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Exercise-induced airflow changes in horses with asthma measured by electrical impedance tomography

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Abstract

Background: Equine asthma (EA) causes airflow impairment, which increases in severity with exercise. Electrical impedance tomography (EIT) is an imaging technique that can detect airflow changes in standing healthy horses during a histamine provocation test.

Objectives: To explore EIT-calculated flow variables before and after exercise in healthy horses and horses with mild-to-moderate (MEA) and severe equine asthma (SEA).

Animals: Nine healthy horses 9 horses diagnosed with MEA and 5 with SEA were prospectively included.

Methods: Recordings were performed before and after 15 minutes of lunging. Absolute values from global and regional peak inspiratory (PIF, positive value) and expiratory (PEF, negative value) flows were calculated. Data were analyzed using a mixed model analysis followed by Bonferroni's multiple comparisons test to evaluate the impact of exercise and diagnosis on flow indices.

Results: Control horses after exercise had significantly lower global PEF and PIF compared to horses with SEA (mean difference [95% confidence interval, CI]: 0.0859 arbitrary units [AU; 0.0339-0.1379], $P < .001$ and 0.0726 AU [0.0264-0.1188], $P = .001$, respectively) and horses with MEA (0.0561 AU [0.0129-0.0994], $P = .007$ and 0.0587 AU [0.0202-0.0973], $P = .002$, respectively). No other significant differences were detected.

Conclusions and Clinical Importance: Electrical impedance tomography derived PIF and PEF differed significantly between healthy horses and horses with SEA or MEA after exercise, but not before exercise. Differences between MEA and SEA were not observed, but the study population was small.

KEYWORDS
heaves, inflammatory airway disease, pulmonary function test, recurrent airway obstruction

Abbreviations: ACVIM, American College of Veterinary Internal Medicine; BALF, bronchoalveolar lavage fluid; CoV, center of ventilation; EA, equine asthma; EIPH, exercise-induced pulmonary hemorrhage; EIT, electrical impedance tomography; GREIT, modified Granz consensus reconstruction algorithm for EIT; HOARSI, horse owner assessed respiratory signs index; MEA, mild-to-moderate equine asthma; PEF, peak expiratory flow; PIF, peak inspiratory flow; ROI, region of interest; SAA, serum amyloid A; SEA, severe equine asthma; $VT_{\Delta Z}$, tidal volume-related electrical impedance tomography factor.

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1 | INTRODUCTION

Equine asthma (EA) is a unifying description for chronic noninfectious inflammatory lower airway diseases in horses.[1-3] It has been recognized previously as the second most common cause of poor performance in racing Thoroughbreds.[4] Inflammation leads to bronchoconstriction, mucus accumulation and impairment of gas exchange.[1,5-9] Horses with the uncontrolled severe form of equine asthma (SEA) show increased respiratory effort at rest because of these impairments of gas exchange. However, if asthma is controlled, clinical signs might not be present at rest and lung function impairments might only be seen with exercise. In asthmatic horses, most airway narrowing occurs only in the most distal airways, accounting for only a small part of the total airway resistance. Thus, milder degrees of airway narrowing go undetected at rest using most currently available tools.[10,11] It has been shown however that impairment of gas exchange occurs in horses with MEA.[10,11] Lung function impairment might not be detectable at rest but could become evident during or after exercise in horses with MEA.

To confirm clinical suspicion of EA in the field, several diagnostic modalities, such as owner questionnaires, arterial oxygen tension, tracheal endoscopy, bronchoalveolar lavage fluid (BALF) cytology or a combination thereof, are used.[1] Although these diagnostic tests can establish a diagnosis of EA, and in conjunction with clinical signs can differentiate between SEA and MEA, none of these tests can evaluate lung function in the field.[12] A method by which practitioners can easily evaluate lung function in the field would be a valuable addition.

Electrical impedance tomography (EIT) is a noninvasive, radiation-free, real-time imaging technique that assesses gas volume shifts in the lungs based on measurement of impedance changes by placing an electrode belt around the thorax.[13] Small alternating high frequency and low amplitude electrical currents are applied through a pair of the electrodes, while the resulting surface potentials are measured by the remaining electrodes.[14] Based on this relationship between impedance change and gas volume, EIT measurements allow evaluation of global and regional lung volume during inspiration and expiration. The impedance change of all pixels within the EIT image can be used as a surrogate of tidal volume ($VT_{\Delta Z}$). From the assumption that the impedance change represents a volume signal, peak inspiratory (PIF) and peak expiratory (PEF) global flow can be evaluated by calculating the first derivative of the global EIT volume signal.[15] All EIT variables including these flow indices are expressed in arbitrary units (AU).

Electrical impedance tomography has been used in equine medicine to monitor distribution of ventilation under general anesthesia,[16-18] and in standing horses.[19] A recent study showed that EIT can detect airflow changes caused by bronchoconstriction in healthy sedated horses during a histamine provocation test based on changes in flow variables.[15] The effect of exercise on EIT-calculated variables that represent exercise-induced airflow changes in healthy horses or horses with EA has not yet been described.

Our objective was to explore differences in pre- and postexercise indices of EIT-derived airflow indices between healthy horses and horses with EA. The hypothesis was that EIT flow variables would increase after exercise and a difference could be detected between healthy horses and horses with EA.

2 | MATERIAL AND METHODS

This study had ethical and institutional approval (ZH 166/15). All owners signed an informed consent form.

2.1 | Animals

Horses were enrolled from the teaching horse herd of the University of Bern (n = 5), the Swiss National Horse Center (n = 6), horses owned by staff members of the University of Zurich (n = 2) and the patient population of the University of Zurich between May 2017 and 2019 (n = 14; Table 1). Inclusion criteria for the control horses (Figure 1) were: no history or physical examination findings of respiratory disease (ie, cough, respiratory rate >16 breaths per minute [bpm], nostril flaring or increased abdominal effort) or exercise intolerance, and a BALF neutrophil proportion <10%, mast cell proportion <2%, and eosinophil proportion ≤1%. Inclusion criteria for horses with EA were a history or physical examination findings of respiratory disease (cough, respiratory rate >16 bpm, nostril flaring or increased abdominal effort) or exercise intolerance and an increased percentage of at least 1 type of granulocyte (neutrophil proportion ≥10%, mast cell proportion ≥2%, or eosinophil proportion >1%) on evaluation of BALF cytology. Differentiation into MEA and SEA was based on clinical signs of respiratory disease at rest (respiratory rate >16 bpm, nostril flaring or increased abdominal effort), "horse owner assessed respiratory signs index" (HOARSI), PaO_2 and tracheal mucus score (Figure 1) and confirmed by BALF cytology (neutrophil proportion >20%). Exclusion criteria for horses from both groups were a history of exercise-induced pulmonary hemorrhage (EIPH) or treatment with corticosteroids or bronchodilators within 2 weeks before presentation.

2.2 | Owner questionnaire

All owners were asked to complete the HOARSI, which is a validated standardized questionnaire based on clinical signs (cough frequency, nasal discharge, breathing at rest, and during work).[20,21] A HOARSI of 3 was considered suggestive for horses with moderate to severe signs (MEA or SEA), and a HOARSI of 4 for horses with severe clinical signs of respiratory disease, compatible with SEA. This questionnaire was used in conjunction with the other variables to classify horses with EA as MEA or SEA (Figure 1).

2.3 | Clinical respiratory tract examination, including EIT measurements

The respiratory tract examination of the horses consisted of a general clinical examination including rebreathing bag examination. An arterial

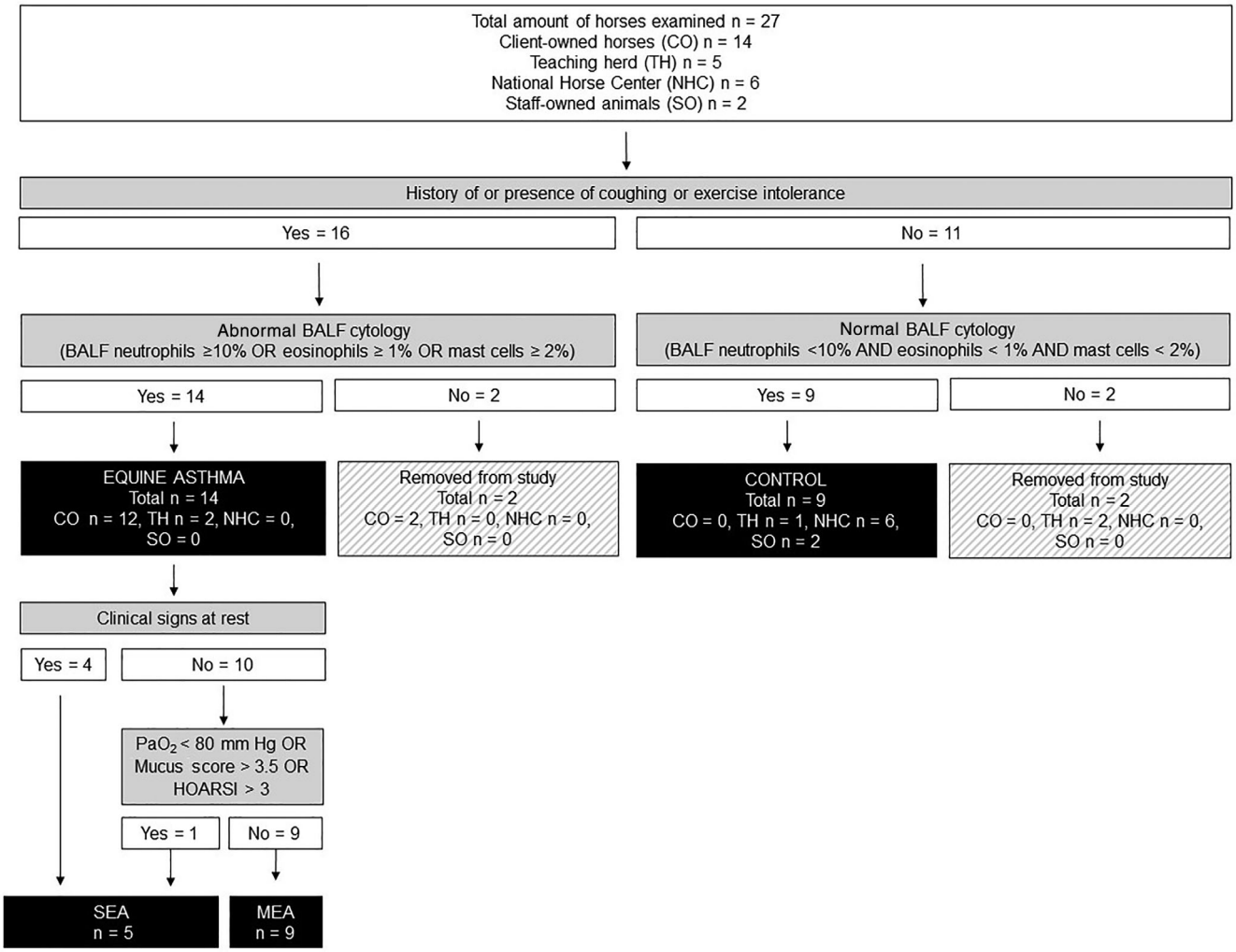

FIGURE 1 Flow chart for classification of study horses into controls, and MEA- and SEA-affected horses. BALF, bronchoalveolar lavage fluid; MEA, mild-to-moderate equine asthma; SEA, severe equine asthma

TABLE 1 Population characteristics of the groups

	Controls (n = 9)		MEA (n = 9)		SEA (n = 5)	
	Median	Range	Median	Range	Median	Range
Age (years)	12	(10-15)	12	(10-27)	16	(9-21)
Weight (kg)	540	(500-575)	525	(385-565)	500	(375-580)
HOARSI score	1	(1)	2.5	(1-3)	4	(4)
PaO_2 at rest (mm Hg)	97	(93-98)	89.5	(80-97.1)	76.9	(68.1-79.7)
Mucus score	1	(0-1)	2	(1-4.5)	2.5	(2, 3)
BALF cytology						
Neutrophils (%)	5	(2.5-9)	9	(0-13.5)	28.5	(23.5-35)
Mast cells (%)	1	(0-1.5)	3	(0-11.5)	1	(0-4)
Eosinophils (%)	0	0	0	(0-6)	0	(0-0)
Lymphocytes (%)	50	(33.5-66)	45	(24.5-72)	44	(38-59)
Macrophages (%)	46	(27.5-60)	47	(18-67)	31	(16.5-32)

Note: Numerical values are reported as median and range.
Abbreviations: BALF, bronchoalveolar lavage fluid (differential cell count performed on 200 cells); HOARSI, horse owner assessed respiratory signs index; MEA, mild-to-moderate equine asthma; PaO_2, arterial oxygen tension; SEA, severe equine asthma.

blood sample then was obtained from the carotid artery using an 18 g needle and drawn into a 1 mL heparinized syringe. The arterial blood sample was immediately analyzed using a blood gas analyzer (RAPIDPoint 500, Siemens Healthcare). The arterial oxygen pressure (PaO_2) at rest was considered decreased when paO_2 was <80 mm Hg (Equine hospital Zurich: 526 m over sea level). The EIT measurements then were made using an EIT device running software recording EIT raw impedance data (BBVet, SenTec, Landquart, Switzerland) before and after 15 minutes of lunging (5 minutes at a walk, 5 minutes at a trot, and 5 minutes at canter). For EIT data collection, an electrode belt (elastic rubber belt with 32 stainless steel electrodes equidistantly mounted) was placed around the thorax in a transverse plane, between the 5th and 6th intercostal space. The coat of the horses was not clipped but moistened by application of water and gel to maximize skin contact with the electrodes and optimize conduction of current. A mark was made in the haircoat using a clipper when the belt was in the desired location, to allow for repeated positioning of the belt in the same location after exercise. Ten to 12 consecutive respiration cycles of good quality without movement artifacts were recorded while the horse was standing quietly. Thereafter, the belt was taken off and the horse was lunged for 15 minutes. After lunging, the EIT belt was placed in the same position as for baseline measurements and recording was performed in the same manner as before exercise. To avoid detecting airflow changes associated with differing respiratory rates, recording was started when the respiratory rate had reached the equivalent of the resting respiratory rate. The respiratory rate was determined by visual assessment of flank movement and counted over 1 minute. After EIT recording, the belt was taken off for the remainder of the examination.

Horses then were sedated using detomidine (20-40 μg/kg, IV; Domosedan ad us. vet., Provet AG, Lyssach b. Burgdorf, CH) and butorphanol (10-20 μg/kg, IV; Alvegesic 1% forte ad us. vet., Virbac Switzerland AG, Glattbrugg, CH) for endoscopic evaluation of the respiratory tract using a 1.8-m long endoscope (Karl Storz GmBH, Tuttlingen, Germany) passed through the nasal passage into the trachea. Pictures were recorded and accumulation of mucus was graded by the attending veterinarian as previously described.[22] Bronchoalveolar lavage then was performed using a standard protocol with a bronchoalveolar lavage (BAL) catheter (large animal BAL catheter; Mila International Inc, Florence, Kentucky) that was passed through the nose into the bronchus until it was wedged. While passing the catheter, 20 mL lidocaine 2% (Lidocaine 2%; Streuli Pharma AG, Switzerland) mixed with 30 mL sterile saline (0.9% NaCl, 1000 mL, Laboratorium Dr G. Bichsel, Interlaken) was injected through the catheter to decrease the cough reflex. After the catheter was wedged, a BAL was performed by instilling 250 mL of sterile saline (0.9% NaCl, 1000 mL, Laboratorium Dr G. Bichsel, Interlaken, Switzerland), in 5 boluses of 50 mL each. After the boluses, the fluid was reaspirated by gentle suction using the same syringe until no additional fluid was obtained. Cytopreparations from unfiltered BALF were stained with Wright-Giemsa. Differential counts of 200 inflammatory cells were performed by a board-certified clinical pathologist at the Veterinary Medical Laboratory of the University of Zurich.

2.4 | Blood sampling for hematology and biochemistry analysis

Blood was collected by venipuncture from the jugular vein into potassium ethylenediaminetetraacetic acid (Vacuette, K3EDTA, 4 mL, Greiner bio-one, St Gallen, Switzerland), lithium heparin (Vacuette, Lithium Heparin, 6 mL, Greiner bio-one) and sodium citrated tubes (Vacuette, Coagulation sodium citrate 3.2%, 2 mL, Greiner bio-one) tubes. Complete blood count (Sysmex XT 2000iV, Sysmex Cooperation, Kobe, Japan; multispecies automated hematology analyzer, validated for equine samples), serum biochemistry (Cobas 6000 <501> System, Roche Diagnostics, Rotkreuz, Switzerland), including serum amyloid A protein (SAA; automated turbidimetric immunoassay, LZ Test Eiken SAA; on Cobas 501 system; Roche Diagnostics) and fibrinogen Clauss (STart Max, DIAGNOSTICA STAGO, France) assays were performed by the Veterinary Medical Laboratory of the University of Zurich within 1 hour after sampling.

2.5 | EIT analysis

Ten to 12 stable consecutive breaths were analyzed by exporting the recorded EIT raw impedance data to Matlab (Mathworks). Based on a modified Granz consensus reconstruction algorithm for EIT (GREIT)[23] that was adapted for horse anatomy, individual images of impedance changes were generated. The regions of interest (ROI) representing the lung area were first divided into halfs and the impedance change (ΔZ) evaluated for each region: ventral (ΔZ_{Vent}) vs dorsal (ΔZ_{Dors}) and left (ΔZ_{Left}) vs right (ΔZ_{Right}).

The EIT tidal volume-related factor ($VT_{\Delta Z}$ expressed in AU) was calculated. Based on this calculation, the global peak expiratory (PEF_{Global}) and inspiratory (PIF_{Global}) flows were calculated (expressed in AU) from all pixels within the total ROI area for each breath. The peak flows then were evaluated for each half and further reported as left, right, ventral, and dorsal peak expiratory flows (PEF_{Left}, PEF_{Right}, PEF_{Vent}, and PEF_{Dors}, respectively) and the corresponding inspiratory flows (PIF_{Left}, PIF_{Right}, PIF_{Vent}, and PIF_{Dors}). Based on the direction of ΔZ, inspiratory values were defined as positive, whereas expiratory values were negative. Absolute values of PEF and PIF were considered for statistical analyses. The shift in distribution of the gas in the lungs measured by EIT is expressed as a single variable known as "center of ventilation" (CoV). Its position is expressed as a percentage of the ventro-dorsal extension of the lung region, with 0% referring to the most ventral part of the lung and 100% to the most dorsal part (CoV_{VD}). The position of the CoV between the right and left lung can be further defined as a percentage (0% referring to the most lateral part of the right lung and 100% to the most lateral part of the left lung, CoV_{RL}). The CoV_{VD} and CoV_{RL} were calculated for each horse before and after exercise.

2.6 | Data analysis

Normality of the data was assessed using the Kolmogorov-Smirnov test. To evaluate the effects of exercise and disease on global PEF

and PIF, as well as all regional PEF and PIF results, a repeated-measures mixed-effect model analysis (restricted maximum likelihood approach) was used. The fixed factors included "diagnosis" (control, MEA, and SEA), "exercise" (before and after lunging) and the interaction "diagnosis*exercise." "Horse" was considered a random variable. If a significant effect of interaction was identified, a Bonferroni post hoc test was performed for multiple comparisons.

Analyses were performed using Prism 8.03.1 (GraphPad Software, Inc, California), and $P < .05$ was considered significant. Normally distributed data are reported as mean ± SD, otherwise data are reported as median (range).

3 | RESULTS

3.1 | Animals

Based on classification criteria, 9 healthy controls and 14 horses with EA (9 MEA and 5 SEA, Figure 1) were included in the study. The healthy control group consisted only of Warmbloods; 2 were female and 7 were geldings. The asthma group consisted of 3 Quarter Horses and associated breeds, 4 Warmbloods, 1 Irish Cob, 1 Canadian, 2 Icelandic, 1 Connemara, 1 Andalusian, and 1 Haflinger. There were 4 females, 9 geldings, and 1 stallion.

History and clinical signs of all horses are shown in Supporting Information S1.

Four of the controls and 1 of the horses with EA could not be exercised because of lameness, resulting in 4 controls and 13 EA horses with complete data sets (Supporting Information S1). One client-owned horse in the SEA group did not have results from BALF cytology available because the sample was nondiagnostic.

3.2 | EIT-based expiratory flow variables

Indices of global expiratory flow (PEF_{Global}) are shown in Figure 2A. There was a significant interaction effect of diagnosis and exercise on PEF_{Global} ($P = .01$). Compared to healthy horses, PEF_{Global} was significantly higher in horses with MEA (mean difference [95% CI]: 0.0561 AU [0.0129-0.0994], $P = .01$) and in horses with SEA (mean difference [95% CI]: 0.0859 AU [0.0339-0.1379], $P < .001$) after exercise. There was no difference between SEA and MEA (mean difference [95% CI]: 0.0298 AU [−0.0169 to 0.0765], $P = .35$) after exercise or in any comparisons before exercise (all $P > .99$). In contrast to healthy horses, PEF_{Global} increased significantly after exercise in horses with MEA and SEA (mean difference [95% CI]: 0.0445 AU [0.0086-0.0805], $P = .013$ and 0.0729 AU [0.0213-0.1245], $P = .005$, respectively).

Regional PEF variables are shown in Figure 3. There was a significant interaction effect between diagnosis and exercise for PEF_{Left} ($P = .03$), PEF_{Right} ($P = .04$) and $PEF_{Ventral}$ ($P = .004$), but not in PEF_{Dorsal} ($P = .07$). Compared to healthy horses, PEF_{Left} and PEF_{Right} were significantly higher in horses with MEA (mean difference [95% CI]: 0.0326 AU [0.0017-0.0634], $P = .04$ and 0.0327 AU [0.0051-0.0603], $P = .02$, respectively) and in horses with SEA (mean difference [95% CI]: 0.0439 AU [0.0068-0.0809], $P = .02$ and 0.0401 AU [0.0069-0.0732], $P = .01$, respectively) after exercise. Compared to controls, $PEF_{Ventral}$ was significantly higher in horses with SEA (mean difference [95% CI]: 0.0675 AU [0.0245-0.1105], $P = .001$), but not in horses with MEA (mean difference [95% CI]: 0.0352 AU [−0.0007 to 0.0711], $P = .06$) after exercise. There was no difference between MEA and SEA after exercise (mean difference [95% CI]: 0.0323 AU [−0.0071 to 0.0717], $P = .14$) and no differences between any groups before exercise (all $P > .999$). In contrast to healthy horses, $PEF_{Ventral}$

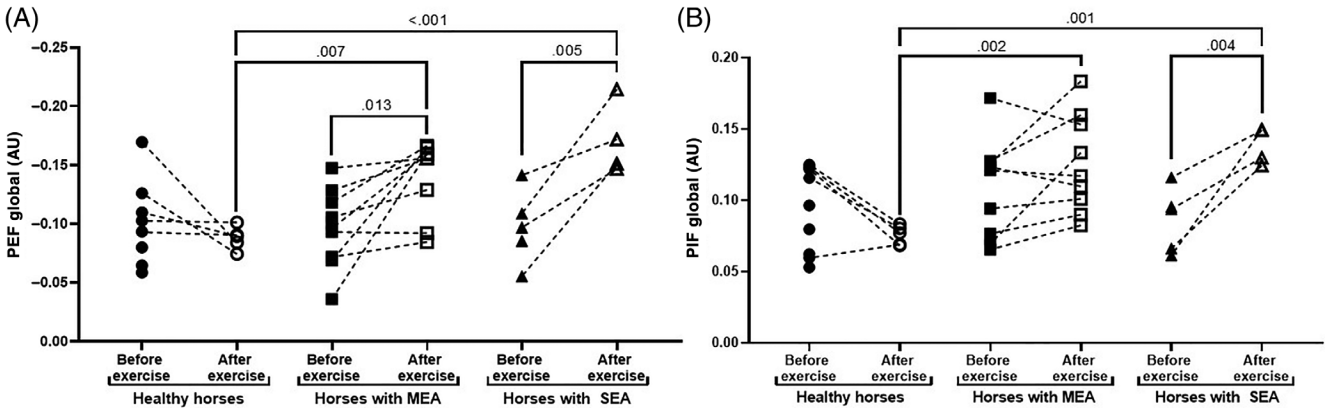

FIGURE 2 Global peak expiratory and inspiratory flow in healthy horses and horses with mild-moderate equine asthma (MEA) and severe equine asthma (SEA), before and after exercise. Values of global peak expiratory flow (PEF_{Global}; A) and inspiratory flow (PIF_{Global}; B) in controls and asthmatic horses, before and after exercise. Each symbol denotes a single animal, and points from the same animal with were connected with lines. The *P*-values, when significant, are indicated in the figure

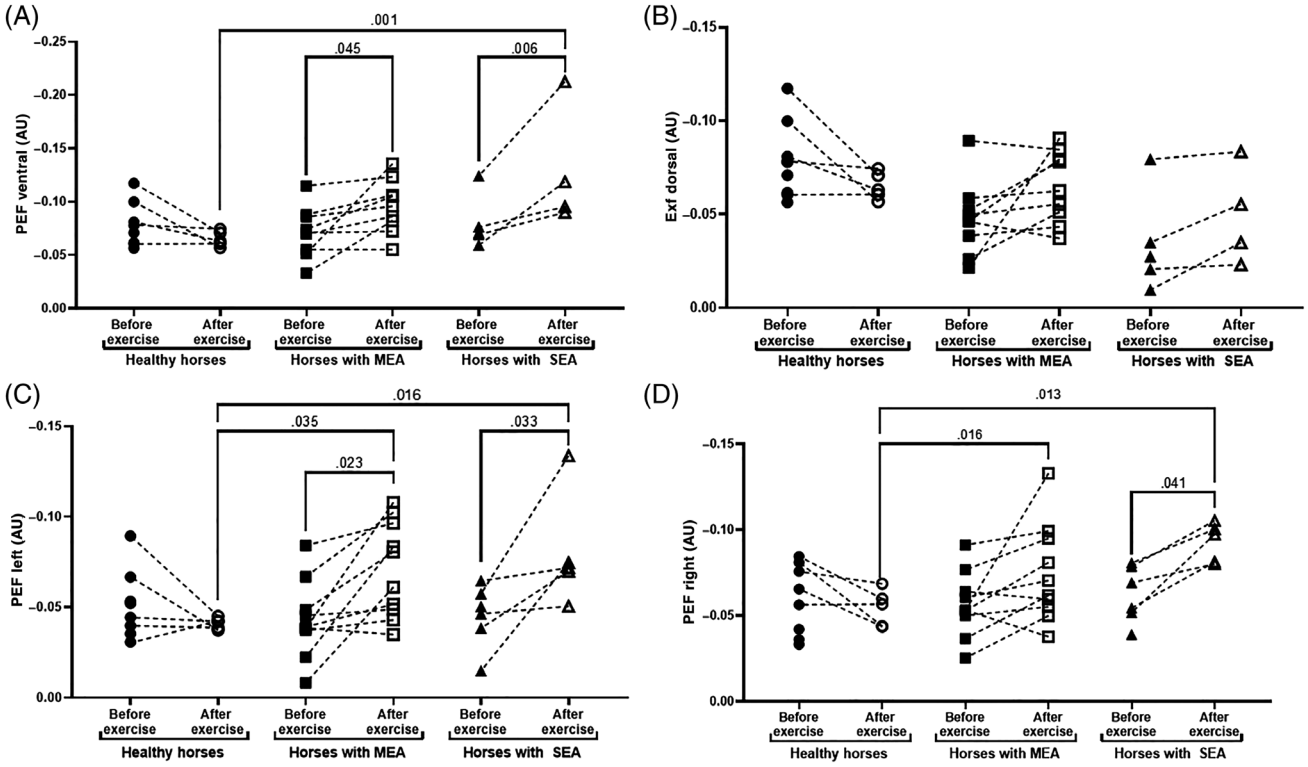

FIGURE 3 Regional peak expiratory flows in healthy horses and horses with mild-moderate equine asthma (MEA) and severe equine asthma (SEA), before and after exercise. Values of ventral ($PEF_{Ventral}$, A), dorsal (PEF_{Dorsal}, B), left (PEF_{Left}, C), and right (PEF_{Right}, D) expiratory peak flows in controls and asthmatic horses, before and after exercise. Each symbol denotes a single animal, and points from the same animal with were connected with lines. The *P*-values, when significant, are indicated in the figure

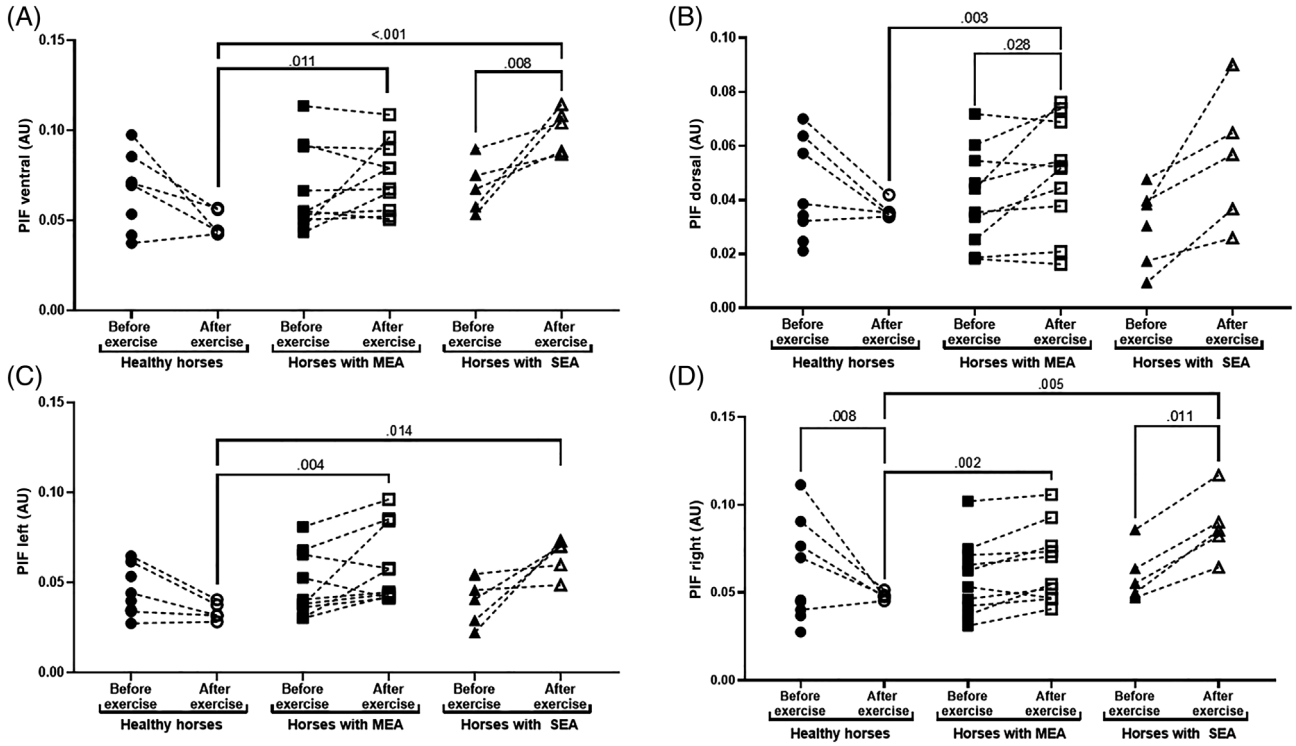

FIGURE 4 Regional inspiratory flows in healthy horses and horses with mild-moderate equine asthma (MEA) and severe equine asthma (SEA), before and after exercise. Values of ventral ($PIF_{Ventral}$, A), dorsal (PIF_{Dorsal}, B), left (PIF_{Left}, C), and right (PIF_{Right}, D) inspiratory peak flows in controls and asthmatic horses, before and after exercise. Each symbol denotes a single animal, and points from the same animal with were connected with lines. The *P*-values, when significant, are indicated in the figure

TABLE 2 Center of ventilation and tidal volume measured by electrical impedance tomography for all horses in each group (before and after exercise)

Other parameters			
Horses	**CoV ventral to dorsal (%)**	**CoV right to left (%)**	**$VT_{\Delta Z}$**
Controls—Before exercise (n = 9)	57.81	53.53	3.32
±SD	±5.98	±6.06	±0.80
Controls—After exercise (n = 5)	60.62	52.13	3.12
±SD	±2.13	±3.17	±1.24
MEA—Before exercise (n = 10)	52.44	49.83	4.31
±SD	±8.52	±4.85	±1.14
MEA—After exercise (n = 10)	51.69	49.37	4.19
±SD	±5.42	±7.34	±1.16
SEA—Before exercise (n = 6)	60.25	49.31	2.59
±SD	±9.42	±7.24	±0.63
SEA—After exercise (n = 5)	63.07	49.97	3.03
±SD	±8.97	±4.13	±0.67

Note: Numerical values are reported as mean ± SD or median (range). Significance was set at $P < .05$.
Abbreviations: CoV (%), center of ventilation; MEA, mild-to-moderate equine asthma; SEA, severe equine asthma; $VT_{\Delta Z}$, estimate of tidal volume from electrical impedance tomography.

and PEF_{Left} significantly increased after exercise in horses with MEA and SEA (mean difference [95% CI] of $PEF_{Ventral}$: 0.0243 AU [0.0004-0.0481], $P = .04$ and 0.0482 AU [0.0133-0.0830], $P = .006$, respectively; PEF_{Left}: 0.0266 AU [0.0033-0.0499], $P = .02$ and 0.0363 AU [0.0025-0.07], $P = .03$, respectively). In horses with SEA, PEF_{Right} significantly increased after exercise (mean difference [95% CI]: 0.0307 AU [0.001-0.0604], $P = .04$), but not in horses with MEA (mean difference [95% CI]: 0.0188 AU [−0.0017 to 0.0392], $P = .08$).

3.3 | EIT-based inspiratory flow variables

Indices of global inspiratory flow are shown in Figure 2B. There was a significant interaction effect of diagnosis and exercise on PIF_{Global} ($P = .002$). Compared to healthy controls, PIF_{Global} was significantly higher after exercise in horses with MEA (mean difference [95% CI]: 0.0587 AU [0.0202-0.0973], $P = .002$) and in horses with SEA (mean difference [95% CI]: 0.0726 AU [0.0264-0.1188], $P = .001$). There was no difference between SEA and MEA (mean difference [95% CI]: 0.0139 AU [−0.0564 to 0.0286], $P > 0.99$) after exercise or in any comparisons before exercise (all $P > 0.5$). In horses with SEA, PIF_{Global} significantly increased after exercise (mean difference [95% CI]: 0.0528 AU [0.0168-0.0888], $P = .004$).

Regional PIF variables are shown in Figure 4. There was a significant interaction effect between diagnosis and exercise for all regional PIF (mean difference [95% CI] of $PIF_{Ventral}$: $P = .002$, PIF_{Dorsal}: $P = .01$, PIF_{Left}: $P = .02$, and PIF_{Right}: $P < .001$). Compared to healthy controls, PIF_{Dorsal} was significantly higher in horses with MEA (mean difference [95% CI]: 0.0303 AU [0.0092-0.0514], $P = .003$), but not in horses with SEA (mean difference [95% CI]: 0.0155 AU [−0.0408 to 0.0098], $P = .4$) after exercise. Compared to healthy controls, $PIF_{Ventral}$, PIF_{Left} and PIF_{Right} were significantly higher in horses with MEA (mean difference [95% CI]: 0.0322 AU [0.0063-0.0582], $P = .01$; 0.0297 AU [0.0081-0.0512], $P = .004$; and 0.0409 AU [0.0136-0.0682], $P = .002$, respectively) and horses with SEA (mean difference [95% CI]: 0.0545 AU [0.0234-0.0856], $P < .001$; 0.0310 AU [0.0052-0.0569], $P = .01$; and 0.0441 AU [0.0114-0.0767], $P = .005$, respectively). After exercise, none of the EIT-calculated inspiratory variables were significantly different between horses with MEA and horses with SEA (all $P > .35$). Before exercise, there was no difference between any groups (all $P > .9$). In horses with SEA, $PIF_{Ventral}$ and PIF_{Right} were significantly increased after exercise (mean difference [95% CI]: 0.0528 AU [0.0168-0.0888], $P = .004$ and 0.0269 AU [0.0058-0.0481], $P = .01$, respectively). In horses with MEA, PIF_{Dorsal} was significantly increased after exercise (mean difference [95% CI]: 0.0156 AU [0.0015-0.0296], $P = .03$). In healthy horses, PIF_{Right} was significantly decreased after exercise (mean difference [95% CI]: 0.0247 AU [0.0061-0.0432], $P = .008$).

3.4 | Center of ventilation and VT_{EIT}

There was no significant effect of the interaction "exercise*diagnosis" on $VT_{\Delta Z}$ ($P = .13$) or on the position of the CoV_{RL} ($P = .67$) or CoV_{VD} ($P = .78$) in this population. Data are shown in Table 2.

4 | DISCUSSION

Results from our study show that EIT flow variables can detect differences in lung function between healthy horses and horses with asthma after exercise. No differences at rest were seen between the 2 study groups. No significant difference was found for flow variables between MEA and SEA horses after exercise.

4.1 | EIT utilization in horses without sedation and directly after exercise

Pulmonary function tests are important, routinely-used tools in the diagnosis of obstructive or restrictive lung disease in human medicine.[12,24] Their application in equine medicine is largely limited to referral centers because of the need for specialized equipment such as facemasks, high-speed treadmills for horses, and esophageal manometers. Sedation often is required, which impacts pulmonary function and complicates interpretation of results.[25,26] We investigated the use of EIT, a noninvasive imaging technique based on electrical impedance in horses with EA. Measurements were easily performed in all horses without sedation and required a minimal amount of time for data collection. The EIT measurements could be repeated shortly after exercising the horse, without any impairment by behavior or adverse effects.

4.2 | Bronchoconstriction and EA

Regardless of the severity of EA, PEF_{Global} was significantly increased as compared to healthy horses after exercise and significantly higher within each asthma group after exercise compared to before exercise. During inspiration, a significant difference in PIF_{Global} was seen in asthmatic horses as compared to healthy horses after exercise. However, an increase in PIF_{Global} was seen only in SEA-affected horses, and not in MEA-affected horses, when comparing before and after exercise. The increase in global flow indices after exercise in severely asthmatic horses was more pronounced during expiration with an increase of 94% compared to inspiration during which an increase of 83% was observed. For MEA, the increase was similar for both flows (60% for expiration and 66% for inspiration). These findings are expected because EA is a lower airway disease, and affects expiration to a greater extent than inspiration.[27] The fact that only SEA horses had increased postexercise PIF_{Global} may be attributed to more severe airway narrowing or may be a consequence of the small number of horses in the study. Recent studies showed that EIT can detect airflow changes in standing sedated adult healthy horses during a histamine provocation test and after the administration of salbutamol.[15,28] In these studies, EIT flow calculations correlated well with plethysmography measurements (including flow-volume loops), which is a validated method to measure different degrees of airway narrowing or dilatation.[15,28] The same EIT algorithm was used in our study to calculate peak inspiratory and expiratory flow changes, based on impedance variations measured by the EIT. No changes in $VT_{\Delta Z}$, represented by the total impedance change of all pixels over a breathing cycle, were seen between any group of horses or time points. This observation is important to note because flow changes can only be attributed to changes in airway diameter at constant tidal volumes (Poiseuille's law). This confirms that the changes seen in PEF and PIF are a consequence of exercise-induced airway narrowing instead of changes in tidal volume as could be argued. Differences between healthy horses and horses with SEA at rest also would be expected because horses with SEA have clinical signs of bronchoconstriction at rest. The absence of this difference in our study is likely a result of low power, with only 5 horses in the SEA group.

The distribution of ventilation represented by the CoV in our study was not different between healthy horses and horses affected by MEA or SEA, neither before nor after exercise. Changes in the distribution of ventilation were reported to be heterogeneous in humans suffering from asthma,[29] with the phenomenon of air trapping in severe asthma occurring mostly in the lower pulmonary lobes.[30] This finding indicates that CoV might not be the best variable to use for EIT-based lung function evaluation in asthmatic horses.

4.3 | Regional flows and distribution of ventilation

One advantage of EIT compared to other lung function tests is that it also allows regional assessment of airflow. The EIT was able to detect more difference in the ventral area of the lungs after exercise as compared to the dorsal area. During expiration, a significant effect of exercise was identified in both asthmatic groups, whereas only SEA-affected horses had increased inspiratory ventral flow. This finding is in agreement with the aforementioned fact that asthma affects expiration more than inspiration.[27] To our knowledge, no data indicate that the ventral area of the lungs may be more affected by asthma in humans or any animal species. This observation however may be explained by the anatomy of the equine lungs. Both lungs have a similar shape with a ventral cardiac notch that separates a smaller cranial portion from a larger caudal lung portion.[31] In the standing horse, most of the lung is dorsal to the diaphragm[31]; therefore, the pulmonary volume is smaller ventrally than dorsally. The CoV_{VD} in our study also was located slightly dorsal in healthy horses, supporting the smaller ventral pulmonary volume. It could be hypothesized that the smaller ventral pulmonary volume may facilitate detection of mild to severe airflow changes in that area. A previous study reported that the CoV_{VD} in healthy horses was located ventrally.[19] This discrepancy may be associated with a difference in the definition of the CoV. The previously mentioned study evaluated CoV_{VD} position based on thoracic height and not based on the ROI of the lungs as done in our study.

A postexercise increase in airflow in the left and right lungs was noticed in both asthmatic groups in comparison to healthy horses. During expiration, more changes were noticed in the left lung in comparison to the right lung.

Similar to prior studies, the position of the CoV_{RL} at rest was located slightly more to the right in healthy horses and in horses with EMA and SEA.[15,19] This finding might be attributed to equine pulmonary anatomy, as the right lung is larger because of the presence of the accessory lobe.[31] The CoV_{RL} was not significantly different between healthy horses and horses with EA, suggesting that air is not redistributed in EA. The presence of airflow changes in both lungs in horses with EA after exercise supports the fact that both lung halves are affected equally. This observation is in agreement with the systemic component of EA.[32-34] Additional studies with higher case

numbers are needed to evaluate whether these variables or a combination of them are useful for the diagnosis of EA and differentiating between MEA and SEA.

4.4 | EIT calculation and future approach to lung function testing

Mild-to-moderate EA is known to decrease performance in racehorses,[35] but only causes subtle respiratory clinical signs at rest.[1] These features make it difficult to diagnose MEA without additional diagnostic testing. Airway hyperreactivity is recognized as the cause of exercise-induced bronchoconstriction.[10,11] This necessitates a dynamic diagnostic measurement immediately after exercise, such as forced expiratory volume over 1 second, which is used in human medicine.[36] In equine medicine, other lung function tests are used such as oscillometry,[37] plethysmography combined with bronchoprovocative challenge[38,39] and forced expiratory maneuvers,[40] but these diagnostic tests are not practical for use in the field. Electrical impedance tomography measurements can be obtained in the field, but none of the flow variables used in our study showed a significant difference between MEA and SEA. On the other hand, our study was not specifically designed to distinguish between MEA and SEA, but to explore a difference between healthy and EA-affected horses before and after exercise. Therefore, additional studies with larger numbers of horses will be needed to assess whether a difference in regional or global PEF or PIF could distinguish between horses with MEA and SEA.

Another future approach would be to use other algorithms in addition to expiratory and inspiratory flow to evaluate EIT measurements. The EIT-derived flow-volume loops may prove useful in naturally-occurring EA because it has been shown recently that indices derived from the shape of flow-volume loops change after histamine bronchoprovocation in healthy horses.[28] This study compared EIT changes to plethysmography and found a similar progression of all variables during histamine challenge and subsequent bronchodilatation using albuterol. Flow-volume loops should be evaluated in horses with EA in the future to determine if these variables could distinguish between MEA and SEA.

Contrary to our hypothesis, no difference was found between healthy and SEA-affected horses at rest. This may be explained by the fact that the severely asthmatic horses were not in acute crisis during examination, and the remodeling of the airways might not have been pronounced enough to induce detectable airflow changes at rest. However, the study population was small, and therefore additional studies would be beneficial.

4.5 | Limitations

The main limitation of our study was the small number of horses in each group, which was the case for several reasons. We elected to exclude horses that had been treated with corticosteroids or bronchodilators 2 weeks before presentation, which is the case for many of our patients. We also elected to use stringent criteria including both clinical signs and BALF findings. Several controls could not be exercised and therefore only data at rest were available. However, even with the relatively low numbers of horses in both groups, we found significant differences in our main outcome variables. We anticipated low numbers of healthy controls from our patient population and teaching herd, and therefore staff members and the Swiss National Horse Center were contacted to provide horses in active training without a history of cough or perceived exercise intolerance for inclusion in the study in addition to horses from the university teaching herd. The different origin of horses also could have contributed some bias to the study.

Another limitation was the total number of cells counted for the BALF cytology. The samples were evaluated by clinical pathologists of the University of Zurich according to the standard clinical protocol, which include the evaluation of 200 cells and not the 400 cells recommended in American College of Veterinary Internal Medicine (ACVIM) consensus statements.[1] This difference may have had an impact on the classification of horses. However, the number of cells counted for the BALF differential affected mostly mast cells, and to a lesser extent neutrophils and eosinophils.[12] The ACVIM consensus statements indicate that up to 5% neutrophils in BALF cytology are consistent with healthy controls, but that >10% indicate MEA.[1] Including the 5% to 10% "gray zone" in the healthy group may have biased our study, but forming more distinct groups was not possible based on the number of horses.

A general limitation of EIT is that 1 plane of electrodes is used and therefore only a lens-shape section of the lung is measured. Recently, image reconstructions of a wider 3-dimensional part of the equine thorax have been completed by using 2 EIT electrode planes[41] and may offer more extensive lung examinations in the future.

Established lung function measurements were not performed for comparison to EIT-measurements for practical reasons. In a previous study, EIT changes were compared to plethysmography in healthy horses during a histamine bronchoprovocation test and showed that EIT and plethysmography flow variables had similar progression during histamine challenge.[15] Ours was an exploratory study to observe if EIT can detect flow changes before and after exercise. In future studies, the magnitude of EIT flow change should be compared to changes evaluated using traditional measurement techniques of respiratory mechanics.

4.6 | Conclusions

In conclusion, we showed that EIT measurements can be easily performed in standing, nonsedated horses before and after exercise and may provide a practical diagnostic tool for use in the field. More studies using larger cohorts and additional EIT-derived variables are needed to further evaluate the diagnostic ability of EIT in horses with EA.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC of Zurich University, ZH155/16.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supplementary Information 1. Clinical signs and results from diagnostic testing of all horses included in the study

Group	Origin	Cough		Exercise intolerance		RR at rest	Abnormal lung sounds	Dys-pnea	Recovery time of RR after exercise in minutes	HOARSI	PaO_2 mmHg	Blood work	Mucus Score	BALF cytology (%)				
		Hx	PE	Hx	PE									Neutro-phils	Eosino-phils	Mast cells	Lympho-cytes	Macro-phages
CONTROL	TH	No	No	No	No	8	No	No	15	1	95	Yes	1	5	0	0	55	40
	NHC	No	No	No	NE	12	No	No	NE	1	98	Yes	0	2,5	0	1,5	50	46
	NHC	No	No	No	NE	8	No	No	NE	1	97	Yes	0,5	5	0	1	34	60
	SO	No	No	No	NE	12	No	No	NE	1	98	Yes	1	3	0	1,5	66	27,5
	NHC	No	No	No	No	16	No	No	10	1	NA	No	NA	5	0	1,5	65,5	28
	SO	No	No	No	NE	8	No	No	NE	1	93	Yes	1	9	0	1	35	55
	NHC	No	No	No	No	12	No	No	10	1	NA	No	NA	8,5	0	1	60,5	29,5
	NHC	No	No	No	No	8	No	No	10	1	NA	No	NA	8	0	1,5	33,5	57
	NHC	No	No	No	No	8	No	No	10	1	NA	No	NA	5	0	1	40	54
MEA	CO	Yes	No	Yes	No	16	No	No	10	3	97,1	Yes	1	3,5	1,5	11,5	36,5	47
	CO	Yes	No	Yes	Yes	14	No	No	25	2	89	Yes	2	12	0	0	54,5	34
	CO	Yes	No	No	Yes	18	No	No	20	3	86,4	Yes	4,5	0	0,5	7,5	25	67
	TH	No	No	No	Yes	8	No	No	25	1	91	Yes	1	8,5	6	3	24,5	50,5
	TH	No	No	No	Yes	12	No	No	25	1	88	Yes	1	13,5	0	0	37,5	49
	CO	Yes	Yes	Yes	Yes	18	No	No	20	3	92	Yes	3	9	0	4	64	23
	CO	Yes	Yes	Yes	Yes	22	No	No	≥ 20	3	NA	Yes	3	9,5	0	0	72,5	18
	CO	No	No	Yes	Yes	12	No	No	≥ 20	2	90	Yes	1	0,5	0	3	45	51,5
	CO	Yes	No	No	No	12	No	No	5	3	85	Yes	3	9	0	4	64	23
SEA	CO	Yes	Yes	Yes	Yes	24	Wheezes	Yes	25	4	74,3	Yes	3	23,5	0	1	59	16,5
	CO	Yes	No	Yes	NA	18	No	No	NA	4	79,7	Yes	3	28	0	4	40	28
	CO	Yes	No	Yes	Yes	28	Wheezes	Yes	≥ 20	4	79,5	Yes	2,5	29	0	1	38	32
	CO	Yes	No	Yes	NE	24	Wheezes	Yes	NE	4	NA	Yes	2	35	0	0	48	17
	CO	Yes	Yes	Yes	Yes	24	Wheezes	Yes	≥ 30	4	68,1	Yes	3,5	NA	NA	NA	NA	NA

BALF, bronchoalveolar lavage fluid (differential cell count performed on 200 cells); CO: client-owned; HOARSI, Horse Owner Assessed Respiratory Signs Index; Hx, historical; MEA, Mild-to-moderate Equine Asthma; NA, not available; NE, not exercised; PaO_2, arterial oxygen tension; PE, physical exam; RR, respiratory rate; SEA, Severe Equine Asthma; NHC: National Horse Center; SO: staff-owned; TH: teaching herd