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Distribution of *Crenosoma vulpis* and *Eucoleus aerophilus* in the lung of free-ranging red foxes (*Vulpes vulpes*)

Alicia Nevárez, Alfonso López¹, Gary Conboy, William Ireland, David Sims

Abstract. *Crenosoma vulpis* and *Eucoleus aerophilus* are nematode parasites that can cause verminous pneumonia in wild carnivores. There is a paucity of information regarding the distribution of parasites in the lungs and the relationship between histopathological and parasitological diagnoses in naturally infected foxes. The objectives of this study were: first, to study the lobar and airway distribution of *C. vulpis* and *E. aerophilus* in wild red foxes and second, to investigate the relationship between fecal and histopathological diagnoses. Samples from 6 sites of the lung and fecal contents were obtained from 51 wild foxes in Prince Edward Island. By fecal examination, 78.4% of wild foxes tested positive for *C. vulpis* and 68.6% for *E. aerophilus*. In contrast, 66.6% and 49% of foxes had histopathological evidence of *C. vulpis* and *E. aerophilus* in the lungs, respectively. Anatomically, *C. vulpis* was observed in the small bronchi and bronchioles of all pulmonary lobes whereas *E. aerophilus* was restricted to the large bronchi and the caudal lobes. Affected airways exhibited severe epithelial glandular hyperplasia and bronchiolar mucous metaplasia. It was concluded that *C. vulpis* is widely distributed in airways of all pulmonary lobes, whereas *E. aerophilus* is mainly restricted to the bronchi of caudal lobes. Also, this study showed that histological examination of lung underestimates the infection with *E. aerophilus*.

Key words: COPD; *Crenosoma vulpis*; *Eucoleus aerophilus*; fox; lungworm; verminous pneumonia.

Crenosoma vulpis and *Eucoleus aerophilus* (formerly—*Capillaria aerophila*) are nematodes that parasitize the lungs of wild and domestic canids and various other carnivores in many regions of the world.^{6,11} *Crenosoma vulpis* infects the bronchi, bronchioles, and trachea and is endemic in red fox populations in Europe and the northeastern parts of North America, particularly Atlantic Canada.^{10,11,14,15,17} Recently, crenosomosis has also been recognized as an important cause of chronic respiratory disease in dogs in Atlantic Canada.^{1,5} Little is known concerning the pathogenicity of *C.*

vulpis infection in wild red foxes; however, infection was believed to cause poor fur quality and to potentially lead to mortality in young ranched silver foxes.⁸ The larval migratory pathways and the progression of lesions caused by *C. vulpis* have been established in experimental studies.¹⁶ However, this information emanated from canine experimental models raising the question whether such findings can be extrapolated to free-living foxes.

Eucoleus aerophilus is another important pulmonary nematode found in the trachea, bronchi, and bronchioles of domestic and wild carnivores and is enzootic in wild foxes in many parts of the world.^{11,15} One epidemiological study in Canada showed that as many as 67.2% of foxes in New Brunswick and Nova Scotia and 44% in Ontario were parasitized with *E. aerophilus*.^{3,14} Infected animals suffered chronic respiratory disease with poor growth and decreased fur quality. Heavy infections could lead to significant mortality because of bronchopneumonia.⁹

To the authors' knowledge, a systematic sampling to study

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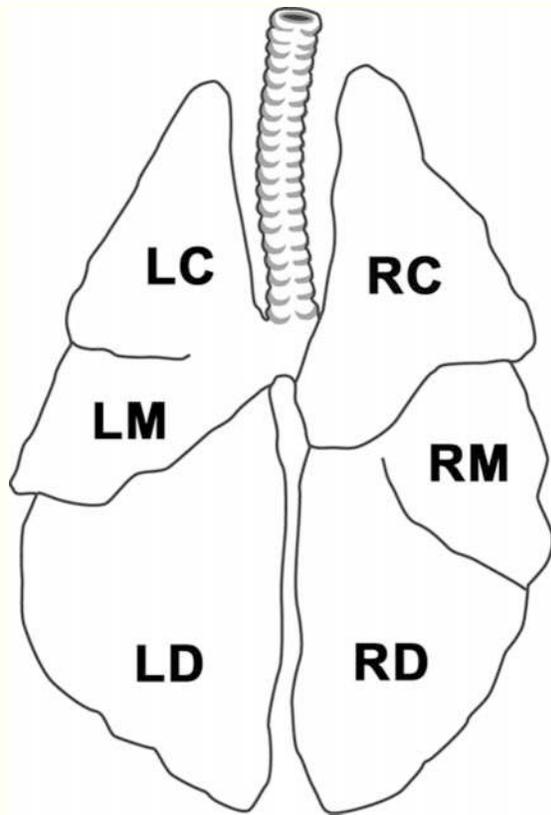


Figure 1. Anatomical sites of the lung sampled for histopathological examination. LC = left cranial; LM = left middle; LD = left caudal; RC = right cranial; RM = right middle; RD = right caudal.

the distribution of *E. aerophilus* and *C. vulpis* in the lung has never been conducted in wild foxes. Also, it is unclear to what degree fecal detection of *E. aerophilus* and *C. vulpis* correlates with the microscopic findings observed in the lungs of wild foxes. The objectives of this study were 2-fold: first, to study the lobar and airway distribution of *C. vulpis* and *E. aerophilus* in wild foxes and second, to investigate the relationship between fecal and histopathological diagnoses.

Fifty-one wild red foxes (*Vulpes vulpes*) were trapped in Prince Edward Island (46°17'N, 63°8'W) in accordance with the Wildlife Conservation Act and the General Regulations as outlined in the Fish and Game Protection Act of Prince Edward Island, Canada. Once trappers removed the pelts, the unopened carcasses were transported within 24 hours to the Atlantic Veterinary College.

Fecal material obtained from the rectum was examined for the presence of helminth larvae and eggs, using the Baermann technique and zinc sulfate centrifugal flotation.² Specific identification of *C. vulpis* first-stage larvae and *E. aerophilus* eggs was based on size and morphology.^{4,6} Postmortem examination with particular attention to the thoracic viscera was performed according to standard procedures.¹² Foxes showing extensive pulmonary hemorrhages caused by trapping were excluded from this study. Lung samples were systematically obtained from 6 pulmonary sites as shown in

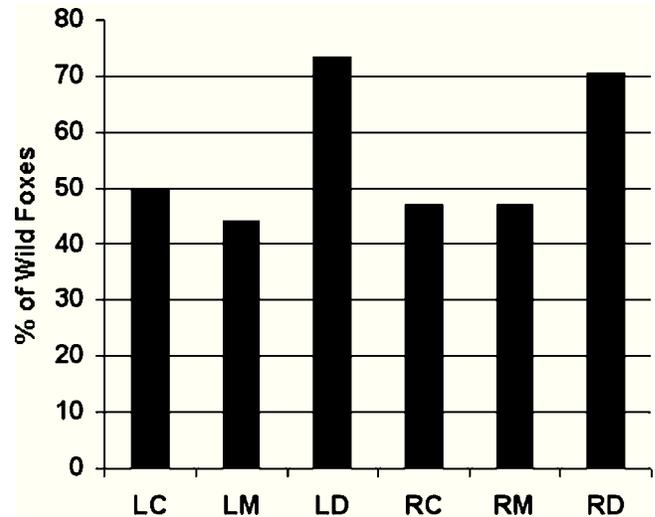


Figure 2. Lobar distribution of *Crenosoma vulpis* in the 6 lung sites of wild foxes. LC = left cranial; LM = left middle; LD = left caudal; RC = right cranial; RM = right middle; RD = right caudal.

Fig. 1. Lung samples were fixed in 10% buffered formalin, processed, and sectioned at a thickness of 5 μ m. Tissue sections were stained with hematoxylin and eosin and periodic acid–Schiff (PAS)–Alcian blue. The following parameters were microscopically evaluated for each pulmonary site: 1) presence or absence of *C. vulpis* or *E. aerophilus*, 2) distribution of parasites in bronchi, bronchioles, and alveoli, and 3) histopathological changes in the lung. Because repetitive counting of coiled parasites could overestimate the number of worms in tissue sections, the presence of *C. vulpis* and *E. aerophilus* was simply expressed as a dichotomous variable (i.e., parasites were either present or absent).

Fecal examination by the Baermann technique revealed that 78.4% of the sampled foxes in Prince Edward Island

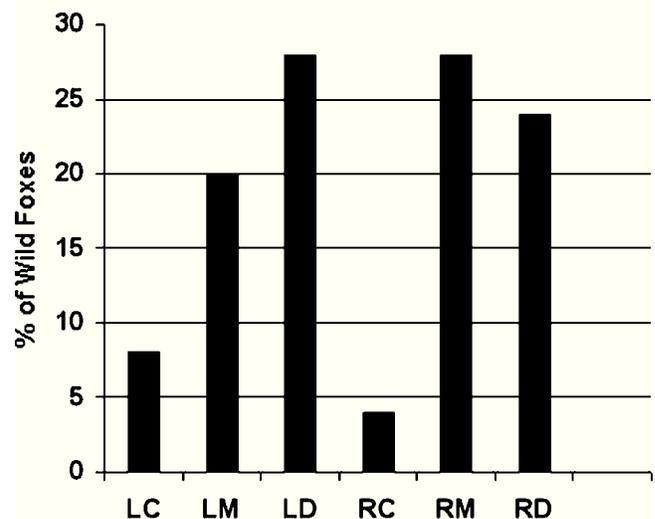


Figure 3. Lobar distribution of *Eucoleus aerophilus* in the 6 lung sites of wild foxes. LC = left cranial; LM = left middle; LD = left caudal; RC = right cranial; RM = right middle; RD = right caudal.

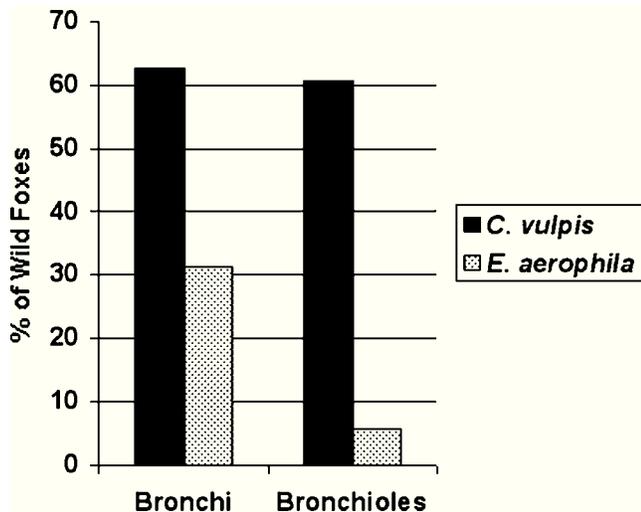


Figure 4. Percentage of foxes with *Crenosoma vulpis* and *Eucoleus aerophilus* in bronchi and bronchioles.

tested positive for *C. vulpis*, whereas 68.6% were positive for *E. aerophilus*. In contrast, histopathological examination showed that 66.6% and 49% of sampled foxes had evidence of *C. vulpis* and *E. aerophilus* in the lungs, respectively.

The distribution of *C. vulpis* and *E. aerophilus* in the different sites of the lung is summarized in Figs. 2, 3. According to airway distribution, 62.7% of the wild foxes had *C. vulpis* in the bronchi and 60.7% in bronchioles (Fig. 4). In the case of *E. aerophilus*, 31.3% of foxes had this nematode in the bronchi and only 5.8% had in bronchioles. Microscopic examination of the lungs revealed that *C. vulpis* was consistently free in the bronchial and bronchiolar lumen, whereas *E. aerophilus* was typically embedded in the airway mucosa. Both of these nematodes were closely associated with mucosal edema and eosinophilic infiltrates in the lung.

Microscopic lesions associated with *C. vulpis* and *E. aerophilus* were typically centered around the bronchi and bronchioles and to a much lesser extent around the alveoli. The bronchi frequently contained *C. vulpis* or *E. aerophilus* alone or admixed with serocellular exudate. The bronchial mucosa showed variable degrees of edema and hyperplasia and also contained numerous eosinophils infiltrating the submucosa.

The lungs showing microscopic evidence of lungworms had notable hyperplasia of the bronchial associated lymphoid tissue (BALT) characterized by large aggregates of lymphocytes and plasma cells in the bronchial mucosa and around the bronchial wall. The severity of BALT hyperplasia varied from mild to severe. Another prominent microscopic change associated with *C. vulpis* was a moderate to severe hyperplasia of the submucosa bronchial glands. The acini of affected glands were mildly to moderately distended and often contained amorphous, pale, eosinophilic, PAS and Alcian blue-positive fluid.

Foxes parasitized with *C. vulpis* or *E. aerophilus* had mild to moderate hypertrophy of bronchial and bronchiolar smooth muscle. However, the most remarkable change was the severe mucous bronchiolar metaplasia. Many bronchioles were lined almost exclusively by goblet cells, and their lumens were plugged with mucous secretions. Eosinophils and

mononuclear cells were occasionally infiltrating the bronchiolar lamina propria and peribronchiolar connective tissue. There were no significant changes in alveoli, pulmonary vessels, or pleura.

This investigation confirmed that *C. vulpis* and *E. aerophilus* are highly prevalent in the wild fox population of Prince Edward Island and supports the view that Atlantic Canada has 1 of the highest prevalences in the world.^{3,10,15,17} There was a small discrepancy between the prevalence rates for *C. vulpis* estimated by lung microscopy and fecal examinations (66.6% vs. 78%) indicating that lung microscopy is a reliable method to detect this nematode in fox lung. There was a slightly larger disparity in the rate at which *E. aerophilus* was detected microscopically in lung tissue as compared with fecal examination (49% vs. 68%). It is concluded that postmortem and histopathological examination of lung may underestimate the true prevalence rate of *E. aerophilus* even when as many as 6 different sites of lung are microscopically examined.

There was a slightly higher tendency for caudal lobes to harbor *C. vulpis*, supporting the general view that lungworms primarily parasitize the caudal lung lobes.^{7,12} However, as many as 40% of cranial and intermediate lung lobes of wild foxes had *C. vulpis*. This finding suggests that unlike *Dictyocaulus* spp. in ruminants and *Metastrongylus* spp. of pigs,⁷ *C. vulpis* tends to be more diffusely distributed in the lungs, parasitizing not only the caudal lobes but the intermediate and cranial parts of the lung as well. Not clear however is whether this difference between foxes and ruminants is because of intrinsic anatomic characteristics, biology of parasites, or a combination of both. The higher lungworm prevalence reported for caudal lobes not only may reflect the preferential site where gross lesions are most commonly found in affected animals but also the historical pathologist's bias to sample these sites of the lung on postmortem examinations.^{7,12,16}

To the authors' knowledge, this is the first report documenting the all inclusive distribution of *C. vulpis* in naturally infected foxes. For routine examination, the authors recommend that 3 or more samples of lungs, including cranial and intermediate areas, be submitted for histopathology, even in cases where gross lesions may have been found only in caudal lobes of foxes. Lung sampling is an important factor that needs to be considered while performing routine diagnostic pathology. As many as 36 foxes (75%) had both *C. vulpis* and *E. aerophilus*, indicating that combined lungworm infections are common. Nine foxes (18.7%) were infected with *C. vulpis* alone, whereas only 3 (6.2%) were exclusively infected with *E. aerophilus*. However, it was not clear whether combined infections have an additive effect on the severity of lung lesions or on the parasitic burden in the live fox.

The ample distribution of *C. vulpis* in the lung contrasted with the much more restricted distribution of *E. aerophilus*. Whereas *C. vulpis* was present in all pulmonary lobes, *E. aerophilus* was predominantly located in the caudal lobes. This preferential caudal distribution of *E. aerophilus* was in agreement with the overall distribution of lungworms in ruminants and pigs.^{7,12} Results of the histological examination also showed that there was no difference in the lungworm prevalence between the left and right lungs.

Crenosoma vulpis was found with equal frequency in bronchi and bronchioles of the foxes whereas *E. aerophilus* was predominately located in the bronchi. Both *C. vulpis* and *E. aerophilus* have been reported to occur in the trachea, bronchi, and bronchioles, but *E. aerophilus* tends to be found higher in the respiratory tract than is *C. vulpis*. This finding was consistent with a study that reported 66% of the *E. aerophilus* worm burden was recovered from the trachea of infected red foxes.³ The possibility exists that postmortem parasitic migration may have biased the location in which the 2 parasites were microscopically seen in airways.¹³ Post-mortem migration was not a likely concern for *E. aerophilus* because this nematode was typically embedded in the bronchial mucosa as reported previously by others.^{7,14}

The pulmonary lesions associated with lungworms are a combination of inflammatory and reactive changes expressed as bronchial hyperplasia and bronchiolar mucous metaplasia.^{7,16} Goblet cell metaplasia with mucous plugging of bronchioles was remarkable and reminiscent of those changes seen in the lungs of human beings and animals with chronic obstructive pulmonary disease (COPD) and recurrent airway obstruction.¹²

It was concluded from this investigation that *C. vulpis* is widely distributed in airways of all pulmonary lobes, whereas *E. aerophilus* is restricted to the bronchi of caudal lobes. Pathologists should be aware that microscopic examination of lung often underestimates true infection. Future studies should investigate whether bronchiolar and bronchial lesions induced by lungworms have true clinical significance for wild foxes and also whether the bronchiolar metaplastic changes could be used as a model to study COPD in parasitized foxes.

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