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## ***Paragonimus kellicotti* INFECTION IN WILD CARNIVORES IN SOUTHWESTERN ONTARIO: II. HISTOPATHOLOGIC FEATURES\***

JAUL J. A. PRESIDENTE\*\* and ROBERT O. RAMSDEN

**Abstract:** Pulmonary lesions associated with naturally acquired *Paragonimus kellicotti* infection were studied in mink (*Mustela vison*), striped skunks (*Mephitis mephitis*), red foxes (*Vulpes vulpes*) and a coyote (*Canis latrans*). In mink a fibrous capsule was formed around mature flukes in dilated bronchioles or bronchi, and there was mild focal interstitial pneumonitis adjacent to fluke eggs in alveoli and migrating parasites. A thick wall, infiltrated heavily with mononuclear cells and eosinophils, surrounded mature *P. kellicotti* in skunks and an extensive inflammatory reaction was found around fluke eggs. In red foxes the wall was thin, hemorrhagic and contained little collagen; necrosis was associated with migrating parasites. Thick capsules formed by marked fibroplastic changes in the lamina propria of affected bronchi were found in the coyote.

In raccoons with pleural adhesions massive fibroplasia with eosinophil infiltration beneath the pulmonary pleura suggested a reaction to a migrating parasite entering the lung from the thoracic cavity. It was thought that immature *P. kellicotti* may have caused the lesion.

### **INTRODUCTION**

Although a number of wild and domestic mammals may harbor the lung fluke *Paragonimus kellicotti* Ward 1908,<sup>1</sup> the histopathologic changes in the lung have been mainly described in infected cats and dogs.<sup>2-5,7,10,15</sup> Gross lesions associated with this infection in mink have been reported.<sup>1,9,14</sup> In Michigan, *P. kellicotti* was thought to be pathogenic in red foxes, but histopathologic features were not described.<sup>13</sup> Prevalence, intensity of infection and size of flukes recovered from wild mammals in Georgia were used as criteria to determine their relative suitability as hosts; however, the pulmonary lesions in these animals were not described or compared.<sup>6</sup>

Since there is limited information available in the literature on pathologic changes associated with *P. kellicotti* in-

fection in wild mammals, a comparison of the histopathologic features in naturally infected species would be useful to wildlife disease investigators.

### **MATERIALS AND METHODS**

From a recent examination of wild carnivores collected in southwestern Ontario,<sup>9</sup> formalin fixed tissues in good condition were obtained from 11 *P. kellicotti*-infected mink, five striped skunks, eight red foxes and one coyote, as well as from seven raccoons with pleural adhesions. Tissues from 22 of 32 mammals (including four raccoons) were collected during the month of November. The remaining specimens were obtained during the latter part of October and first part of December. These tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E).

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## RESULTS

### Mink

Most nodules containing *P. kellicotti* were formed by altered lamina propria of dilated bronchioles. Necrotic debris, blood and fluke eggs were found in the lumina around flukes (Fig. 1).

Several stages in the formation of a fibrous capsule around large flukes were evident. Erosion of the bronchiolar epithelium to the lamina propria with necrosis and diffuse hemorrhage were the earliest changes seen. Fibroplasia of the lamina propria formed a wall up to 1000  $\mu\text{m}$  thick, characterized by moderate infiltrations of mononuclear cells, neutrophils and eosinophils. An outer layer of collagen with mild cellular infiltration had formed. At a later stage, the lamina propria (600  $\mu\text{m}$  thick) was not as hemorrhagic or cellular, and the bronchiolar epithelium was intact in some areas, hyperplastic or eroded in others. Pigment laden macrophages, cells containing lipid vacuoles and spicules of metaplastic bone were frequently seen in this layer. When smaller bronchi were affected, mild hyperplasia of the bronchial glands and marked hypertrophy of the acinar units occurred (Fig. 2). The outer band of dense collagen was 100-500  $\mu\text{m}$  thick. In a chronic stage, the bronchiolar wall was a dense fibrous capsule (600+  $\mu\text{m}$  thick) with a mild plasma cell infiltration. There was necrosis of the inner region of the wall and numerous fibroblasts were evident in this zone.

Lumina of displaced bronchioles located around the perimeter of *Paragonimus* nodules contained eosinophils, mononuclear cells and mucus. There was a moderate mononuclear cell infiltration in the lamina propria and surrounding these bronchioles. Mild hyperplasia of bronchial epithelium was occasionally seen in bronchi located in the adjacent parenchyma and there was hypertrophy of goblet cells as well as hypersecretion of bronchial glands. Squamous metaplasia of bronchial epithelium was evident in one mink. Peribronchiolar and perivascular lymphoid cuffs were common.

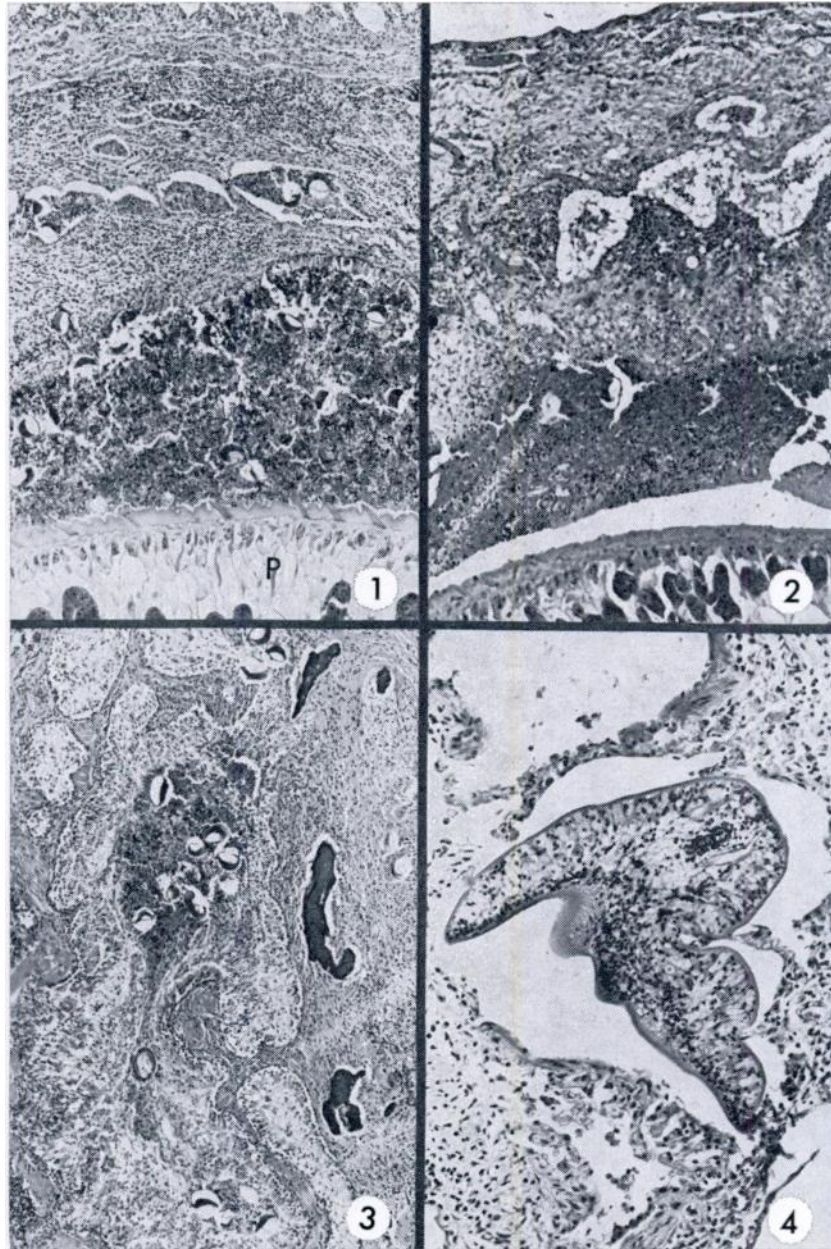
The surrounding parenchyma was atelectatic, congested and hemorrhagic with

thickened alveolar walls infiltrated with mononuclear cells. Proteinaceous fluid was occasionally seen in alveoli and interstitial spaces. Fluke eggs were diffusely distributed in alveoli in many areas. The reaction around egg masses varied from mild chronic inflammatory cell infiltration to fibrosis with metaplastic bone formation in one mink (Fig. 3). Severe inflammation was associated with eggs located immediately beneath the pulmonary pleura. Changes in the pulmonary pleura were confined to the region overlying nodules where it was thickened (100  $\mu\text{m}$ ) with collagen, or near immature flukes where fibrin strands were seen on the surface. Six immature *P. kellicotti* (90-170  $\mu\text{m}$ ) were found in dilated alveolar sacs in two mink. There was only a mild cellular infiltration around these flukes (Fig. 4).

Concurrent infections with other parasites were evident in the pulmonary parenchyma of several *P. kellicotti* infected mink. In five animals, sections through numerous thin nematodes in dilated bronchioles were common. There were larger nematodes located in the lamina propria of a large bronchus in one mink. Microgranulomas that surrounded protozoan schizonts or merozoites engulfed by macrophages were found in five mink.

### Striped Skunk

The structure of dilated bronchioles or bronchi containing *P. kellicotti* was similar to that described for mink. An inner zone (200  $\mu\text{m}$  thick) of necrotic debris and fluke eggs was characterized by massive infiltrations of mononuclear cells, neutrophils and eosinophils. There was fibroplasia of the lamina propria with large numbers of plasma cells and eosinophils in the middle zone (1400  $\mu\text{m}$ ). Hyperplasia of bronchial glands occurred when bronchi were affected; the glands formed a continuous layer 500  $\mu\text{m}$  thick and although typically acinar in shape, epithelial cells were pale and disrupted internally. The nuclei were generally clumped or irregularly distributed because of accumulated product in their





cytoplasm. A thick layer of dense collagen (200-800  $\mu\text{m}$ ) formed the third zone. In some skunks displaced bronchioles around the affected bronchial wall formed an additional layer 1500  $\mu\text{m}$  thick (Fig. 5). Changes in these bronchioles varied from loss of nuclear polarity in the epithelium to extensive bronchiolar hyperplasia.

Prominent changes in the adjacent parenchyma included severe bronchiolitis and peribronchiolitis, bronchitis with necrotic debris and fluke eggs in their lumina, perivascular cuffing and chronic

interstitial pneumonitis with fibrosis. Extensive areas of granulomatous pneumonitis with giant cells had formed around aggregations of fluke eggs (Fig. 6). The pulmonary pleura was generally thickened and often covered with fibrin strands. Pigment laden macrophages and mononuclear cells were found beneath the pleura.

There was a large mass of fluke eggs within a thick fibrous capsule in adipose tissue adjacent to the liver in one skunk. An immature fluke was seen in section through the hepatic parenchyma.

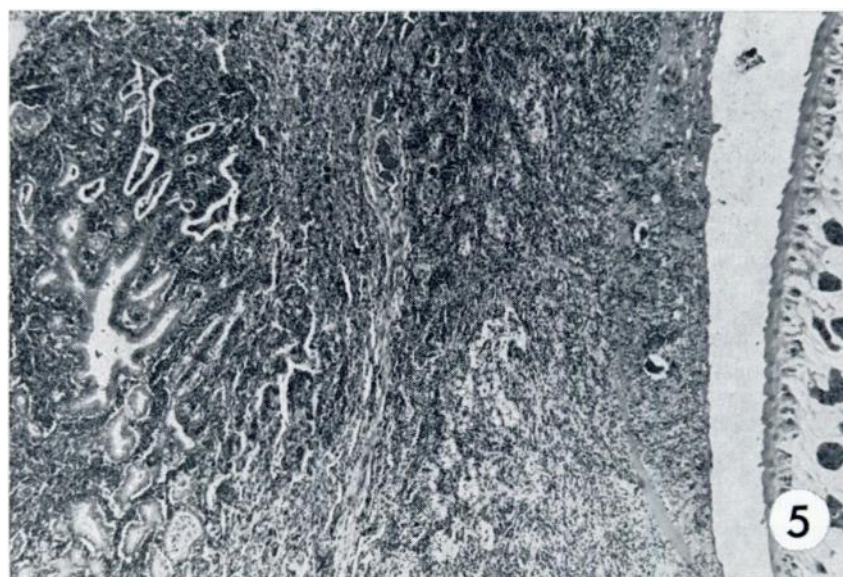


FIGURE 5. Thickened bronchial wall surrounding *P. kellicotti* in a striped skunk. There was an inner zone of necrotic debris and fluke eggs, then a region of mononuclear cell infiltration with hyperplasia and mild hypertrophy of bronchial glands, and an outer layer consisting of displaced bronchioles with hyperplastic changes in the epithelium. H&E x 130.

FIGURE 1. Exudate containing fluke eggs, nuclear debris and blood adjacent to *Paragonimus kellicotti* (P) in a dilated bronchiole. There were inflammatory and fibroplastic changes in the lamina propria with formation of a thin layer of collagen. Lung from a mink. H&E x 160.

FIGURE 2. Hypertrophy of bronchial glands in the lamina propria of a dilated bronchus containing *P. kellicotti*. Lung from a mink. H&E x 200.

FIGURE 3. Focal chronic granulomatous reaction with metaplastic bone formation around fluke eggs in the pulmonary parenchyma of a mink. H&E x 160.

FIGURE 4. Immature *P. kellicotti* in a dilated alveolar sac with mild mononuclear cell infiltration in thickened alveolar septa. Lung from a mink. H&E x 380.

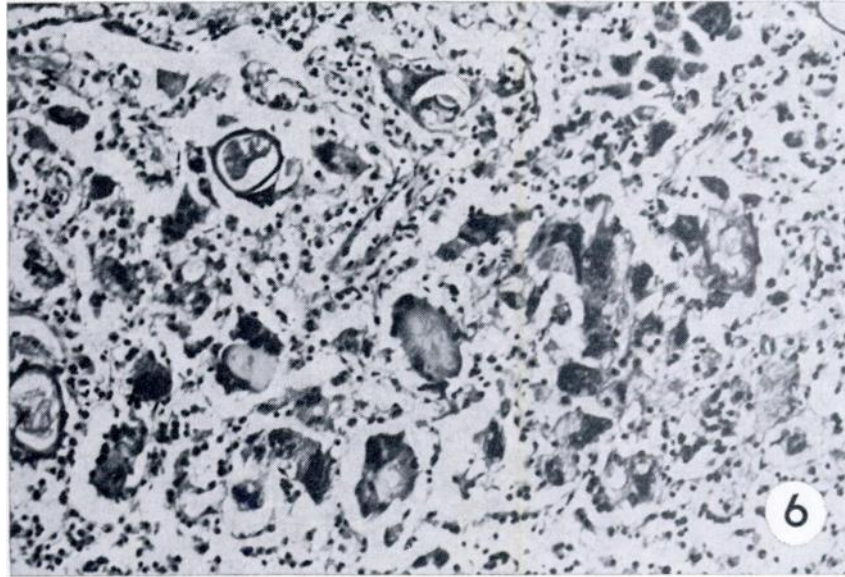


FIGURE 6. Granulomatous pneumonitis with giant cell formation around fluke eggs in the pulmonary parenchyma of a striped skunk. H&E x 510.

#### Red Fox

The wall of dilated bronchi containing *P. kellicotti* was thin (300-500  $\mu$ m) and characterized by severe erosion to the bronchial gland region with hemorrhage and infiltrations of plasma cells, mononuclear cells and eosinophils. There was moderate hyperplasia and hypertrophy of bronchial glands and some fine collagen strands in the outer zone, but a fibrous capsule was not formed.

Changes in the surrounding parenchyma included extensive hemorrhage, congestion, interstitial pneumonitis with

edema and diffuse fibrosis, and perivascular and peribronchiolar lymphoid cuffing. Erythrocytes, eosinophils and mononuclear cells were seen in adjacent bronchial lumina and there was hyperplasia of the epithelium. Eosinophils were evident in the lamina propria and hypertrophy of bronchial glands occurred. In the parenchyma most flukes were immature and apparently had been migrating. Trauma caused by a fluke that penetrated the pleura was found in one fox (Fig. 7). Fluke tracks were common and these were characterized by necrosis and diffuse hemorrhage with fibroplasia

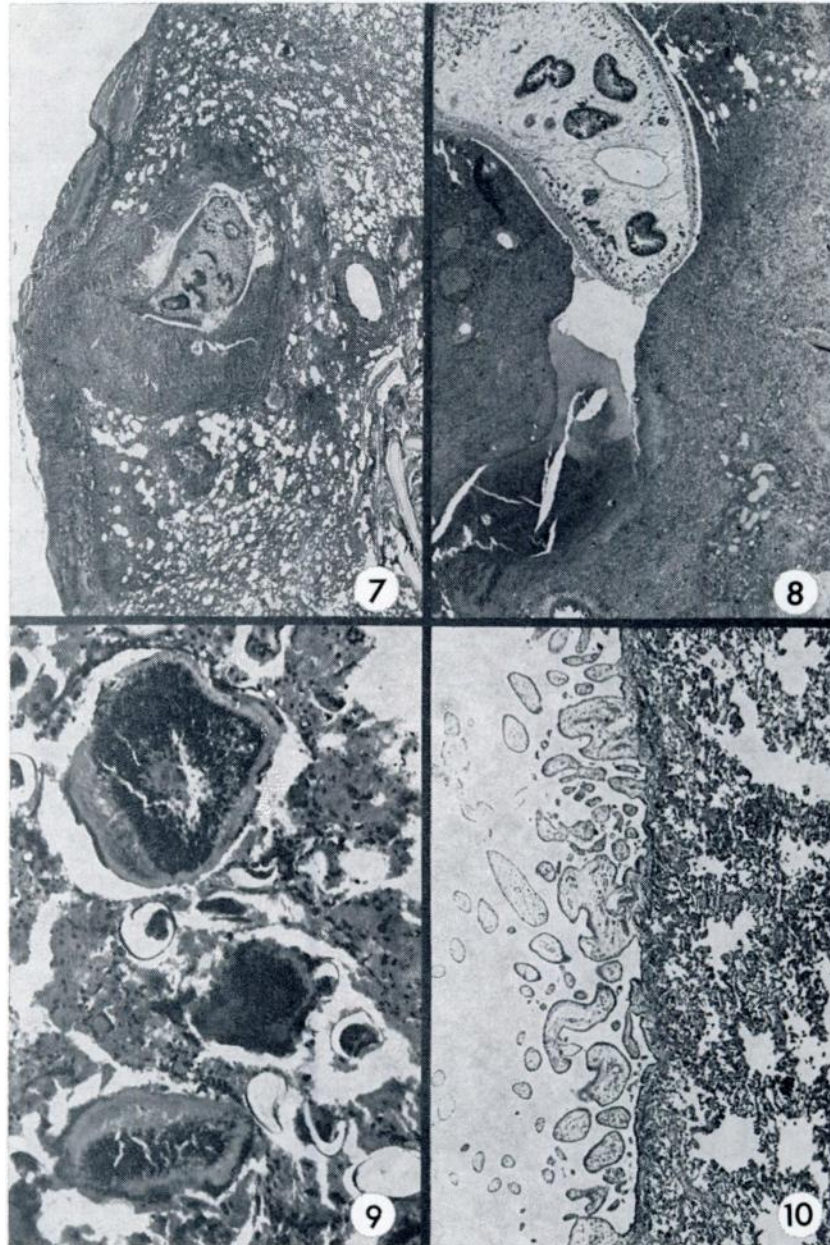
FIGURE 7. Trauma associated with a migrating *P. kellicotti* that penetrated the pulmonary pleura into the parenchyma of the lung of a red fox. H&E x 10.

FIGURE 8. A track caused by a migrating fluke in the pulmonary parenchyma of a red fox. Necrosis and diffuse hemorrhage with inflammatory cell infiltration and fibroplasia characterized the margin of the track. H&E x 11.

FIGURE 9. Chronic granulomatous reaction around fluke eggs in the pulmonary parenchyma of a red fox. These structures resemble the "club colony" formed around certain mycotic and bacterial agents. H&E x 380.

FIGURE 10. Hyperplastic changes in the pulmonary pleura of a red fox. The mesothelial cells were cuboidal or columnar and disposed into filaments containing collagen. H&E x 100.





and infiltrations of plasma cells and eosinophils along their margins (Fig. 8). Areas of focal necrosis and hemorrhage were also associated with masses of fluke eggs. Accumulation of debris around some eggs resulted in formation of eosinophilic ray or club-shaped structures similar to those found around some mycotic and bacterial agents (*Actinomyces*, *Coccidioides*, *Actinobacillus*, *Staphylococcus*) (Fig. 9). Extensive areas of granulomatous pneumonitis and fibrosis characterized the chronic stage of the reaction to fluke eggs. Mesothelial cells of the pulmonary pleura were cuboidal to columnar and disposed into thick filaments in many areas (Fig. 10). The pleura was thickened and covered with fibrin strands or thick fibrous tags.

#### Coyote

Marked fibroplastic changes in the lamina propria of affected bronchi resulted in formation of a fibrous capsule 1500  $\mu\text{m}$  thick. The bronchial epithelium was intact in some areas and the proliferating zone of the wall (400  $\mu\text{m}$  thick) contained numerous dilated capillaries as well as moderate numbers of eosinophils and plasma cells (Fig. 11). A middle zone of dense collagen (700-1000  $\mu\text{m}$ ) contained few inflammatory cells and isolated bronchial glands. Chronic interstitial pneumonitis with diffuse fibrosis and hemorrhagic or congested areas characterized an outer zone of compression atelectasis (300-500  $\mu\text{m}$ ) (Fig. 11). Compressed bronchioles, trapped fluke eggs and large quantities of black pigment were also evident. Necrotic debris, mucus and fluke eggs were found in

lumina of some affected bronchi, while in others connective tissue extended from the lamina propria across the lumina, and formed a solid mass of collagen.

In lumina of adjacent bronchi there was excess mucus and hypertrophy of goblet cells in the epithelium was evident. The lamina propria was fibroplastic and infiltrated with eosinophils and plasma cells (Fig. 12). Lumina of bronchial glands were distended and marked hypertrophy of the epithelium occurred in some areas. Fluke eggs were occasionally seen in alveoli and a diffuse fibrous reaction occurred around these. The pulmonary pleura was disposed into short thick folds that contained collagen. The pulmonary parenchyma was normal beyond the immediate vicinity of the nodules.

#### Raccoon

The lesions thought to be associated with *P. kellicotti* infection were confined to the pulmonary pleura and underlying parenchyma. A subpleural focus of necrosis and inflammation was found in one raccoon and a thick band of proliferating connective tissue extended for several mm along the pleura (Fig. 13). Fibroplastic changes in the pleura and underlying parenchyma formed a layer 2200  $\mu\text{m}$  thick in another animal. Eosinophil infiltration occurred in the deeper layers of collagen as well as in thick fibrous projections of the pulmonary pleura. A granuloma with a core of necrotic eosinophils and fibrous capsule 500  $\mu\text{m}$  thick was located beneath the pleura in another raccoon (Fig. 14). Occasional eosinophils were seen in peribronchial areas in a few animals.

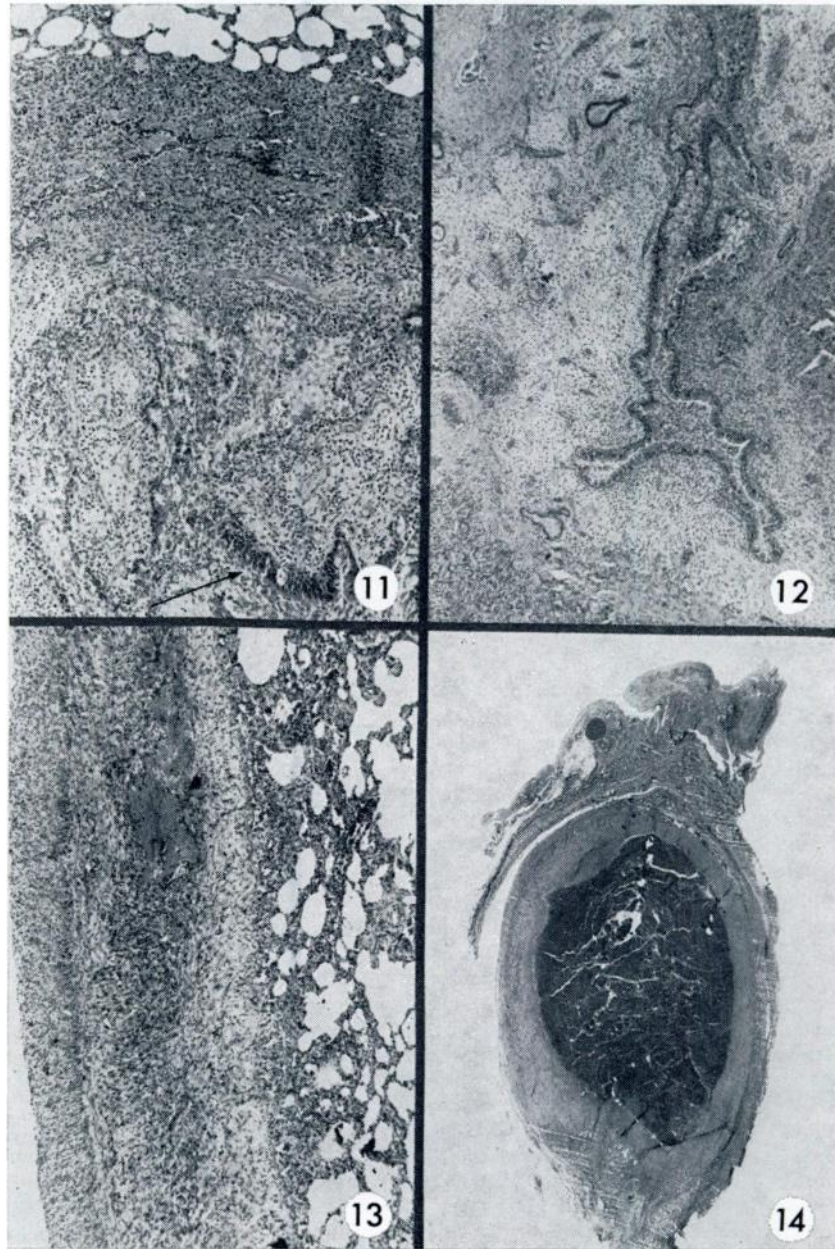
FIGURE 11. Marked fibroplasia in the lamina propria of a dilated bronchiole from the *P. kellicotti* infected coyote. The epithelium was intact in some areas (arrows) and there was compression atelectasis of adjacent alveoli. H&E x 160.

FIGURE 12. Extensive proliferation of connective tissue in the lamina propria of a bronchiole from the infected coyote. Mononuclear cells, eosinophils and mucus were evident in the lumen. H&E x 100.

FIGURE 13. Focal necrosis with inflammation and early fibroplasia beneath the pulmonary pleura of a raccoon. H&E x 160.

FIGURE 14. A subpleural granuloma with a core of necrotic eosinophils in the lung of a raccoon. H&E x 5.





#### Comparative Features of the Reaction to *P. kellicotti* Infection

Differences in the histologic appearance of the wall of altered bronchi in the four infected species of carnivore are shown (Figs. 15-18). A thin fibrous capsule surrounded flukes in mink (Fig. 15), while the thick wall in the striped skunk consisted of inflammatory cells, hyperplastic bronchial glands and displaced bronchioles (Fig. 16). Severe erosion with hemorrhage and inflammation and little collagen characterized the relatively thin wall formed in the red fox (Fig. 17). Marked fibroplasia with dense collagen formed in affected bronchial walls of the coyote (Fig. 18). Although lung flukes were not recovered from raccoons, pleural fibroplasia with eosinophil infiltration (Fig. 13) and granuloma formation suggested penetration and destruction of parasites migrating from the thoracic cavity to the pulmonary surface.

#### DISCUSSION

Only limited comparisons can be made among these carnivores since rate and number of metacercariae ingested and duration of infection are not known. However, most carcasses were collected at approximately the same time of year and may render the stage of infection comparable.

Fibrous capsule formation around *P. kellicotti* in altered bronchioles and bronchi, with only a mild inflammatory reaction around immature flukes and most egg aggregations, indicated that in mink the parasite is well adapted to this host. Metaplastic bone formation around some

flukes and egg granulomas was not evident in the pulmonary parenchyma of the other carnivores examined. The nematodes in bronchioles of several mink were probably *Perostrongylus pridhami*.<sup>12</sup> Perivascular and peribronchiolar lymphoid cuffs were more prevalent in mink with concurrent *P. pridhami* infection than in the other *P. kellicotti* infected carnivores. This reaction may have been primarily associated with *P. pridhami* infection in mink.<sup>11</sup> The larger nematode located in the lamina propria of a large bronchus was identified as *Filaroides martis*.<sup>12</sup> The protozoan schizonts in the parenchyma of five animals were similar to those of *Hepatozoon* spp.<sup>8</sup>

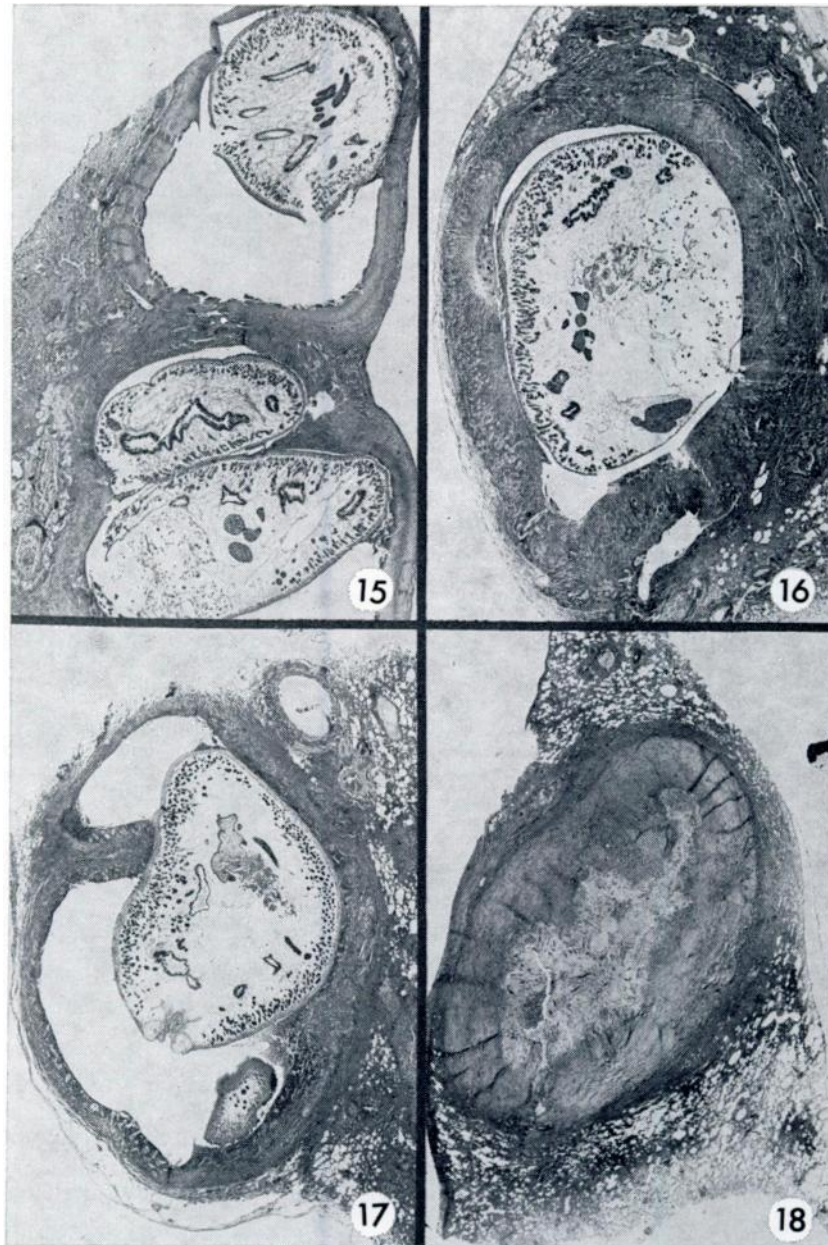
Inflammatory and proliferative changes in bronchial walls surrounding *P. kellicotti* in striped skunks were more severe than in mink. Granulomatous pneumonitis with giant cell formation around fluke eggs was not seen in the other species examined. This finding is similar to the reaction described in infected dogs.<sup>8,7</sup> Although the striped skunk is a suitable host for *P. kellicotti*,<sup>6</sup> the strong inflammatory reaction suggests that the host-parasite relationship is poorly developed.

Most of the lung flukes in infected red foxes appeared to have been migrating and even mature ones were surrounded only by a thin wall that contained little collagen. Necrosis and diffuse hemorrhage in the pulmonary parenchyma and hemothorax<sup>9</sup> indicated that *P. kellicotti* was probably most pathogenic to red foxes. Our findings support the statement by Stuht and Youatt<sup>18</sup> that the lung fluke may be a significant pathogen in this carnivore.

Recovery of *P. kellicotti* from one coyote constituted a new host record for the lung fluke.<sup>9</sup> Extensive fibrosis of

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FIGURES 15 - 18. Comparison of the histologic features of altered bronchial walls in four species of *P. kellicotti* infected carnivore. FIGURE 15. Thin fibrous capsules formed around flukes in a mink. H&E x 7. FIGURE 16. Thick wall formed around a fluke in a striped skunk. It consisted of inflammatory and proliferative changes in the lamina propria of the affected bronchus and displaced hyperplastic bronchioles. H&E x 7. FIGURE 17. Inflammation, mild hyperplasia and focal hemorrhage in the thin wall formed around flukes in a red fox. H&E x 7. FIGURE 18. Thick fibrous wall resulting from extensive fibrosis of the bronchial wall in the coyote. H&E x 4.





bronchial walls with occlusion of their lumina and only small numbers of fluke eggs in the pulmonary parenchyma suggested that the patent period of the infection was of short duration. It was concluded that the coyote is probably an aberrant and unsuitable host for *P. kellicotti*.

In raccons fibroplasia with eosinophil infiltration and granuloma formation in the region of the pulmonary pleura suggested lesions in response to a parasite penetrating the lung from the thoracic cavity. Few parasites migrate directly from the intestinal lumen through the peritoneal and thoracic cavities to the lungs. Experimental infection studies in mink have established that *P. pridhami* migrates by this route,<sup>11</sup> but the susceptibility of the raccoon to this nematode is not known. The lung fluke also attains the lungs by direct migration, and it was thought that immature *P. kellicotti* may have induced the lesions. Subsequent to analysis of raccoon tissues collected in 1973 an immature *P. kellicotti* (200  $\mu$ m) was found in lung sec-

tion from a carcass received in 1974. The fluke was located in a dilated alveolar sac beneath the pulmonary pleura. This finding in association with the site of pulmonary lesions tends to support our suggestion. Postmortem examination of a raccoon fed *P. kellicotti* metacercariae revealed pleural adhesions, but the histopathologic features were not described.<sup>1</sup> Additional experimental infection studies are necessary to establish whether these lesions are in response to migrating lung flukes.

Pathologic changes associated with *P. kellicotti* infection in these wild mammals, particularly those in mink and striped skunks, are similar to lesions described in cats and dogs.<sup>2-5,7,10,15</sup> These changes are in agreement with many of the features recorded in man and other hosts with *Paragonimus westermani* infection.<sup>16,17</sup> Experimental infection studies are essential to fully elucidate the nature of the host-parasite relationships among the various species of wild mammal that may acquire *P. kellicotti* infection.

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#### ADDENDUM

Since the time this paper went to press, a muskrat from Huron County was submitted for necropsy. There were four dark nodules on the lungs and from these ten *P. kellicotti* were recovered. Five intact flukes measured 13-15 X 8-9 mm in size and contained eggs.

Although tissues were severely autolyzed, the nodules were identified as dilated bronchi with thick walls (600-1000  $\mu$ m). The epithelium was eroded in some areas, and there was squamous metaplasia in others. Most of the wall consisted of bronchial glands that were hyperplastic and hypertrophic; dilated glands contained an excessive quantity of mucus. Collagen and small numbers of mononuclear cells were also seen. A zone of compression atelectasis surrounded these altered bronchi and there were aggregations of eggs in adjacent alveoli. Chronic pneumonitis, characterized by interstitial thickening, leukocyte infiltration and many macrophages containing black pigment, was associated with some fluke eggs.

The changes in the bronchial wall were most similar to those seen in some mink (Fig. 2) but the reaction was more extensive and severe. The presence of black pigment in the pulmonary parenchyma was like that described in the coyote.

As an incidental finding in this animal, focal aggregations of eggs resembling *Capillaria hepatica* were seen in the hepatic parenchyma.