Age Effects on Blood Gas, Spirometry, Airway Reactivity, and Bronchoalveolar Lavage Fluid Cytology in Clinically Healthy Horses

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Background: Despite the increasing number of geriatric horses attended by veterinarians, there is a lack of understanding of aging-related changes on the respiratory system of horses.

Objective: To identify aging-related changes on the respiratory function and bronchoalveolar lavage fluid (BALF) cytology of horses.

Animals: Fifteen healthy young adult (2–11 years) and 16 healthy aged ($\geq$20 years) horses.

Methods: The respiratory system was examined by measurement of arterial blood gases (ABG), use of respiratory inductive plethysmography (RIP) for assessment of breathing pattern and ventilatory parameters, histamine bronchoprovocation, and BALF cytology.

Results: No significant differences were detected with regard to values obtained by ABG or bronchoprovocation of young adult and aged healthy horses. In aged horses, there were significant differences in mean $\pm$ SD of the following parameters when compared to young horses: prolonged expiratory time (Te) measured by RIP (3.9 $\pm$ 1.5 s versus 3.0 $\pm$ 0.6 s), decreased percentage of alveolar macrophages (40.6 $\pm$ 11.3% versus 53.5 $\pm$ 9.6%), and increased percentage of lymphocytes (53.4 $\pm$ 9.5% versus 43.9 $\pm$ 11.0%). No correlations between airway reactivity and ventilatory parameters, ABG, or BALF cytology were found in this asymptomatic population.

Conclusions: These results suggest that aging does not cause changes in the results obtained by ABG, most RIP-derived variables, and bronchoprovocation in the horse. A decreased percentage of macrophage and an increased percentage of lymphocytes in the BALF cytology may be expected in the asymptomatic geriatric horse and may be a result of aging.

Key words: Aging in horses; Arterial blood gases; Geriatric; Pulmonary function testing.

W ith the high prevalence of older horses and potential for increasing lifespan of elderly horses presented to veterinarians,[1,2] a thorough understanding of normal aging-related changes is of paramount importance. Aging results in structural and functional modifications in all mammals. These changes must be recognized as normal features of aging rather than pathologic changes.

Aging-related changes in pulmonary function are well characterized in human medicine. Aging of the lungs is associated with decreased pulmonary elastic recoil, chest wall compliance, and respiratory muscle strength, as well as an increase in alveolar dead space.[3,4] The basis for these functional changes includes loss of elastin content and resultant increase airspace dimensions. Functional consequences of the morphologic alterations in the lungs of older people include increased residual volume and functional

Abbreviations:

ABG	arterial blood gas
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BCS	body condition score
CBC	complete blood count
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
PC	provocative concentration of histamine
PEF	peak expiratory flow
PIF	peak inspiratory flow
RIP	respiratory inductive plethysmography
Te	expiratory time
Ti	inspiratory time
V_E	minute ventilation
V_T	tidal volume
Δ_{flow}	delta flow

residual capacity, decreased maximal expiratory flow rate and forced vital capacity, lower diffusion capacity, and higher ventilation/perfusion mismatch.[5] Although no clinical signs are seen in healthy subjects as a result of these functional changes, there is a decrease in the reserve capacity of the lung in the presence of disease and these changes may impact exercise capacity.[3] In conjunction with this decline in lung function, over 45% of elderly humans demonstrate an increase in airway responsiveness to nonspecific agonists such as histamine.[6] Moreover, elderly humans have been shown to have increased airway inflammation.[7,8]

In the horse, aging-related changes in pulmonary structure and function are poorly characterized. To

our knowledge, only 2 studies have been performed that addressed aging-related changes to the respiratory system of horses. These studies indicate that older horses experience both functional and biochemical changes with age. One study showed that horses > 20 years of age have lower arterial oxygen and carbon dioxide partial pressures and higher alveolar to arterial pressure gradient compared with horses 3–8 years of age.[9] The other study included horses of 6–25 years of age and showed that surfactant phospholipid content decreased with age in horses.[10] The age-related changes in lung function (ie, spirometry), airway reactivity, and BALF cytology in mature horses have not been investigated previously. The purpose of this study was to investigate the influence of aging on arterial blood gases, lung function, response to histamine bronchoprovocation, and cytology of BALF.

Material and Methods

Animals

Clinically normal horses between the ages of 2 and 11 years (young adults) and horses ≥20 years of age (aged horses) were selected for this study. Horses were excluded from this study if they were currently pregnant or if they had clinical signs (eg, cough, nasal discharge) or a diagnosis of inflammatory or infectious respiratory disease in the previous 2 years. Animals that had received steroidal anti-inflammatory drugs, bronchodilators, antibiotics, or some combination of these during the 2 weeks preceding the study also were excluded. All horses were free of clinical signs of pituitary pars intermedia dysfunction. All procedures were performed on resident farms of the horses, without prior fasting. Written client consent was obtained for all horses before enrollment in the study and all procedures were approved by the Tufts University Clinical Sciences Research Committee, the Tufts University Institutional Animal Care and Use Committee, and the University of Connecticut Institutional Animal Care and Use Committee.

Design

Prospective cross-sectional study. A complete history, physical examination, and CBC were obtained before study enrollment to exclude horses with any evidence of preexisting clinical respiratory disease. Horses were given a body condition score (scale 1–9) and body weight was estimated using a flexible measuring tape. Ambient temperature and humidity were recorded before histamine bronchoprovocation. The respiratory system of each horse was evaluated by the measurement of arterial blood gas, RIP, histamine bronchoprovocation, and BALF cytology during a single farm visit.

Arterial Blood Gas

Horses were manually restrained with a lip twitch and 2 mL of arterial blood was obtained by puncture of the transverse facial artery, using a 25-gauge needle and a 3-mL plastic syringe[a] with no anticoagulant added, while the horse was breathing room air. The sample was immediately sealed to avoid air exposure and analyzed using a hand-held analyzer.[b] Partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were measured in whole blood using a stall-side commercial cartridge-based system.[c] Rectal temperatures on the horses ranged between 36.6 and 38°C. Blood gas values were not corrected for the patient's rectal temperature.

RIP and Pneumotachography

Respiratory inductive plethysmography was performed using a commercial system[d] in accordance with the methods previously described for horses.[11] Briefly, each horse was sedated with detomidine[e] (0.01–0.02 mg/kg BW, IV) and fitted with a face mask, pneumotachograph, and abdominal and thoracic inductance bands. Airflow and thoracic and abdominal volume changes were simultaneously measured during spontaneous breathing. Airway obstruction (delta flow, Δ_{flow}), thoracoabdominal synchronicity (phase angle, Φ), and ventilatory parameters were obtained.

Histamine Bronchoprovocation

This procedure was performed using a commercial flowmetric plethysmography system[d] as previously described.[12] Briefly, after measurement of baseline Δ_{flow}, 0.9% saline (negative control) was nebulized for 2 minutes and measurement of Δ_{flow} was immediately repeated. Then, incremental concentrations of histamine[f] (4, 8, 16, and 32 mg/mL) were administered by nebulization for 2 minutes until a ≥75% increase in Δ_{flow} was recorded or the maximum concentration (32 mg/mL) was reached. All nebulization was performed using a low dead space face mask and a portable air compressor[g] and nebulizers[h] that generate particles with a median mass aerodynamic diameter of <3 μm. Data collection was initiated immediately after nebulization with recording periods of 3 minutes. A dose–response curve was generated by the computer software, and the provocative concentration of histamine resulting in a 35% increase (PC35) and a 50% increase (PC50) over the postsaline Δ_{flow} was obtained. Horses with PC35 ≤8 mg/mL were classified as hyper-reactive for categorical comparisons between groups.

BAL and Evaluation of BALF

720 μg of inhaled albuterol[i] was administered via a commercial aerosol delivery device[j] to each horse after histamine bronchoprovocation and before BAL. Past studies have shown that histamine bronchoprovocation does not alter BAL cell differentials.[13] The animals were sedated using detomidine[e] (0.01 mg/kg BW, IV) and the BAL performed using a commercial cuffed BAL tube[k] that was inserted into 1 nostril down to a subsegmental bronchus. A total of 60 mL of 0.3% lidocaine[l] was used to desensitize the larynx, trachea, and bronchi during passage of the tube before wedging. Two aliquots of 250 mL warmed saline were instilled into the animal's lung and drawn back by suction at 10 cm H_2O pressure. The 2 samples were pooled, the total BALF volume recovered was quantified, and 10 mL of the BALF were added to a glass tube containing EDTA, which was submitted within 12 h of BALF collection for total cell count and cytocentrifugation for slide preparation. The slides were stained with Romanowsky Stain[m] and Toluidine Blue, the latter specifically for enumeration of mast cells. Cells (n = 400) were classified by one of the authors (MRM) as the percentage of total cells that were macrophages, lymphocytes, neutrophils, eosinophils, and mast cells (400× magnification).

Statistical Analysis

Data are reported as mean ± SD. Data were assessed for normality using the Shapiro–Wilk test. The effect of age (young versus old) on continuous variables was analyzed by using an

independent sample, nonparametric Mann-Whitney U-test, and categorical baseline characteristics were assessed using a Fisher's exact test. Correlations between airway reactivity (PC35 and PC50) and results of ABG, RIP, and BALF cytology were evaluated using a Spearman's Rho Correlation. A significance level of $P < .05$ was used. All statistic analyses were performed with commercial software.[n]

Results

Study Population Description

Fifteen young adult horses (range, 2–11 years; median, 7 years old) and 16 aged horses (range, 20–28 years; median, 22 years old) were included in this study. Results of physical examination and CBC did not identify any abnormalities. Baseline characteristics of the horses in each group are summarized in Table 1. Horses from 6 different farms were included in this study. Equal numbers of young and aged horses were obtained from each farm with the exception of 2 farms, 1 where we tested 3 old horses and 1 young horse and the other farm where we tested 4 old horses and 5 young horses. The bedding material and hay used were the same for all horses at a given farm. Ambient conditions (eg, temperature, humidity) during pulmonary function testing did not differ between groups.

Respiratory Function Parameters

No significant differences were observed in the PaO_2 or $PaCO_2$ between aged horses (PaO_2, 97.9 ± 10.1 mmHg; $PaCO_2$, 43.3 ± 3.2 mmHg) and

young horses (PaO_2, 99.2 ± 9.7 mmHg; $PaCO_2$, 42.8 ± 3.9). Two RIP-derived ventilator parameters were significantly different between the young and old horses. Minute ventilation was significantly lower in the older horses and expiratory time (Te) was significantly higher in old horses (3.9 ± 1.5 s) compared to young horses (3.0 ± 0.6 s). There was no significant difference in respiratory frequency, Ti, Te/Ti, or the other RIP-derived ventilatory parameters evaluated (Table 2). Airway reactivity was not measurable in 1 young and 1 aged horse. No difference in airway reactivity was seen between aged horses (PC35, 18.0 ± 11.1 mg/mL; PC50, 20.3 ± 11.3 mg/mL) and young horses (PC35, 16.1 ± 10.5 mg/mL; PC50, 18.9 ± 10.7 mg/mL). Based on criteria that were used to categorize the severity of airway reactivity (see Methods), 4/14 and 4/15 horses exhibited airway hyper-reactivity in response to histamine challenge in the group of young and aged horses, respectively. There was no difference in total BALF cell count or absolute number of macrophages or lymphocytes. However, BALF cytology identified a significantly lower mean percentage of macrophages and a reciprocal higher mean percentage of lymphocytes in old horses (macrophages, $40.6 \pm 11.3\%$; lymphocytes, $53.4 \pm 9.4\%$) when compared to young horses (macrophages, $53.5 \pm 9.6\%$; lymphocytes, $43.9 \pm 10.9\%$; Table 3).

There was no statistically significant correlation between the variables airway reactivity (PC35 and PC50), arterial blood gas values, baseline parameters obtained by RIP, or BALF cytology results in either group or the pooled study population.

Discussion

To our knowledge, this is the first study to investigate changes in respiratory function and BALF cytology of horses that may be a function of the aging process. The diagnostic tests used in our study were

Table 1. Mean (SD) baseline characteristics of young (n = 15) and aged (n = 16) horses.

	Young	Aged	P values
Age (years)	6.3 (3.2)	22.7 (2.4)	.000
Breed			
Quarter Horse	4	5	.15
Morgan	5	1	
Thoroughbred	1	5	
Standardbred	1	2	
Paint Horse/Paint cross	3	0	
Warmblood	1	1	
Mustang	0	1	
Sex			
Mares	11	10	.519
Geldings	4	6	
Weight (lb)	1025 (116.4)	1034.5 (90.1)	.922
BCS			
4/9	2	3	.546
5/9	12	13	
6/9	1	0	
Ambient temperature (°F)	54.7 (5.0)	54.2 (5.1)	.599
Turnout schedule			
<6 h/d	2	3	.919
≥6 but <24 h/d	8	8	
24 h turnout	5	5	

Table 2. Mean (SD) RIP-derived ventilatory parameters in aged (n = 16) and young (n = 15) horses.

	Aged	Young	P values
Respiratory rate (breaths/min)	11.0 (4.4)	13.2 (3.1)	.80
Tidal volume (L)	5.6 (0.8)	5.4 (0.7)	.473
Minute ventilation (L/min)	58 (16)	67.3 (12)	.038
Delta flow (L/s)	0.86 (0.4)	0.89 (0.4)	.822
Phase angle (°)	17.7 (14)	14.9 (6.3)	.637
Ti (s)	2.4 (0.6)	2.1 (0.5)	.070
Te (s)[a]	3.9 (1.5)	3.0 (0.6)	.025
Te/Ti	1.61 (0.3)	1.47 (0.3)	.257
PIF (L/s)	3.8 (0.85)	4.0 (0.6)	.240
PEF (L/s)	4.9 (1.4)	5.6 (1.2)	.240
PEF/PIF	1.3 (0.3)	1.41 (0.3)	.525

[a]Denotes values that are statistically different (P value listed in table).

Table 3. Mean (SD) BALF characteristics in aged (n = 16) and young (n = 15) horses.

Units	Aged	Young	P Value
Volume recovered			
mL	275.9 (43.0)	259 (84.3)	.654
Cell count			
cells/μL	215.2 (117.2)	247.8 (169.3)	.922
Macrophages			
%[a]	40.6 (11.3)	53.5 (9.6)	.03
cells/μL	83.6 (43.9)	136.0 (107.1)	.129
Lymphocytes			
%[a]	53.4 (9.4)	43.9 (10.9)	.017
cells/μL	115.8 (69.6)	104.5 (68.8)	.682
Neutrophils			
%	6.1 (4.8)	3.79 (2.0)	.423
cells/μL	15.5 (18.1)	9.1 (8.7)	1.000
Mast cells			
%	1.92 (1.5)	2.67 (1.3)	.151
cells/μL	4.2 (3.3)	7.0 (6.4)	.318
Eosinophils			
%	0.18 (0.4)	0.18 (0.5)	.984
cells/μL	0.21 (0.5)	0.86 (3.1)	.953

[a]Denotes values that are statistically different (P value listed in table).

selected based on their portability and usability in field conditions to better recruit healthy horses. Therefore, this study was aimed at providing information to the equine practitioner who is called to investigate clinical signs of respiratory disease in the geriatric horse. Although it is acknowledged that this is a small cross-sectional study, the findings suggest that differences between young and old horses are not likely to be biologically relevant for the endpoints examined. Thus, clinical changes in an aged horse with regard to its respiratory system should raise concern that these changes are more likely to be disease related rather than aging related.

In contrast to a previous report,[9] our study showed no difference between the ABG results of young and aged horses. A possible explanation of this contradiction is the different age ranges of the horses included in each study. In the Aguilera-Tejero et al study, the age range was narrower in the young group (3–8 years versus 2–11 years in this study) and wider in the aged group (20–45 years versus 20–28 years in this study) when compared to our study. It would be interesting to evaluate horses >30 years of age to see if extreme age changed the ABG results. Also, the Aguilera-Tejero study corrected ABG results to rectal temperature, whereas ours did not. This could account in part for the different conclusions of these 2 studies. Temperature correction of ABG results is a controversial issue in human medicine[14,15] and is likely unnecessary when the patient's temperature is measured within 1°C of 37°C[14] which was the case of the horses in our study (mean ± SD, 37.3 ± 0.3°C; range, 36.6–38°C). Studies in geriatric dogs have not shown a significant difference in PaO_2 or $PaCo_2$ results compared to younger dogs.[16,17] There is a well-characterized age-related

decrease in the PaO_2 of humans that reaches a plateau as humans reach their early-mid 70s.[18,19]

The slightly greater Te observed in aged horses observed in this study is likely of no clinical relevance, because this value was within reference ranges previously reported for healthy horses[20,21] and Te/Ti ratio was not different between groups. The higher Te may be a result of slightly lower respiratory rate (not statistically significant) observed in aged group after administration of sedation for RIP. The lower minute ventilation also may be explained in this manner. The absence of differences in the remainder of RIP-derived parameters is in agreement with reports in humans, where RIP showed no difference in the breathing pattern or ventilatory parameters between healthy young and healthy geriatric subjects.[22,23]

There were no differences in the PC35 and PC50 values or the number of hyper-reactive horses in each of the groups. These findings are in contrast to the conclusion of a review article on the influence of age on airway reactivity in humans,[6] which showed a positive association between age and airway hyper-reactivity. Several studies included in the review point to a bimodal distribution of airway reactivity, which appears to be highest in the early (children and adolescents) and late (elderly) phases of life. A trend for bimodal distribution of airway reactivity in the horse was not observed in the population studied as none of the 5 youngest horses (2–3 year old) or the 2 oldest horses (28 years old) from our study was hyper-reactive; however, no foals or horses >28 years of age were included in this study. In an earlier study, airway reactivity in foals (48–92 days old) was measured using more classical methods (resistance-compliance).[24] Mean provocative dosage of histamine that induced a 35% decrease in dynamic compliance (comparable to PC50)[11] was considerably lower for foals on average (5.4 ± 1.74 mg/mL) than adults in this study, which strongly resembles the patterns in humans. Interestingly, young cats (1–2 years) have much higher airway reactivity than geriatric cats (12–13 years).[25] It was thought that age-related changes in airway reactivity in cats may explain the higher rate of spontaneous asthma-like conditions in younger cats.[25] In the horse, inflammatory airway disease is more common in the young to middle-aged horse, but recurrent airway obstruction occurs more often in the older horse,[26] implying that the functional and cytologic changes in these diseases are not directly related to aging.

The percentage of lymphocytes was higher and the percentage of macrophages was lower in the BALF of aged horses, with the mean percentages of lymphocytes being slightly higher than the reference values of 30–50% lymphocytes previously reported for the horse.[27] Higher percentage of lymphocytes and a lower percentage of alveolar macrophages also have been reported in the geriatric human.[7,28] The higher lymphocyte percentage in BALF of aged subjects may be caused by the cumulative antigenic stimulation over many years and the lower macrophage percentage is probably the reciprocal to the higher lymphocyte

percentage and higher but not significant neutrophil percentage.

Asymptomatic airway hyper-reactivity has been documented in humans and seems to precede the occurrence of asthma usually by several years.[6] The clinical relevance of the hyper-reactivity observed in several of the asymptomatic horses in our study is unknown, because no longitudinal studies with hyper-reactive horses have been performed to identify if they will develop inflammatory airway disease or recurrent airway obstruction. The lack of correlation between airway reactivity and BALF cytology previously has been reported in healthy humans.[29] However, when asthmatic human subjects in remission were submitted to bronchoprovocation after exposure to an allergen, the increase in airway responsiveness was associated with an influx of eosinophils and lymphocytes in the bronchial lumen.[29] In symptomatic horses, the only type of BALF cells that correlated with airway reactivity was mast cells as demonstrated in previous studies.[30,31]

Environmental factors contribute to the development of lower airway inflammation in the horse.[26] A limitation of our study is that environmental conditions that may have impacted pulmonary function or cytology were not standardized. To minimize the effect of possible environmental factors on the results of the tests, similar numbers of aged and young horses from each farm were included in the study. We also analyzed the amount of time spent in turnout and ambient conditions during the test period, both of which were similar for both groups. However, we cannot exclude the possibility that the data were biased by specific differences in the particulate matter inhaled by 1 group versus the other because there still could be differences in particulate exposure even between neighboring stalls.[32,33] Future studies that carefully document particulate exposure at the personal breathing level would augment studies which attempt to examine the effects of age or other cohorts.

The results of this study suggest that the only aging-related changes in the horse detected with the diagnostic tests performed are a higher percentage of lymphocytes and a lower percentage of alveolar macrophages in the BALF. In conclusion, it is likely that any abnormalities detected on ABG values, RIP-derived variables, or bronchoprovocation results in a clinically affected aged horse are related to the disease process and not directly a result of aging, within the age categories studied here. Additional studies involving larger number of horses within a wider age range are warranted to validate these findings.

Conflict of Interest Declaration: Authors disclose no conflict of interest.

Footnotes

[a] Medi-Pak Performance, McKesson Medical-Surgical, Richmond, VA
[b] VetScan I-Stat, Abaxis, Union City, CA
[c] I-STAT CG4+ Cartridge, Abaxis
[d] Open Pleth, Ambulatory Monitoring Inc, Ardsley, NY
[e] Dormosedan, Pfizer, New York, NY
[f] Histamine diphosphate monohydrate, MP Biomed, Solon, OH
[g] ProNeb Turbo, Model 38B0201, Pari, Midlothian, VA
[h] LC Plus, Pari
[i] Ventolin HFA, GlaxoSmithKline, Research Triangle Park, NC
[j] Aerohippus, Trudell Medical International, London, ON, Canada
[k] Broncho-Alveolar Lavage Catheter (BAL300), Mila International, Erlanger, KY
[l] Lidocaine Injectable, Sparhawk Laboratories Inc, Lenexa, KS
[m] Protocol Hemaspray Stain, Fisher Scientific Co, Kalamazoo, MI
[n] SPSS, version 12, SPSS Inc, Chicago, IL

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