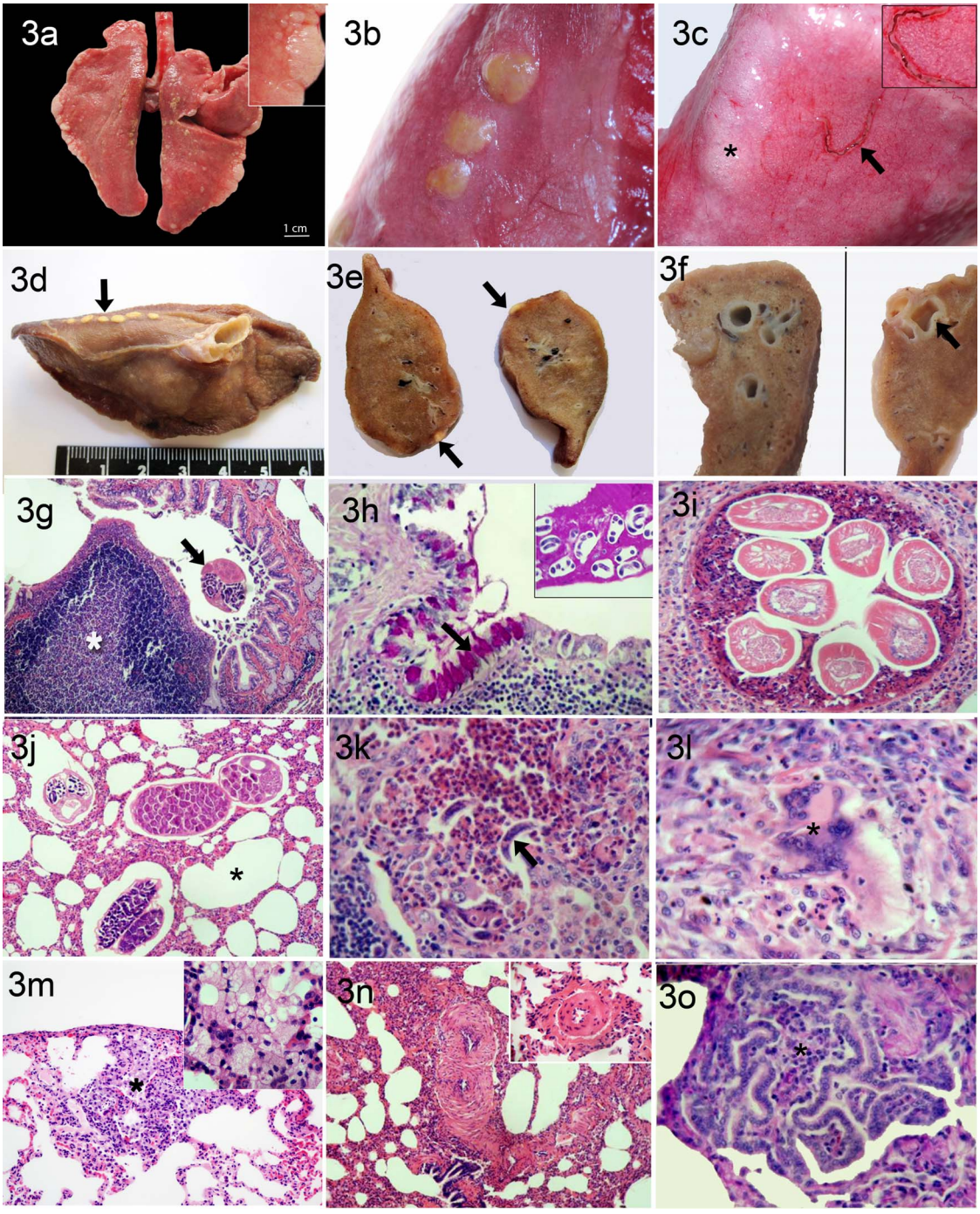

Figure 3. **a.** Fresh lungs showing focal to tan nodules and locally extensive area of emphysema along the border of the pulmonary lobes (arrow). Inset: Close-up of the emphysema. **b.** Fresh lung. Three distinct tan nodules on the pleural surface of a caudal lung lobe. The surrounding pulmonary parenchyma is unremarkable. **c.** Fresh lung. A focal area of emphysema (asterisk) closely associated with a serpiginous parasitic tract (arrow). Inset: View of *D. hayesi* under a dissecting microscope. **d.** Fixed lung. Row of several subpleural tan (lipoic) nodules (arrow). **e.** Fixed lung; cut surface. Well-demarcated lipoic nodules (arrows) on the pleural and subpleural tissue. The surrounding pulmonary parenchyma is unremarkable. **f.** Fixed lung; cut surface. Left side: normal intrapulmonary bronchus; right side: distended bronchus with irregular borders (arrow). **g.** Histologic section of

Table 1. Distribution of microscopic lesions in the lung of opossums (*D. virginiana*) infected with *D. hayesi*.

Microscopic finding	Anatomic region of the lung			
	Bronchus	Bronchiole	Alveoli	Blood vessels
Adult nematodes	+	+	+	−
Larvae	+	+	+	−
Lymphoid hyperplasia	+	+	−	+
Glandular hyperplasia	+	−	−	−
Goblet cell hyperplasia	+	−	−	−
Goblet cell metaplasia	−	+	−	−
Smooth muscle hyperplasia[a]	−	−	−	+
Fibrosis	+	+	+	+
Epithelial degeneration	+	+	−	−
Eosinophils	+	+	+	−
Macrophages/giant cells	+	+	+	−

[a] Hyperplasia and hypertrophy.

nism by which nematodes induce necrosis of type I cells is incompletely understood. Mechanistically, however, it could be the result of direct cell injury caused by the larvae or their secretory products, or of indirect injury triggered by the enzymes and free radicals released by the infiltrating eosinophils.[3,15,24] In contrast to *Capillaria didelphis* infection, the lungs of opossums infected with *D. hayesi* did not have pulmonary necrosis or mineralization.[30]

Alveolar bronchiolization was another type of alveolar lesion found in the opossums that consisted of clusters of bronchiolar cells lining the alveolar basement membrane. This nonspecific microscopic change is also known in human pathology as peribronchiolar metaplasia or lambertosis.[9,32] Lambertosis implies an erratic remodeling in which bronchiolar cells migrate and populate the alveoli following alveolar injury and fibrosis.[9,17] This unusual microscopic lesion is often mistaken for pulmonary adenoma, an attention-grabbing observation since "extensive adenomatoid proliferation of alveolar epithelium" was reported many years ago in opossums with verminous pneumonia.[5,26] In all likelihood, opossums are prone to develop alveolar bronchiolization after pulmonary injury, not necessarily adenomas as suggested by other investigators.[29,33]

Lungworms frequently cause hypertrophy and hyperplasia of the pulmonary vasculature, but these changes are mainly microscopic and rarely result in gross pulmonary abnormalities. Arterial muscular changes often seen in verminous pneumonia are the consequence of vasoactive substances released locally in the lung.[6,9] The clinical significance of muscular hypertrophy and hyper-

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intrapulmonary bronchus. Bronchial lumen contains a cross section of an adult *D. hayesi* admixed with cell debris (arrow). Also, note severe hyperplasia of the bronchial associated lymphoid tissue (asterisk). Hematoxylin-eosin (H&E) stain, ×100. **h.** Histologic section of intrapulmonary bronchus. Bronchial mucosa is showing goblet cell hyperplasia (arrow) and lymphoplasmacytic aggregates in the submucosa. Periodic acid–Schiff stain, ×40. Inset: Periodic acid–Schiff–positive mucus contains numerous *D. hayesi* larvae. Periodic acid–Schiff stain, ×400. **i.** Histologic section of pulmonary bronchiole. Cross section of several *D. hayesi* surrounded by abundant eosinophilic exudate. Note that the bronchiolar lumen is completely blocked and the pale appearance of the nematodes suggests that these parasites are dead. H&E stain, ×400. **j.** Histologic section of lung. Adult *D. hayesi* in alveoli. There is increased cellularity in the alveolar interstitium causing thickening of the alveolar walls. Also, some alveoli appear notably distended and possibly emphysematous (asterisk). H&E stain, ×400. **k.** Histologic section of lung. Parasitic larvae in alveoli (arrow) associated with an intense infiltration of eosinophils and macrophages. H&E stain, ×400. **l.** Histologic section of lung. Multinucleated giant cell (asterisk). H&E stain, ×400. **m.** Histologic section of lung. Focus of alveolar histiocytosis (macrophages) in the subpleural region (asterisk). H&E stain, ×100. Inset: Close-up of alveolar macrophages showing vacuolated cytoplasm. H&E stain, ×400. **n.** Histologic section of pulmonary vasculature. Note marked thickening of the smooth muscle in a medium size pulmonary artery. H&E stain, ×100. Inset: Similar change is affecting a small pulmonary artery. H&E stain, ×100. **o.** Histologic section of pulmonary alveoli. Focus of alveolar metaplasia showing aggregates of bronchiolar cuboidal cells lining the alveolar wall. Some eosinophils are visible in these alveolar lumen (asterisk). H&E stain, ×400.

plasia in the parasitized lung has been put into question, particularly in cats harboring *Aelurostrongylus abstrusus*.[6,17]

All the opossums, even those exhibiting scores of lungworms in the bronchi, appeared clinically healthy and in good body condition. There was no emaciation at necropsy in any of the opossums, suggesting that *D. hayesi* has little or no effect on the general health status of this marsupial. This notion is also supported by experimental studies in which opossums infected with *D. hayesi* did not show significant weight loss.[26] On the other hand, some researchers have indeed implicated *D. hayesi* with emaciation and clinical signs.[10,16] However, these assumptions should be taken with caution because many of these opossums also had proven parasitic coinfection and other comorbidities.[11,16,20]

It is challenging to discriminate parasitic lesions in animals with dual parasitic infections or comorbidities,[7,11,23] a fact that could lead to incorrect diagnostic interpretations. For instance, *D. hayesi* was once reported as the cause of a fatal lungworm infection in an opossum, yet on necropsy, the lungs of this animal exhibited cranioventral consolidation.[10] This type of lesion and distribution is inconsistent with verminous pneumonia but rather indicative of bacterial pneumonia.[6,17] Not surprisingly, *D. hayesi* has been incriminated in predisposing the lung to secondary bacterial pneumonia,[16,30] yet none of the 20 parasitized opossums in the study reported here had gross or microscopic evidence of a superimposed bacterial infection.

Dual parasitic infections also make the interpretations of lung lesions problematic.[5,11,20] For instances, *D. hayesi* and *Eucoleus aerophilus* (formerly *Capillaria aerophilus*) commonly occur together in opossums of the United States,[11,30] making it difficult to distinguish which parasitic lesions belongs to each parasite. None of the opossums captured in Colima, Mexico, had microscopic evidence of *E. aerophilus* or any other parasite that could make problematic the interpretation of microscopic lesions. Thus, the absence of lung capillariasis in this study directly incriminates *D. hayesi* as the primary cause of lung lesions in the opossums. As a corollary, the severe granulomatous reaction reported in opossums with mixed verminous pneumonia can likely be attributed to *E. aerophilus* and to a much lesser extent to *D. hayesi*.[26]

Lipid pneumonia was another lesion grossly and microscopically observed in opossums infected with *D. hayesi*. The relationship between *D. hayesi* and lipid plaques was reported previously in opossums and other animals infected with lungworms.[5,12,28] The underlying pathogenesis of endogenous lipid pneumonia implies an imbalance between the synthesis of surfactant lipids and lipid clearance in the lung.[9,17] Based on this notion, there are three plausible mechanisms by which *D. hayesi* could cause lipid pneumonia: 1) Excessive lipid production in the lung arising from proliferation (hyperplasia) of type II pneumocyte, the chief lipid-producing cell in the alveoli. 2) Reduced lipid clearance resulting from mechanical obstruction of the airways by *D. hayesi*. 3) A combination of the two above. Based on the relatively mild hyperplasia of type II pneumocyte in the lungs of the opossums, the lipid accumulation was most probably the result of airway obstruction caused by the lungworms. Obstructive airway disease is one of the putative causes of endogenous lipid pneumonia in dogs and cats.[14,28]

In conclusion, *D. hayesi* is a highly prevalent parasite in the opossum population of Colima, Mexico. Fixation enhances gross pulmonary lesions, and the poorly collapsible lung most likely results from interstitial inflammation, airway obstruction, and emphysema. Gross observation of the pleura and airways is useful in detecting adult lungworms, but proper microscopic identification of *D. hayesi* is necessary. The chief microscopic lesions consist of bronchointerstitial eosinophilic pneumonia and goblet cell hyperplasia and metaplasia of the bronchus and bronchiole respectively. Bronchial gland hyperplasia and vascular smooth muscle hypertrophy and hyperplasia are also frequently observed in parasitized lungs. Larvae and dead nematodes result in giant cell granulomatous inflammation. Endogenous lipid pneumonia and alveolar lambertosis are rare but noteworthy findings in parasitized opossums. Finally, *D. hayesi* appears well adapted to the host without causing visible clinical signs, debilitating disease, secondary bacterial pneumonia, or diffuse lung injury.

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