Airway Reactivity, Inflammation, Iron, and Iron-Associated Proteins in Urban versus Rural Horses

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Horses are increasingly housed in urban environments, and are thus exposed to greater amounts of particulate air pollution particles. Iron has been documented to be an important component of particulate air pollution: horses from urban environments had greater amounts of iron and the iron-binding protein, ferritin, in bronchoalveolar lavage fluid, but less airway reactivity, than did horses from rural environments. Authors' addresses: Lung Function Laboratory, Tufts University School of Veterinary Medicine, North Grafton, MA (Mazan, Hoffman); National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Chapel Hill, NC (Ghio). © 2001 AAEP.

1. Introduction
The equine population is no longer purely rural: from police horses in large cities to pleasure horses in suburbia, horses are subject to the effects of air pollution on a daily basis. Exposure to air pollution particulate matter (PM) has been associated with higher human respiratory morbidity, respiratory-related hospital admissions, and mortality,1–3 and declines in lung function in individuals with pre-existing respiratory disease,4,5 as well as mild pulmonary inflammation in healthy individuals.6 Small airway inflammatory disease (SAID), similar to asthma in humans, causes significant morbidity in the equine population,7 and is characterized by airway hyperreactivity (AWHR) and evidence of inflammation in bronchoalveolar lavage fluid.8,9 Air pollution due to particulate matter may play a role in the initiation and maintenance of SAID and other lung injury.

Horses may be even more susceptible than are humans to the adverse respiratory effects of air pollution: they are exposed to the ambient air on a continuous basis, and have a remarkably large minute volume, resulting in high flow rates and the potential for greater exposure of airways and lung tissue to particulate matter in the atmosphere.

Multiple studies have shown that transition metals, especially iron, may be largely responsible for the airway injury and inflammation that are induced with exposure to air pollution particles, due to their ability to initiate lipid peroxidation through production of oxygen-based free radicals.10–14 Increased levels of ferritin, an iron storage protein, and lactoferrin, an iron-binding protein decrease the oxidative stress to the individual.15 Despite the clear association between air pollution and exacerbations of respiratory disease in humans, the connection to AWHR associated with asthma and SAID
remains nebulous. If air pollution can be established to have an adverse affect on equine respiratory health and well-being, then it will be important to monitor and modify as best as possible the environment of those horses living in high pollution areas, such as police horses, urban race horses, and city carriage horses. This retrospective study was conducted to determine if horses from urban environments with concomitantly greater exposure to air pollution particles show evidence of airway inflammation in bronchoalveolar lavage fluid (BALF), airway hyperreactivity on lung function testing and histamine bronchoprovocation, and increased levels of iron and iron transport and storage proteins in BALF, compared to horses from rural environments.

2. Materials and Methods
The Institutional Animal Care and Use Committee at Tufts University School of Veterinary Medicine approved all procedures.

The study population comprised horses that had been presented to the Large Animal Hospital at Tufts University School of Veterinary Medicine for evaluation of poor performance over the summer of 1998. Exclusion criteria included a recent history of infectious disease or EEIPH, or evidence of EEIPH on BAL, as defined by free red cells or hemosiderophages seen on cytology. Urban horses were defined as living less than, and rural horses were defined as living more than, 40 miles from a major city or manufacturing center. Horses from urban (U; n = 13, mean age 6.9 yr ± 5.5) and rural environments (R; n = 11, mean age = 7.6 yr ± 5.5) underwent baseline lung function testing and histamine bronchoprovocation to establish the presence of airway obstruction or AWRH, and BAL to evaluate airway inflammation.

Lung Function and Histamine Bronchoprovocation
Total respiratory system resistance (R_{RS}) was measured using the forced oscillatory system and histamine bronchoprovocation was performed as previously described. Briefly, after measurements of baseline R_{RS}, a succession of doubling concentrations of histamine, starting with 1 mg/ml, were nebulized until R_{RS} at 1 Hz doubled from baseline to establish a PC_{100R_{RS}}.

Bronchoalveolar Lavage
Airway inflammation was determined by measuring inflammatory cells in BALF. Cells were classified as alveolar macrophages, lymphocytes, neutrophils, metachromatic (mast) cells, or eosinophils, and were expressed as percentages of the total count.

Measurement of Iron, Iron-related, and Inflammatory Proteins
L-ferritin and transferrin concentrations were measured using commercially available kits (an enzyme immunoassay and an immunoprecipitin analysis respectively), controls, and standards from Microgen-ics Corporation and INCSTAR Corporation. These assays were modified for use in the Cobas Fara II centrifugal spectrophotometer. Transferrin receptor and lactoferrin were measured with commercially available ELISA kits. Iron concentrations in the supernatant were quantified using inductively coupled plasma emission spectroscopy. Lavage protein and albumin concentrations were determined using the Pierce Coomassie Plus Protein Assay Reagent and an immunoprecipitin assay.

Statistics
Data are expressed as mean values ± SD. Differences between groups R and U; horses with lower airway inflammation (PMN > 5% or mast cells > 2%, n = 15) vs. controls (n = 9); and horses with airway hyperreactivity (PC_{100R_{RS}} < 6 mg/ml histamine, n = 19) vs. controls (n = 5), were examined using t tests of independent means. Significance was assumed at p < 0.05.

3. Results
Urban horses had markedly greater iron levels in BALF (210.8 ± 199.7 ppb) than rural horses (106.6 ± 28.0), approaching statistical significance (p < 0.08). Urban horses also had a trend toward greater levels of ferritin in the BALF (111.9 ± 72.7 ng/ml vs. 82.0 ± 27.8, p < 0.12). Rural horses were markedly more reactive than urban horses (PC_{100R_{RS}} = 2.9 ± 2.0 mg/ml histamine, vs. 7.1 ± 5.7, p < 0.05). There was no evidence of airway obstruction in either group (R horses R_{RS} = 0.60 ± 0.27 cm H_{2}O/L/s; U horses 0.64 ± 0.21). There were no other significant differences among any groups.

4. Conclusions
Many horses now live an urban life, and are exposed to the pollutants that abound in urban ambient air. Because horses are a highly athletic, outdoor species, they are sampling the ambient air, along with the pollutants that it holds, on a continual basis. Many horses suffer from SAID, which can severely inhibit performance. It is hypothesized that human asthma can be caused or exacerbated by air pollution; a similar mechanism may exist with SAID in horses. Interestingly, we found that airway reactivity was higher in rural than in urban horses, despite normal baseline respiratory resistance in both groups. This suggests an innate cause of airway reactivity in the rural group, rather than being attributable purely to airways geometry. It seems likely that environmental triggers other than air particulate matter determined AWRH in this group of horses. This confirms similar findings from studies of human asthmatics. We found higher, although not statistically significant, iron levels in the BALF of urban horses, which may be caused by air pollution particles. Alternatively, the iron may be the result of extravasation from the serum due to airway in-
flammation; however, horses from urban environments had significantly less airway reactivity when tested with histamine bronchoprovocation, and had no greater evidence of airway inflammation in the bronchoalveolar lavage fluid. As we excluded horses with a history or evidence of EIPH, we do not consider this to be a confounding variable. Higher ferritin levels in horses from urban environments may reflect a protective mechanism against oxidative stress by sequestering iron, and may thus explain the lack of greater airway inflammation in urban horses. It is possible that there were too few horses in this study to establish definitively whether increased exposure to particulate air pollution (as suggested by increased iron and ferritin in the BALF of urban horses) is associated with airway inflammation. Although we did not find statistically significant differences between iron and ferritin levels in horses from urban versus rural environments, this is likely due to the small number of horses in the study. The trend toward increased iron and ferritin in BALF in urban horses supports further study in urban versus rural horses. It is only by understanding the role that particulate air pollution plays in the development of lung injury in horses that we can make progress in treating or even preventing lung injury in horses in urban environments. Moreover, horses, by continually sampling the outside air, have the potential to function as a valuable sentinel and model for the role of the environment in airway disease in other species such as humans.

References and Footnotes


aSigma Chemical Company, St Louis, MO 63101.
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fCalbiochem, La Jolla, CA 92037.
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