J Vet Intern Med 2014;28:918–924

Environmental Exposures and Airway Inflammation in Young Thoroughbred Horses

K.M. Ivester, L.L. Couëtil, G.E. Moore, N.J. Zimmerman, and R.E. Raskin

Background: Inflammatory airway disease (IAD) in horses is a widespread, performance-limiting syndrome believed to develop in response to inhaled irritants in the barn environment.

Objectives: To evaluate changes in bronchoalveolar lavage fluid (BALF) cytology and exposure to particulates, endotoxin, and ammonia during horses' first month in training.

Animals: Forty-nine client-owned 12- to 36-month-old Thoroughbred horses entering race training.

Methods: In this prospective cohort study, a convenience sample of horses was assigned to be fed hay from a net (n = 16), whereas the remaining horses were fed hay from the ground (n = 33). BALF was collected at enrollment and after 14 and 28 days in training. Respirable particulate, inhalable particulate, respirable endotoxin, and ammonia concentrations were measured at the breathing zone of each horse weekly.

Results: Median respirable particulates were significantly higher when horses were fed from hay nets than when fed hay from the ground (hay net 0.28 mg/m^3, no hay net 0.055 mg/m^3, $P < .001$). Likewise, inhalable particulate (hay net 8.3 mg/m^3, no hay net 3.3 mg/m^3, $P = .0064$) and respirable endotoxin (hay net 173.4 EU/m^3, no hay net 59.2 EU/m^3, $P = .018$) exposures were significantly higher when horses were fed from hay nets. Feeding hay from a net resulted in significantly higher BALF eosinophil proportions over time ($P < .001$). BALF eosinophils were significantly related to respirable particulate exposure (14 days in training $r_s = 0.37$, $P = .012$, 28 days in training, $r_s = 0.38$, $P = .017$).

Conclusions and Clinical Importance: Pulmonary eosinophilic inflammation develops in response to respirable particulate exposure in young Thoroughbreds, indicating a potential hypersensitivity to inhaled particulate allergens.

Key words: Bronchoalveolar lavage; Cytology; Endotoxin; Eosinophils; Particulates.

Inflammatory airway disease (IAD) in horses is a widespread syndrome in which inflammation of the lower airways results in impaired gas exchange and poor performance.[1] As the most common chronic airway disease of equine athletes, the prevalence of IAD in racing 2-year-olds has been estimated to be as high as 80%.[2] IAD is the second most common cause of lost use and need for veterinary care in young racehorses.[3] Though particularly prevalent in this population, the disease impacts welfare and performance of equine athletes across all disciplines. The clinical signs of cough, poor performance, and excess mucus in the airways can be subtle and difficult to differentiate from cases of respiratory infection. Diagnosis is confirmed by demonstration of increased percentages of neutrophils, mast cells, eosinophils, or combination of inflammatory cell types in bronchoalveolar lavage fluid (BALF), lower airway obstruction, airway hyperresponsiveness, or impaired gas exchange in the absence of both infection and increased respiratory effort at rest.[1] Phenotype can vary, with young horses often exhibiting increased proportions of eosinophils and mast cells in BALF, suggesting hypersensitivity.[1] In addition, both increased BALF mast cell and eosinophil percentages are associated with airway hyperresponsiveness and poor performance.[4–6] Different IAD phenotypes are likely to reflect differences in etiology and pathophysiologic mechanisms.

Abbreviations:

BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
EU	endotoxin units
IAD	inflammatory airway disease

Exposure to airborne dust and other irritants present in the barn environment appears to play a major role in pathogenesis of IAD. Development of airway inflammation in otherwise healthy horses occurs upon introduction to barn confinement,[7,8] and higher dust environs increase the degree of airway inflammation,[9,10] as do higher respirable endotoxin concentrations.[9] Furthermore, challenge by inhalation of endotoxin recruits neutrophils to the alveolar space in a dose-dependent manner.[11] Experimentally, exposure to gaseous ammonia also induces airway inflammation in the horse,[12] and naturally occurring exposures greater than 2 ppm increase the risk of tracheal neutrophilic inflammation.[9] While the barn environment has thus been strongly implicated in the development of IAD, the pathogenesis of the disease remains largely unknown.[1,5,7] As the carriers of aeroallergens, particulates could be expected to induce eosinophilic and mastocytic airway inflammation if this phenotype does indeed arise as a consequence of hypersensitivity; however, research directly linking changes in

From the Department of Veterinary Clinical Sciences (Ivester, Couëtil), the Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine (Moore, Raskin), and the School of Health Sciences (Zimmerman), Purdue University, West Lafayette, IN.

Corresponding author: Kathleen M. Ivester, Veterinary Clinical Sciences, Purdue University, 625 Harrison Street, West Lafayette, IN 47907; e-mail: kivester@purdue.edu

Submitted August 21, 2013; Revised December 6, 2013; Accepted January 21, 2014.

DOI: 10.1111/jvim.12333

BALF cytology to measures of natural environmental exposure is lacking. Attempts to develop prevention and treatment strategies are hindered by incomplete knowledge of the etiology and pathophysiology of the syndrome.

Therefore, the objectives of the study were to evaluate changes in BALF cytology in Thoroughbred horses over the course of their first month in training while measuring individual horse exposure to particulates, endotoxin, and ammonia at the breathing zone in order to test the hypotheses that (1) IAD is highly prevalent in young Thoroughbreds, with relative increases in BALF mast cells and eosinophils occurring most commonly; (2) individual horse exposure to airborne particulates can be influenced by the method by which hay is fed; and (3) airway inflammatory phenotype is associated with the type of environmental exposure. Specifically, the percentage of BALF neutrophils correlates with ammonia and endotoxin exposures, whereas eosinophils and mast cells correlate with particulate exposures.

Materials and Methods

Twelve- to 36-month-old Thoroughbreds entering race training were recruited upon arrival at a local facility if they had no evidence of respiratory or other systemic disorder upon physical examination and complete blood count, no prior history of race training, and enrollment and initial evaluation performed within 6 days of arrival.

Upon enrollment, eligible horses had physical examination, blood collection, and BAL performed at the facility. In order to ensure variation in exposure, each horse was then arbitrarily assigned by the assistant trainer to be fed hay exclusively from a hay net or from the ground. Horses assigned to the hay net group were a convenience sample of enrolled horses because of the increased labor required of barn staff to feed hay from a net for the duration of the horses' enrollment in the study. All horses were bedded on sawdust and fed oats and a mixture of grass and alfalfa hay. Physical examination and BAL were repeated on days 14 and 28. Respirable particulate, inhalable particulate, and ammonia concentrations were measured continuously at the breathing zone of each horse 1 day each week over the course of 4–6 hours. On each occasion, sampling was conducted between the hours of 10 AM and 4 PM. Endotoxin content of respirable particulate samples was determined.

IAD was diagnosed on the basis of BALF differential cytology counts. Horses with >5% neutrophils, >2% mast cells, >1% eosinophils, or any combination thereof were classified as IAD.[1]

Blood was collected by direct jugular venipuncture into an EDTA tube for complete blood count on day 0 only. Fresh feces were collected at enrollment, 14 days, and 28 days and submitted for quantitative egg counts.

BAL was performed while horses were sedated with detomidine hydrochloride (0.01–0.02 mg/kg IV) and butorphanol tartrate (0.01 mg/kg; IV). A sterile BAL tube[a] (10 mm outer diameter) was passed through the nose and wedged into a peripheral bronchus. Local anesthesia was achieved with delivery of 60 mL of a 0.4% lidocaine solution during tube passage, and 250 mL of 0.9% NaCl was infused and recovered manually. Manual and automated cell counts were performed on fresh BALF. Cytologic specimens were prepared by cytospin centrifugation and processed with modified Wright stain. Differential cell counts were performed on a minimum of 400 total cells.

Air Quality

Ammonia exposure was determined with ammonia monitor badges[b] secured to the halter, near the nostril of the horse. The badges provided a time-weighted average ammonia concentration with a range of 3–600 ppm × h.

Particulate filter sampling was conducted with personal samplers.[c] The respirable fraction was collected onto 37-mm type AE glass fiber filters with the aluminum cyclone[d] (50% collection efficiency at 4 μm) with a flow rate of 2.5 L/min. The inhalable fraction was collected onto 25 mm PVC filters with the Institute of Occupational Medicine (IOM) personal sampler[d] (50% collection efficiency at 100 μm) with a flow rate of 2.0 L/min. Sampling pumps were calibrated before and after sampling.[e] The cyclone and IOM sampler were secured to the noseband of the halter in order to sample dust at the breathing zone of the horse. Flexible tubing[f] connected samplers to the pumps, which were secured to a surcingle placed around the girth of the horse. The horse was free to move around the stall as usual. Before and after sampling, filters were placed in a desiccator for at least 18 hours before being weighed. The weight of particulates was determined gravimetrically. The weight of particulates was divided by volume of air sampled to obtain airborne particulate concentration in mg per cubic meter of air. Filters were stored at −20°C until endotoxin analysis.

Endotoxin content of the respirable dust was determined by a kinetic chromogenic limulus amebocyte lysate (LAL) technique.[g] Endotoxin extraction from respirable particulates was conducted in a sterilized laboratory hood with 10 mL nonpyrogenic water for elution. Polystyrene sample vials were agitated end-over-end for 1 hour at room temperature, followed by centrifugation at 1,000 *g* for 10 min. Supernatant was analyzed immediately in duplicate. Endotoxin activity was divided by volume of air sampled to obtain respirable endotoxin concentration in endotoxin units per cubic meter of air.

Informed consent was obtained for each horse from the trainer or owner, and the Purdue Animal Care and Use Committee approved all procedures.

Statistical Analysis

Weekly exposure measurements were averaged for each horse. Differences in exposure between groups were evaluated with Wilcoxon rank sums. Correlations among particulate, endotoxin, and ammonia exposures were evaluated by Spearman rank correlation, as were correlations between average exposure and BALF cytology variables at day 14 and day 28, and the change in cytology variables. Those exposure variables correlated with cytology variables with $P < .2$ were chosen for inclusion in a generalized linear mixed model of cell proportions. Generalized linear models were constructed to judge the effect of hay net group assignment and exposures upon cell proportions and total nucleated cell counts (TNCC) over time by the logit link function under a binomial distribution. Mixed models included the random effect of horse upon model intercept and slope parameters. Marginal models without horse effect were constructed to provide estimates of population-averaged response to exposures and hay net assignment. Statistical significance was set at $P < .05$, and significance of pairwise comparisons was controlled by Tukey's posthoc method. Data analysis was performed using statistical software.[h]

Results

Between May 2009 and October 2012, 49 horses were recruited and enrolled into the study (Fig 1). Horses were enrolled a median (range) of 4 (0–6) days after

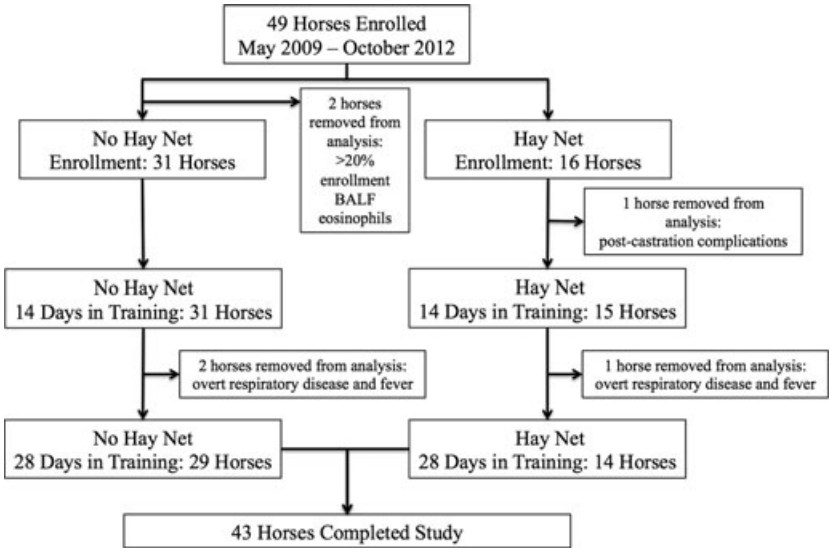

Fig 1. Flow diagram of study subject enrollment and exclusion.

arriving at the facility. Between arrival and enrollment, all horses were fed hay from the ground. Two horses assigned to be fed hay from the ground had proportions of eosinophils in BALF greater than 20% and were removed from all analyses because of the likelihood that these horses had previous respiratory disease or extreme exposures that could confound results. As a result, data from 47 horses were analyzed.

At enrollment, cytology data were not available for 1 horse and 35/46 horses (76%) had IAD. At 14 and 28 days in training respectively, 34/46 (74%) and 33/43 (77%) horses had cytologic differential counts in BALF indicative of IAD (Table 1).

Exposure and cytology measurements for 13 horses fed hay exclusively from hay nets were compared to 28 horses fed hay from the ground. Hay net feeding resulted in significantly higher respirable and inhalable particulate exposures ($P < .001$, $P = .0064$, respectively; Fig 2, Table 2). Similarly, respirable endotoxin exposures were significantly higher in the hay net group ($P = .018$). No difference in ammonia exposure was detected between groups ($P = .36$). While the number of horses with eosinophilic IAD did not differ between groups (Table 1), significant interaction between hay net assignment and time on eosinophil proportions in BALF was demonstrated ($P < .001$, Fig 3). BALF TNCC, mast cell proportions, and neutrophil proportions did not differ between groups at any time point. Inclusion of the 2 previously excluded horses with profound BALF eosinophilia at enrollment had no effect upon model significance and minimal effect upon model parameters (data not shown).

There was a significant correlation between respirable particulate exposure and both inhalable particulate and respirable endotoxin exposures (Table 3). Respirable particulates, inhalable particulates, respirable endotoxin, ammonia, number of days in barn before enrollment as well as the number of days in training, and the random effect of horse were chosen for inclusion in model building. There was no evidence of correlation between fecal ova counts and BALF eosinophils (Table 4). Respirable particulate exposure, number of days in training, and random horse effect remained significant in the exposure model ($P < .001$ for each), and this generalized linear mixed model fit the observed data well. When the random factor of horse was removed from the model, the resulting marginal model describes the population-averaged response to respirable particulates according to the equation below (Fig 4): LN (% Eosinophil/100 − % Eosinophils) = −5.3 + 0.75 (Dust) − 0.009 (Days) + 0.03248 (Days × Dust)

Where LN is the natural log, Dust is the average respirable particulate exposure in mg/m^3, Days is the number of days in training, and Days × Dust is the interaction term between days in training and respirable particulate exposure. Inclusion of the 2 horses with profound BALF eosinophilia at enrollment in the model had no effect upon model significance and minimal effect upon model parameters (data not shown).

Inhalable particulates, respirable endotoxin, ammonia, or number of days in the barn before enrollment satisfied Spearman rank correlation criteria but did not achieve statistical significance or improve model fit. None of the measured exposure variables accounted for significant variation in either BALF mast cells or neutrophils over time.

Discussion

The majority of young Thoroughbred horses were diagnosed with IAD during their first month in training in this study. Elevation of hay in a net resulted in increased exposure to particulates and endotoxin, but did not affect ammonia exposure. The increased particulate exposures of horses fed hay from a net were accompanied by an increase in eosinophil proportions in BALF.

Table 1. Prevalence and cytologic phenotype of inflammatory airway disease (IAD).

Days in Training	Number of Horses		Number (%) [95% CI] Classified as IAD		Number (%) [95% CI] with Increased% Neutrophils		Number (%) [95% CI] with Increased% Mast Cells		Number (%) [95% CI] with Increased% Eosinophils	
0	46*	NoHN: 30*	NoHN: 22 (73) [55–86]	HN: 13 (81) [56–94]	NoHN: 4 (13) [5–30]	HN: 2 (12.5) [2–37]	31 (69) [54–81]	NoHN: 20 (67) [49–81] HN: 11 (69) [44–86]	14 (31) [19–46]	NoHN: 9 (30) [17–48] HN: 5 (31) [14–56]
		HN: 16	35 (76) [62–86]		6 (13) [6–27]					
14	46	NoHN: 31	NoHN: 25 (81) [63–91]	HN: 9 (60) [36–80]	NoHN: 6 (19) [9–37]	HN: 4 (27) [10–52]	29 (63) [49–76]	NoHN: 21 (68) [50–82] HN: 8 (53) [30–75]	14 (31) [19–46]	NoHN: 7 (23) [11–40] HN: 7 (47) [25–70]
		HN: 15	34 (74) [60–85]		10 (22) [12–36]					
28	43	NoHN: 29	NoHN: 21 (72) [54–86]	HN: 12 (86) [59–97]	9 (21) [11–35]	HN: 4 (29) [11–55]	22 (51) [37–65]	NoHN: 15 (52) [34–69] HN: 7 (50) [27–73]	12 (28) [17–43]	NoHN: 7 (24) [12–42] HN: 5 (36) [16–61]
		HN: 14	33 (77) [62–87]							

CI, confidence interval; NoHN, no hay net group; HN, hay net group.
IAD diagnosed by an increase in 1 or more inflammatory cell population.
*Cytology data missing for 1 horse because of nondiagnostic cytologic preparation.

Fig 2. Comparison of particulate exposures between groups. Dark gray = respirable particulates; light gray = inhalable particulates; Line = median; triangle = mean respirable particulates; diamond = mean inhalable particulates; box = interquartile range; whiskers = range; open circles = outliers; ***$P < .001$, **$P = .0064$.

IAD was highly prevalent in the study population, with relative increases in mast cells and eosinophils the predominant abnormality and mast cells >2% in BALF in 31/46 horses at enrollment. These findings are similar to the diagnosis of mastocytic IAD in 10/13 adult sporthorses confined to stalls bedded with straw.[13] While no other studies report IAD prevalence in young racehorses by cytologic analysis of BALF for diagnosis, prevalence of increased tracheal mucus in a similar population of young Thoroughbreds reached only 20%.[2] The impact of mastocytic airway inflammation during the first month of training upon later training and racing performance is unknown. Exercise intolerance,[5,6] increased airway reactivity,[5] and pulmonary dysfunction[14] have been associated with BALF mastocytosis, but it is not known how long airway inflammation persists. Estimates of IAD duration range from 15.5 days when disease is defined as increased tracheal mucus, flocculent tracheal lavage fluid, or both[15] to 8 weeks by a disease definition of increased visual tracheal mucus and increased tracheal lavage neutrophils.[2]

Hay net assignment resulted in significantly different exposures to respirable and inhalable particulates and respirable endotoxin, with higher concentrations measured when hay was fed from a net. Correspondingly, proportions of eosinophils in BALF were significantly higher in the hay net group when compared to the no hay net group after 14 and 28 days in training. The effect of hay net feeding appears to arise from increased respirable particulates, as evidenced by the highly significant effect of respirable particulate exposure upon the proportion of eosinophils in BALF. Comparison of the accuracy with which the mixed model and the marginal model fit the observed data highlights the magnitude of the random effect of horse,

Table 2. Exposure of horses to particulates, endotoxin, and ammonia.

	Respirable Particulates (mg/m^3)	Inhalable Particulates (mg/m^3)	Ammonia (ppm)	Respirable Endotoxin (EU/m^3)
Hay net	0.28 (0.039–2.4)***	8.3 (2.8–19.4)**	2.87 (0.96–3.77)	173.4 (32.4–997.6)*
No hay net	0.055 (ND–1.01)***	3.3 (0.50–9.8)**	3.5 (1.2–12.7)	59.2 (6.9–730.9)*

Median (range). ND, not detectable; limit of detection = 0.028 mg/m^3.
***P < .001, **P = .0064, *P = .018.

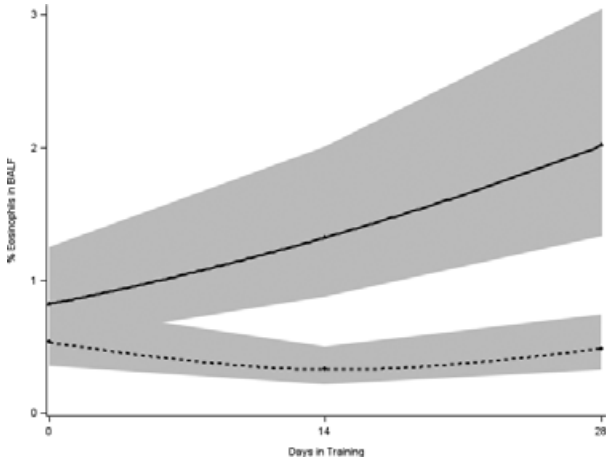

Fig 3. Marginal generalized linear model of predicted % eosinophils in BALF over time. Dotted line = no hay net group; solid line = hay net group; bands = 95% confidence intervals of predicted marginal means. BALF, bronchoalveolar lavage fluid.

Table 3. Spearman rank correlation between concentrations of particles, endotoxin, ammonia, in the breathing zone of horses.

	Respirable Particulates	Inhalable Particulates	Respirable Endotoxin
Respirable Particulates	1		
Inhalable Particulates	**0.56 (0.0016)**	1	
Respirable Endotoxin	**0.65 (< 0.001)**	0.35 (0.072)	1
NH_3	−0.13 (0.35)	−0.12 (0.52)	−0.049 (0.67)

R_s (P-value). Statistically significant correlations are in bold.

likely a reflection of individual variation in susceptibility to eosinophilic airway inflammation.

In humans, airway eosinophilia is considered a hallmark of atopic asthma, and the role of eosinophils as antigen-presenting cells, regulators of the inflammatory response, or destructive effector cells is a topic of active debate and research.[16–19] The recruitment of eosinophils to the airway and surrounding bronchial tissue after allergen challenge in atopic asthmatic subjects has long been recognized.[20,21] In the horse, BALF eosinophilia is associated with clinical signs of respiratory disease and airway hyperreactivity.[4] In yearling Thoroughbred colts, there is an association

Table 4. Spearman rank correlation between measures of exposure to particles, endotoxin, ammonia, and fecal ova counts and proportion of eosinophils in bronchoalveolar lavage fluid (BALF).

	% Eosinophils in BALF	
	14 Days in Training	28 Days in Training
Respirable Particulates [mg/m^3]	0.37 (0.012)	0.38 (0.017)
Inhalable Particulates [mg/m^3]	0.32 (0.085)	0.36 (0.088)
Respirable Endotoxin [EU/m^3]	0.34 (0.020)	0.37 (0.018)
NH_3 [ppm]	−0.29 (0.062)	−0.27 (0.088)
Number of days in barn before enrollment	−0.28 (0.11)	−0.32 (0.10)
Ova [eggs/g]	−0.041 (0.80)	0.29 (0.79)

R_s (P-value).

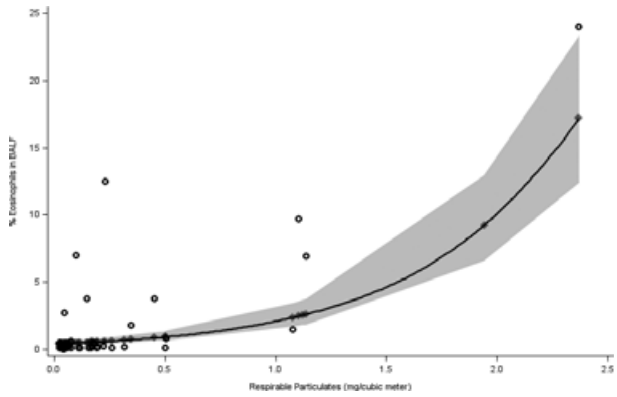

Fig 4. Fit plot of marginal generalized linear model at 28 days in training: % eosinophils in BALF versus respirable particulate exposure. Circles = observed; line = marginal generalized linear mixed model; band = 95% confidence interval of the predicted marginal mean. BALF, bronchoalveolar lavage fluid.

between increased BALF neutrophils, eosinophils, and TNCC and race training but not stabling.[22] No measures of particulate or endotoxin exposures were made, so conclusions cannot be drawn between the severities of exposure compared to this study. In the current report, all horses were entering training and underwent similar physical activity, so the effect of exercise upon the observed relationship between eosinophilic airway inflammation and particulate exposure

cannot be determined. BALF eosinophilia is recognized in cases of pulmonary parasite migration[23] and has also been proposed to be an indicator of intestinal parasitism.[24] There was no evidence of a relationship between BALF eosinophils and intestinal parasite load as judged by quantitative fecal flotation in this study. Eosinophilic inflammation of the airway was significantly associated with respirable particulate exposure.

Contrary to our hypothesis, the proportion of neutrophils in BALF was not related to respirable endotoxin or ammonia exposures. The median respirable endotoxin concentration measured at the breathing zone of horses in the hay net group (21.6 ng/m^3) exceeds that which induces neutrophilic inflammation in otherwise healthy mature control horses (3.95 ng/m^3).[11] While care must be taken when comparing endotoxin concentrations among studies with different sampling, handling, and assay protocols,[25] our results support an age-related difference in the response of our study population, rather than insufficient exposure. The time-weighted average ammonia exposure measured in this study exceeded the 2 ppm threshold associated with neutrophilic inflammation detected by cytology of tracheal lavage fluid.[9] Cytology often differs drastically between the BAL and tracheal wash fluids,[26] and the relationship between IAD as it is currently defined and the syndrome of tracheal neutrophilic inflammation is unknown.[1,27]

The relative importance of respirable particulate exposure over that of inhalable particulate and respirable endotoxin requires further evaluation in an environment with less pronounced correlation between exposures. There is a synergistic effect between particulates and endotoxin in eliciting a neutrophilic inflammatory response from the airway of mature horses exposed to fractionated hay dust suspension.[28] Similar experimental challenge studies in juvenile horses might demonstrate comparable synergy that was not discernable in this observational study.

Proportions of mast cells in BALF showed minimal evidence of response to the environmental exposures measured in this study. Mast cell counts and percentages had little within-horse variation over the course of the first month in training, potentially indicating a resident pulmonary function for this cell in juvenile horses. Postmortem examination of the respiratory tract of healthy adult horses ranging in age from 2 to 12 years has confirmed the presence of mast cells at each level of the respiratory tract, with 35% of mast cells found in the connective tissue surrounding blood vessels, 20% in the airway walls, 15% in alveolar walls, and less than 3% in the alveolar spaces.[29] There is significant association between the Thoroughbred breed and airway inflammation that includes increased BALF eosinophils, mast cells, or both,[30] and this association might partially explain the prevalence of mastocytic and eosinophilic inflammation seen in this study.

In contrast to the current report, Halflinger horses between 6 and 14 years of age exhibit a positive correlation between BALF mast cell percentages and particulate exposure, with significant within-horse variation under differing environmental conditions.[31] The disparity between this study and the current report further emphasizes the importance of age in determining airway response to exposure and highlights a possible effect of breed.

In conclusion, this cohort exposure study of Thoroughbreds entering training confirms that airway inflammation in young horses most commonly manifests as an increase in airway mast cells, eosinophils, or both. Furthermore, in this population, recruitment of eosinophils to the airway is associated with respirable particulate exposure. This finding supports the hypothesis that IAD develops in response to inhaled environmental irritants and offers the first epidemiologic evidence that eosinophilic IAD might represent a hypersensitivity to inhaled particulate allergens.

Footnotes

[a] Mila International Inc, Erlanger, KY
[b] ChromAir badge, Morphix Technologies, Virginia Beach, VA
[c] AirCheck 2000, SKC, Inc, Eighty Four, PA
[d] SKC, Inc, Eighty Four, PA
[e] Defender Bios calibrator, SKC, Inc
[f] Tygon, Saint Gobain, Courbevoie, France
[g] Kinetic-QCL, Lonza, Basel, Switzerland
[h] SAS statistical software, release 9.3, SAS Institute, Inc, Cary, NC

Acknowledgments

The authors thank Donna Griffey, Aurea Lucas, Katie Smith, Casey Cromer, and Kyle Clever for their technical assistance; Whitney Huang for statistical assistance; Dr Bill Hope, Dr Amanda Farr, and Dr Katie Naughton for their help in the collection of clinical samples; Mr James Elliott and Mr Jeremy Stahley for facilitating this study at their facility; and the owners of the horses for allowing the use of these animals in the study. This study was supported by the state of Indiana and the Purdue University College of Veterinary Medicine research account funded by the total wager tax and the SVM Internal Competitive Small and Agricultural Animal Disease Research Funds Program.

Conflict of Interest: Authors disclose no conflict of interest.

References

1. Couetil LL, Hoffman AM, Hodgson J, et al. Inflammatory airway disease of horses. J Vet Intern Med 2007;21:356–361.

2. Wood JL, Newton JR, Chanter N, et al. Inflammatory airway disease, nasal discharge and respiratory infections in young British racehorses. Equine Vet J 2005;37:236–242.

3. Wilsher S, Allen WR, Wood JL. Factors associated with failure of thoroughbred horses to train and race. Equine Vet J 2006;38:113–118.

4. Hare JE, Viel L. Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. J Vet Intern Med 1998;12:163–170.

5. Hoffman AM, Mazan MR, Ellenberg S. Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. Am J Vet Res 1998;59:176–181.

6. Bedenice D, Mazan MR, Hoffman AM. Association between cough and cytology of bronchoalveolar lavage fluid and pulmonary function in horses diagnosed with inflammatory airway disease. J Vet Intern Med 2008;22:1022–1028.

7. Holcombe SJ, Jackson C, Gerver V, et al. Stabling is associated with airway inflammation in young Arabian horses. Equine Vet J 2001;33:244–249.

8. Tremblay GM, Ferland C, Lapointe JM, et al. Effect of stabling on bronchoalveolar cells obtained from normal and COPD horses. Equine Vet J 1993;25:194–197.

9. Malikides N, Hodgson JL. Inflammatory Airway Disease in Young Thoroughbred Racehorses. Rural Industries Research and Development Corporation Publication No 03/089.2003.

10. Wyse CA, Skeldon K, Horchkiss JW, et al. Effects of changes to the stable environment on the exhalation of ethane, carbon monoxide, and hydrogen peroxide by horses with respiratory inflammation. Vet Rec 2005;157:408–412.

11. Pirie RS, Dixon PM, Collie DDS, McGorum BC. Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. Equine Vet J 2001;33:311–318.

12. Katayama Y, Oikawa M, Yoshihara T, et al. Clinicopathological effects of atmospheric ammonia exposure on horses. J Equine Sci 1995;21:99–104.

13. Gerber V, Robinson NE, Luethi S, et al. Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. Equine Vet J 2003;35:491–495.

14. Richard EA, Fortier GD, Denoix J-M, et al. Influence of subclinical inflammatory airway disease on equine repiratory function evaluated by impulse oscillometry. Equine Vet J 2009;41:384–389.

15. Ramzan PHL, Parkin TDH, Shepherd MC. Lower respiratory tract disease in Thoroughbred racehorses: Analysis of endoscopic data from a UK training yard. Equine Vet J 2008;40:7–13.

16. Walsh ER, August A. Eosinophils and allergic airway disease: There is more to the story. Trends Immunol 2010;31:39–44.

17. Lee JJ, Jacobsen EA, McGerry MP, et al. Eosinophils in health and disease: The LIAR hypothesis. Clin Exp Allergy 2010;40:563–575.

18. Akuthota P, Xenakis JJ, Weller PF. Eosinophils: Offenders or general bystanders in allergic airway disease and pulmonary immunity? J Innate Immun 2011;3:113–119.

19. Wegman M. Targeting eosinophil biology in asthma therapy. Am J Respir Cell Mol Biol 2011;45:667–674.

20. Aalbers R, Kauffman HF, Vrugt B, et al. Allergen-induced recruitment of inflammatory cells in lavage 3 and 24 h after challenge in allergic asthmatic lungs. Chest 1993;103:1178–1184.

21. De Monchy JG, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985;131:373–376.

22. Michelotto PV Jr, Muehlmann LA, Zanatta AL, et al. Platelet-activating factor and evidence of oxidative stress in the bronchoalveolar fluid of Thoroughbred colts during race training. J Vet Intern Med 2010;24:414–419.

23. Dixon PM, Railton DI, McGorum BC. Equine pulmonary disease: A case control study of 300 referred cases, Part 3: Ancillary diagnostic findings. Equine Vet J 1995;27:428–435.

24. Riihimäki M, Lilliehöök I, Raine A, et al. Clinical alterations and mRNA levels of IL-4 and IL-5 in bronchoalveolar cells of horses with transient pulmonary eosinophilia. Res Vet Sci 2008;85:52–55.

25. Duquenne P, Marchand G, Duchaine C. Measurement of endotoxins in bioaerosols at workplace: A critical review of literature and a standardization issue. Ann Occup Hyg 2013;57:137–172.

26. Derksen FJ, Brown CM, Sonea I, et al. Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. Equine Vet J 1989;6:23–26.

27. Cardwell JM, Christley RM, Gerber V, et al. What's in a name? Inflammatory airway disease in racehorses in training. Equine Vet J 2011;43:756–758.

28. Pirie RS, Dixon PM, McGorum BC. Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves. 3: Effect of fractionation of HDS. Equine Vet J 2002;34:343–347.

29. Mair TS, Stokes CR, Bourne FJ. Distribution and ultrastructure of mast cells in the equine respiratory tract. Equine Vet J 1988;20:54–58.

30. Nolen-Walston RD, Harris M, Agnew ME, et al. Clinical and diagnostic features of inflammatory airway disease subtypes in horses examined because of poor performance: 98 cases (2004-2010). J Am Vet Med Assoc 2013;242:1138–1145.

31. Ferro E, Ferrucci F, Salimei E, et al. Relationship between the conditions of lower airways in healthy horses, environmental factors and air quality in stables. Pferdeheilkunde 2000;16:579–586.