Journal of Animal and Veterinary Advances 7 (10): 1252-1256, 2008
ISSN: 1680-5593

Hyperplastic and Metaplastic Changes in the Bronchi and Bronchioles of Red Foxes (*Vulpes vulpes*) Naturally Infected with *Crenosoma vulpis* and *Eucoleus aerophilus*

[1]Alicia M. Nevárez-Garza, [1]Alfonso López, [2]Gary Conboy and [3]William Ireland
[1]Department of Pathology (Nevárez-Garza), Faculty of Veterinary Medicine,
Universidad Autónoma de Nuevo León, Mexico
[2]Department of Pathology and Microbiology (López, Conboy),
[3]Department of Biomedical Sciences (Ireland), Atlantic Veterinary College,
University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada

Abstract: *Crenosoma vulpis* and *Eucoleus aerophilus* are 2 important lung nematodes of wild and domestic carnivores. A recent report underscored the severity of bronchial gland hyperplasia and bronchiolar metaplasia in parasitized foxes. The objective of this study was to quantify the degree of bronchial gland hyperplasia and bronchiolar metaplasia in foxes naturally infected with *C. vulpis* and *E. aerophilus*. Fifty-one trapped wild foxes and 12 farmed foxes were necropsied and 6 lung sites were processed for histopathological examination. The degree of bronchial gland hyperplasia was evaluated using the Reid index (bronchial gland to bronchial wall ratio) and the severity of goblet cell metaplasia was determined by morphometric analyses. None of the farmed foxes had microscopic evidence of nematodes in the lung, whereas evidence of *C. vulpis* and *E. aerophilus* was detected in 66.6% (34/51) and 49% (25/51), respectively, of the wild foxes. Lungworms caused a significant increase in the Reid index with 2-fold increase in the size of bronchial glands. Bronchiolar goblet cell metaplasia was present in 60.7% (31/51) of foxes harboring *C. vulpis* and in 23.5% (12/51) of those with *E. aerophilus*. The odds ratio for bronchiolar goblet cell metaplasia was 16.0 ($p<0.001$) for *C. vulpis* and 9.0 ($p<0.001$) for *E. aerophilus*. It was concluded that the severity of airway inflammation and bronchiolar obstruction in foxes parasitized with *C. vulpis* and *E. aerophilus* is similar to that reported in humans with chronic obstructive pulmonary disease. The clinical significance of airway changes in apparently healthy wild foxes needs to be investigated.

Key words: Bronchiolitis, bronchitis, chronic obstructive pulmonary disease, *Crenosoma vulpis*, *Eucoleus aerophilus*, foxes, goblet cell metaplasia, lungworm

INTRODUCTION

Crenosoma vulpis and *Eucoleus aerophilus* are 2 important nematodes that parasitize the lungs of foxes (*Vulpes vulpes*) in many parts of the world, particularly in Atlantic Canada and the eastern United States (Smith, 1978; Zeh *et al*., 1977). The lesions caused naturally and experimentally by *C. vulpis* and *E. aerophilus* have been described and are characterized by chronic bronchitis and bronchiolitis (Stockdale and Hulland, 1970). A recent study showed that while *C. vulpis* is widely distributed in bronchi and bronchioles of all pulmonary lobes, *E. aerophilus* is restricted to the bronchi of the caudal lobes (Nevárez *et al*., 2005).

This study also postulated, that bronchiolar mucous metaplasia is a significant lesion in the bronchioles of foxes.

Enlargement and hyperplasia of bronchial glands has also been reported in animals harboring *C. vulpis* and *E. aerophilus* (Nevárez *et al*., 2005; Stockdale and Hulland, 1970). Over production of mucus resulting from hyperplasia of bronchial glands and bronchiolar metaplasia can result in airway obstruction and airflow limitation. In human and veterinary medicine, the aforementioned changes along with the plugging of airways are collectively referred to as Chronic Obstructive Pulmonary Disease (COPD) or Recurrent Airway Obstruction (RAO) (López, 2007; Rendell, 2006). The

Corresponding Author: Alicia Nevárez, Departamento de Patología, Facultad de Medicina Veterinaria,
Universidad Autónoma de Nuevo León, Ave Lázaro Cárdenas 4600, Monterrey, N. L. México,
Unidad Universitaria Mederos, CP, 64930

pathological implications of COPD-like changes in foxes parasitized with *C. vulpis* and *E. aerophilus* have received little attention. For instance, it is unclear if the degree of bronchial gland hyperplasia and bronchiolar metaplasia is quantitatively different in parasitized foxes as compared to nonparasitized foxes. It is also, yet to be determined if the magnitude of bronchiolar mucous plugging in foxes is comparable to that in patients exhibiting sclinical signs of COPD.

The objectives of the current study were to quantify by morphometric analyses the degree of bronchial gland hyperplasia, bronchiolar goblet cell metaplasia and mucous plugging in the lungs of foxes naturally infected with *C. vulpis* and *E. aerophilus*.

MATERIALS AND METHODS

Fifty-one wild red foxes trapped in Prince Edward Island were used for this study. Trapping was done 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. These foxes were part of a previous study aimed to determine the topographical distribution of lungworms in the lung (Nevárez *et al*., 2005). Twelve farmed foxes raised at the experimental fox farm of the Nova Scotia Agricultural College were used as parasite-free control animals.

For each fox, samples of lung from the Right Cranial (RC), Right Middle (RM), Right Diaphragmatic (RD), Left Cranial (LC) and two sites of Left Diaphragmatic (LD) lobes were fixed in 10% buffered formalin and processed for histopathological examination as previously described (Nevárez *et al*., 2005). Lungs were examined microscopically and the presence of parasites, inflammation, bronchial gland hyperplasia, bronchiolar metaplasia and formation of mucous plugs in airways was recorded. Identification of *C. vulpis* and *E. aerophilus* was based on the morphological features of the parasites (Soulsby and Moniz, 1982).

Morphometric analyses were done in the lungs of 15 parasitized wild foxes and 12 nonparasitized lungs obtained from farmed foxes. The severity of bronchial gland hyperplasia and bronchiolar metaplasia, as well as the degree of airway plugging was quantified using commercial software. The areas of the mucous plug and the bronchiolar lumen were calculated at 6.3×magnification in three randomly selected bronchioles for each pulmonary lobe. The bronchiolar mucous plug and the bronchiolar lumen were manually traced with a red line on a computer monitor using morphometric software. The area for the bronchiolar mucous plug and bronchiolar lumen was expressed as the percentage of lumen occupied by the mucus.

The relative proportion of cells and cell debris in the mucus was determined in 3 bronchioles from each lobe as follow: Bronchioles containing mucous exudate were selected on the computer screen at a magnification of 1.2x. Areas of the mucous plug were selected using the Mattfeldt orientator (Gundersen *et al*., 1988), which consists of a numeric circle on a transparent overlay that is placed on the monitor screen. The relative proportions of cells versus mucus were then calculated using image analysis.

The Reid index or bronchial gland to bronchial wall ratio was calculated in three randomized bronchi in each individual lung lobe and expressed as a percentage of bronchial wall occupied by the glands. The thicknesses of the bronchial walls were traced by image analysis by placing two points on the opposite sides of the wall (Thurlbeck, 1988). Similar measurement was done on the corresponding bronchial gland. The relationship between the presence of lungworms in the lung and bronchial hyperplasia and bronchiolar metaplasia was investigated using the Odds ratio and Chi-square test. Differences in the Reid index and mucous morphometry in parasitized and nonparasitized foxes was statistically tested by one-way Analysis of Variance (ANOVA).

RESULTS

The lungs of 66.6% (34/51) of wild foxes had cross-section evidence of *C. Cvulpis* infection while 49% (25/51) had *E. aerophilus*. The adult and larval stages of *C. vulpis* were identified based on parasite microscopic morphology (Fig. 1). The uterus of female *C. vulpis* was typically filled with developing eggs and developing first-stage larvae. The intestinal cells were multi-nucleated and on the surface, the cuticle of *C. vulpis* showed darkly staining ridges and muscle cells in the

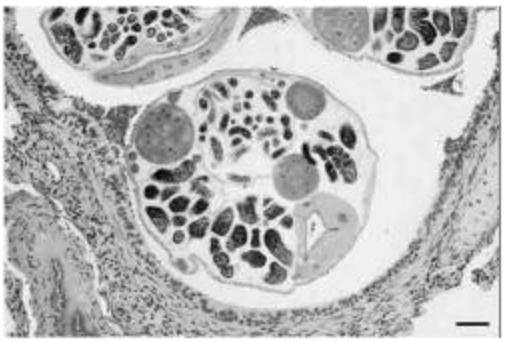

Fig. 1: Bronchus; wild fox. Cross sections of *Crenosoma vulpis* showing the gut (g), ovary (o) and uterus filled with first-stage larvae (L1) and developing eggs. Hematoxylin and eosin Bar = 50 μm

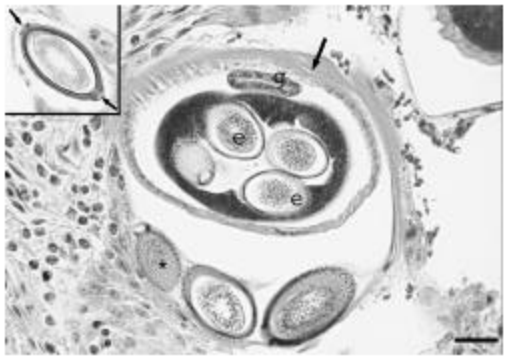

Fig. 2: Bronchus; wild fox. Cross sections of an adult female *Eucoleus aerophilus* in the lumen (arrow). This parasite shows the gut (g) and uterus filled with bipolar plugged eggs (e). Inset: Detail of an egg showing the bipolar plugs (arrows). Hematoxylin and eosin. Bar = 10 μm

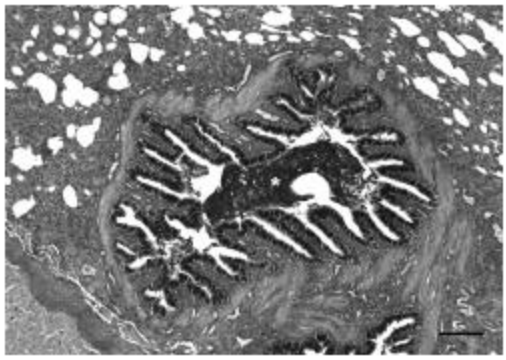

Fig. 3: Bronchiole; wild fox. Severe goblet cell metaplasia. Note bronchiole filled with mucus and cell debris (asterisk). The bronchiolar mucosa is lined predominantly by mucus-producing goblet cells. PAS-Alcian blue stain. Bar = 100 μm

underlying cuticle layers. *Eucoleus aerophilus* were readily distinguished morphologically from *C. vulpis* in histological sections (Fig. 2). In cross section, the uterus of adult female *E. aerophilus* usually contained thick-walled eggs, many of which exhibit the typical bipolar plugs (Fig. 2). The shell wall surface was marked with a pattern of ridges characteristic for *E. aerophilus*. The intestine was typically composed of a series of short columnar cells and the esophagus was composed of stichocytes.

The airways parasitized with *C. vulpis* and *E. aerophilus* had remarkable cellular infiltrates and intraluminal accumulation of mucus. The lamina propria and lumens contained many eosinophils admixed with neutrophils and mononuclear cells. Some airways without parasites in the wild foxes contained eosinophils and other inflammatory cells. Inflammatory cells were typically absent in the airways of farmed foxes. The bronchi containing *C. vulpis* also exhibited moderate goblet cell hyperplasia, but most remarkable, was the severe goblet cell metaplasia in affected bronchioles. Some bronchioles were lined almost exclusively by goblet cells (Fig. 3). None of these changes was observed in the bronchioles of farmed foxes.

Bronchial goblet cell hyperplasia was present in 56.8% (29/51) of wild foxes with *C. vulpis* and in 21.5% (11/51) in foxes with *E. aerophilus*. The odds ratio for bronchial goblet cell hyperplasia was 10.2 ($p<0.001$) for *C. vulpis* and 6.8 ($p<0.001$) for *E. aerophilus*. Bronchiolar goblet cell metaplasia was present in 60.7% (31/51) of foxes with *C. vulpis* and in 23.5% (12/51) of foxes with *E. aerophilus*. The odds ratio for bronchiolar goblet cell metaplasia and *C. vulpis* was 16.0 ($p<0.001$) and 9.0 ($p<0.001$) for *E. aerophilus*. Microscopically, the bronchioles of wild foxes with parasites were lined with abundant goblet cells and the bronchioles often contained large mucous plugs. These changes were present at all levels of the bronchioles. The bronchiolar mucosa of farmed foxes did not contain goblet cells or mucous plugs.

Morphometric analyses revealed that bronchiolar plugs in parasitized foxes occupied 63.8 ± 10.6% of the bronchiolar lumen. In contrast, farmed foxes had only 20.8 ±8.3% of the bronchiolar lumen filled exclusively with cell debris or exfoliated cells. This difference in the percentage of mucous plug size between wild and farmed foxes was significant ($p<0.001$). Morphometric analysis also showed that the relative proportions of mucus to cells in the bronchiolar plugs were significantly larger ($p<0.001$) in wild foxes (52.7±15.7%) compared to farmed foxes (7.0±7.9%). The nonmucous component of the plug in wild foxes was largely composed of eosinophils, neutrophils, macrophages and some fluid and cellular debris. The bronchioles of farmed foxes sporadically contained exfoliated cellular debris but no mucus or inflammatory cells.

The bronchi of wild foxes containing *C. vulpis* or *E. aerophilus* had a noticeable enlargement and some distention of the bronchial glands (Fig. 4). Some glands were only distended, while others contained cellular debris, neutrophils and some eosinophils. The bronchial gland of one wild fox contained the remnants of a parasite, presumably *E. aerophilus*. None of these changes were present in the bronchi and bronchial glands of the farmed foxes. Morphometric studies showed a significant ($p<0.01$) gland enlargement in foxes infected with

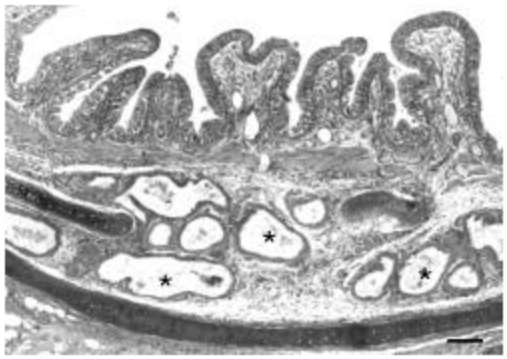

Fig. 4: Bronchus; wild fox. Epithelial hyperplasia and enlarged submucosal gland (asterisks). Hematoxylin and eosin. Bar = 100 μm

lungworms as shown by a Reid index of 44.24±8.26%, compared to only 19.13±2.74% in the nonparasitized control group.

DISCUSSION

The host-parasite interaction in mammals is typically followed by eosinophil infiltration, which is a characteristic microscopic finding in the airways of parasitized foxes (Behm *et al*., 1997). It is interesting to note, however, that eosinophilic infiltrates were not only seen in parasitized bronchi, but also in airways lacking parasites or parasitic larvae. Inflammation without intralesional nematodes suggests perhaps a postmortem movement of lungworms along airway lumen as has been described in other organs such as the stomach and intestine (Brown *et al*., 1993). It is also, feasible that the inflammatory process could have extended from parasitized to nonparasitized airways through spilling of cytokines and other pro-inflammatory mediators (Durham *et al*., 2000). Another possible explanation is that the inflammatory process and immune response remained active long after the nematodes had been expelled from the airways of foxes as reported for other nematodes in the intestine (Behm *et al*., 1997; Kanobana *et al*., 2003).

As previously reported (Nevárez *et al*., 2005; Stockdale and Hulland, 1970), *C. vulpis* and *E. aerophilus* provoke chronic bronchial inflammation microscopically characterized by peribronchial lymphoplasmacytic infiltrates, mucosal hyperplasia and enlargement and hyperplasia of bronchial glands.

In the current study, bronchial gland enlargement in parasitized foxes was remarkable as demonstrated by the two-fold increase in the bronchial to wall ratio (Reid index) in foxes harboring lungworms. The Reid index of 20% present in healthy foxes is quantitatively similar to the index reported for the healthy human lung, while the 40% index in foxes with lungworms is comparable to that seen in humans with chronic bronchitis or COPD (Thurlbeck, 1988). The current investigation clearly shows that the Reid index is an objective method to quantify the degree of bronchial gland hyperplasia in animals and to the authors' knowledge this the first time that this index has been used in veterinary pathology. The Reid index could be valuable for other studies such as Recurrent Airway Obstruction (ROA) in horses and asthma in dogs and cats. Bronchiolar goblet cell metaplasia was a significant microscopic change in the wild foxes. This lesion correlated with the presence of *C. vulpis* in the airways as statistical analyses revealed that foxes with crenosomosis were 16 times more likely to have metaplastic bronchiolitis than noninfected foxes. In contrast, the association of goblet cell metaplasia and *E. aerophilus* was not as strong, probably because this nematode is mainly found in major bronchi rather in the bronchioles (Nevárez *et al*., 2005).

The high prevalence of dual parasitic infections in wild foxes precluded any definitive conclusion as to whether bronchiolar epithelial mucous metaplasia was caused by *C. vulpis* or by *E. aerophilus*. Nonetheless, al fact points to *C. vulpis* and exclude *E. aerophilus* as the putative cause of bronchiolar metaplasia. As previously reported (Morrison and Gier, 1979; Nevárez *et al*., 2005). *E. aerophilus* is mainly found in large bronchi rather than in bronchioles where airway metaplasia takes place. Future studies should evaluate the effect of single-parasite infections on the degree of bronchiolar metaplasia.

Due to the medical importance of human asthma and COPD, there has been a plethora of investigations to elucidate the pathogenesis of bronchiolar goblet cell metaplasia (Thurlbeck, 1988). Bronchiolar epithelial mucous metaplasia is one of several host responses to persistent irritation in the mammalian lung (Jamal *et al*., 1984; Rendell, 2006). For instance, goblet cell metaplasia is characteristically seen in patients with a history of chronic cigarette smoking or after prolonged inhalation of irritant gases such as sulfur dioxide (Basbaum and Jany, 1990). Squamous metaplasia (Basbaum and Jany, 1990), a microscopic change commonly reported in patients with COPD, was not observed in any of the parasitized foxes. Bronchiolar mucous metaplasia in the parasitized wild foxes was so severe that some airways were lined almost exclusively by goblet cells. Despite considerable research in the last 2 decades, the pathogenesis of bronchiolar goblet cell metaplasia is still poorly understood (Basbaum and Jany, 1990; Jamal *et al*., 1984; Williams *et al*., 2006). Recent investigations with

asthmatic and COPD patients have established a link between goblet cell metaplasia and secretion of inflammatory mediators such as IL-4, IL-9 and IL-13 (Basbaum and Jany, 1990). The participation of these and other cytokines in mucous metaplasia in foxes parasitized with *C. vulpis* is yet to be demonstrated.

Morphometric analyses in the current study revealed that 52% of the bronchiolar plug in parasitized foxes was composed of mucus and the remaining 48% of inflammatory and exfoliated cells. It was also interesting to note that the cellular components of the mucous plugs included neutrophils and not only eosinophils as expected in a parasitic disease (López, 2007; Stockdale and Hulland, 1970). The neutrophil is one important effector cell incriminated in bronchiolar injury in horses with recurrent airway obstruction, a condition also characterized by mucous plugging of the bronchioles (López, 2007). Future quantitative studies using bronchoalveolar lavage should clarify if neutrophils are a major contributor to the bronchoalveolar cellularity in foxes harboring lungworms.

An important issue that needs to be investigated is whether the chronic bronchitis-bronchiolitis with mucus plugging has relevant clinical and welfare implications for parasitized wild foxes. The magnitude of mucous plugging in some trapped foxes was comparable in severity to that seen in bronchioles of horses with clinical evidence of recurrent airway obstruction (heaves). This is an important issue, since these wild foxes were presumed to be relatively healthy at the time of trapping.

CONCLUSION

This investigation demonstrated that foxes harboring lungworms develop a chronic bronchitis and bronchiolitis characterized by substantial bronchial mucous gland hyperplasia, but most remarkably, by severe bronchiolar metaplasia with copious accumulation of mucus in the bronchioles. The magnitude of these changes is similar to that reported in human COPD and therefore parasitized foxes could be a useful animal model to study the pathogenesis of bronchiolar epithelial mucous metaplasia.

REFERENCES

Basbaum, C. and B. Jany, 1990. Plasticity in the airway epithelium. Am. J. Physiol. Lung Cell Mol. Physiol., 259: 38-46.

Behm, C.A. and K.S. Ovington, 1997. The role of eosinophils in parasitic helminth infections: Insights from genetically modified mice. Parasitol Today, 13: 202-209.

Brown, C.C., D.C. Baker and I.K. Barker, 1993. Alimentary system. Pathology of domestic animals. In: Maxie, M.G. (Ed.). Academic Press, San Diego, CA., pp: 1-296.

Durham, S.R., S.J. Till and C.J. Corrigan, 2000. T lymphocytes in asthma: Bronchial versus peripheral responses. J. Allergy Clin. Immunol., 106 (5 Suppl): S221-226.

Gundersen, H.J., T.F. Bendtsen and L. Korbo, 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS., 96: 379-394.

Jamal, K., T.P. Cooney, J.A. Fleetham and W.M. Thurlbeck, 1984. Chronic bronchitis: Correlation of morphologic findings to sputum production and flow rates. Am. Rev. Respir. Dis., 129: 719-722.

Kanobana, K., A. Koets and N. Bakker, 2003. T-cell mediated immune responses in calves primary-infected or re-infected with *Cooperia oncophora*: Similar effector cells but different timing. Int. J. Parasitol., 33: 1503-1514.

López, A., 2007. Respiratory System: Pathologic Basis of Veterinary Diseases. In: McGavin, M.D. and J.F. Zachary (Eds.). 4th Edn. Elsevier, St. Louis, MO., pp: 463-558.

Morrison, E.E. and H.T. Gier, 1979. Parasitic infection of *Filaroides osleri*, *Capillaria aerophila* and *Spirocera lupi* in coyotes from the southwestern United States. J. Wildl. Dis., 15: 557-559.

Nevárez, A., A. López and G. Conboy, 2005. Distribution of *Crenosoma vulpis* and *Eucoleous aerophilus* in the lung of free ranging red foxes (*Vulpes vulpes*). J. Vet. Diagn. Invest., 17: 486-489.

Rendell, S.H., 2006. Airway epithelial stem cells and the pathophysiology of chronic obstructive pulmonary diseases. Proc. Am. Thorac. Soc., 3: 718-725.

Smith, H.J., 1978. Parasites of red foxes in New Brunswick and Nova Scotia. J. Wildl. Dis., 14: 366-370.

Soulsby, E.J.L. and H.O. Moniz, 1982. Helminthes, arthropods and protozoa of domesticated animals. 7th Edn. Lea and Febiger, Philadelphia, PA., pp: 282-342.

Stockdale, P.H. and T.J. Hulland, 1970. The pathogenesis route of migration and development of *Crenosoma vulpis* in the dog. Pathol. Vet., 7: 28-42.

Thurlbeck, W.M., 1988. Chronic Airflow Obstruction. Pathology of the Lung. Thurlbeck. In: W.M. (Ed.). Thieme, New York, pp: 519-575.

Williams, O.W., A. Sharafkhaneh and V. Kim, 2006. Airway mucus: From production to secretion. Am. J. Respir. Cell Mol. Biol., 34: 527-536.

Zeh, J.B., W.B. Stone and D.E. Roscoe, 1977. Lungworms in foxes in New York. Fish Game J., 24: 91-93.