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STANDARD ARTICLE

The effect of single pretreatment with salbutamol on recovery of bronchoalveolar lavage fluid in horses with suspected or confirmed severe equine asthma

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Background: Bronchoalveolar lavage (BAL) is a method for the recovery of respiratory secretion from the lower airways.

Objectives: To investigate if the administration of a single dose of a bronchodilatator in horses with a suspected or confirmed severe equine asthma could improve recovery of bronchoalveolar lavage fluid (BALF).

Animals: Twenty-eight horses with severe equine asthma.

Methods: Horses were divided into 2 groups: group "treated" was given salbutamol before endoscopic examination and BALF collection, whereas group "not treated" was not given. BAL was performed with BAL-catheter by instilling 350 mL of sterile saline. Amount of recovered fluid was recorded. Statistical analysis was performed with a two-tailed Student's *t* test.

Results: The average fluid recovery in the horses treated with salbutamol was 52% ± 15% (mean +/− SD), compared with 38% ± 13% for the group of horses not treated with salbutamol ($P = 0.013$).

Conclusions and Clinical Importance: Clinicians should consider administration of salbutamol before performing BAL on horses with asthma.

KEYWORDS

albuterol, bronchi, bronchoalveolar lavage, equidae, lungs, β_2-adrenergic agonist

1 | INTRODUCTION

Severe equine asthma, formerly known as recurrent airway obstruction (RAO), is one of the most common respiratory disorders in older horses.[1,2] Clinical signs in susceptible horses occur after exposure to aeroallergens, such as in moldy hay, straw, and barn dust. It causes airway hyperreactivity, inflammation, bronchoconstriction, and neutrophil influx.[3] Bronchoalveolar lavage (BAL) is the gold standard for diagnosing respiratory diseases such as equine asthma.[4]

BAL is a procedure used in the recovery of respiratory secretions from the lower airways. It is primarily used for cytological evaluation of non-septic conditions and is especially helpful when severe equine asthma, mild equine asthma, or exercise-induced pulmonary hemorrhage is suspected. The procedure can be performed either endoscopically or blindly by equine BAL catheter.[5] Samples are taken from a localized part of the lung; therefore, BAL is only used when diffuse lower airway disease is suspected.[5,6] Single BAL is representative of the whole lung in horses with severe equine asthma.[7]

In some horses, clinicians have observed that the sampled bronchus collapses when vacuum is applied to retrieve the BAL fluid (BALF), thereby reducing the amount of recovered fluid. In human medicine, asthmatic patients with moderate to severe airway obstruction usually have a poor BALF recovery, which might potentially be

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive airways disease; CRT, capillary refill time; CS, clinical score; ES, endoscopy score; MDI, metered-dose inhaler; SD, standard deviation; RAO, recurrent airway obstruction.

because of bronchoconstriction and airway collapse.[8] A positive correlation between the BAL cytological profiles and the recovery of fluid was found, with fluid volumes being significantly lower in horses with severe inflammation.[9] In addition, there were differences in the fluid recovery between the collapse score categories, with fluid recovery being significantly less in the severe collapse group.[9] Horses with severe inflammation in BALF cytology have lower BALF volume return than those with mild, moderate, or no inflammation.[10] Therefore, in human medicine, bronchodilatators enhance the recovery of BALF, and drugs belonging to this group are widely used before BAL procedure.[11,12]

Salbutamol, also known as albuterol, is a short-acting β-adrenergic receptor agonist that causes bronchodilatation. It is used in the treatment of humans with asthma, chronic obstructive airways disease and of horses with severe equine asthma.[13,14] Salbutamol improves pulmonary function by approximately 70% in horses during an episode of airway obstruction.[14] Bronchodilatation before BAL reduces the risk of bronchoconstriction and cough during the procedure.[5] Inhaled salbutamol is preferred over systemic administration because of the reduced dosage regimes which consequently lowers the risk of adverse effects. The metered-dose inhaler system is advantageous over nebulization; it is rapidly administered, gives consistent dose delivery, and there is minimal risk of pulmonary contamination by environmental microorganisms.[15] It is also more efficient than oral administration of β-adrenergic receptor agonists.[16]

Because there is a lack of information on whether salbutamol enhances the recovery of BALF, the objective of our study was to investigate whether the administration of a single dose of a bronchodilatator in horses with suspected severe equine asthma could improve the recovery of BALF.

2 | MATERIALS AND METHODS

2.1 | Horses

The study population consisted of 28 horses, including 3 Stallions, 15 Mares, and 10 Geldings. Horses were either confirmed or suspected cases of severe equine asthma. Horses were randomly divided into 2 groups: group I was given salbutamol before endoscopic examination and BALF collection, whereas group II was not treated with salbutamol before examinations. Group I was made up of 12 horses and group II of 16 horses. The age of the horses ranged from 7 to 26 years and weighed from 400 to 750 kg. All horses were privately owned. Horses came from different farms in the Lower Silesian Voivodship of Poland. Horses were either stabled or out on pasture before examinations. Samples were taken from December 2016 to November 2017. Horses were not currently undergoing treatment for asthma.

2.2 | Clinical examination

Before video endoscopic examination and BALF collection, horses underwent a full clinical examination, including visual inspection, measurement of rectal body temperature, pulse and respiratory rate, evaluation of appearance of mucous membranes, auscultation of the heart including heart rate, evaluation of intestinal peristalsis, and recording of capillary refill time. The weight of the horses was estimated with equine girth tape.

2.3 | Endoscopic examination and BALF collection

All horses were at rest for at least 12 hours before examination. Group I was given a single dose of inhaled salbutamol (VentolinEvohaler, 100 μg/actuation, GlaxoSmithKline, Great Britain) before sedation and 30 minutes before BAL. An EquineHaler (Kruuse, Denmark) was used, in the dosage of 1 actuation per 100 kg body weight (100 μg/100 kg). Endoscopic examination and BAL were performed on horses sedated with 0.5 mg/kg xylazine (Nerfasin Vet, 100 mg/mL, aniMedica, GmBH, Senden-Bösensell, Germany) and 0.01 mg/kg butorphanol (Morphasol, aniMedica). A twitch was used additionally. A 1.8-m long endoscope (Karl Storz GmBH, Tuttlingen, Germany) was passed through the nasal passage into the trachea. Pictures were taken and changes in the airway were graded by a clinician with a modified RAO staging scale, previously described by Tilley et al (Table 1).[17] A BAL catheter (large animal BAL catheter; Mila International Inc., Florence, Kentucky) was passed in the same fashion as the endoscope into the bronchus until it wedged. BAL was performed by instilling 350 mL of sterile normal saline (Fresenius NaCl 0.9%, 500 mL, Fresenius Kabi Polska Sp. z o.o., Warsaw, Poland) at 37°C, in multiple boluses with 50-mL syringes. BALF was then re-aspirated through gentle suction using the same syringes until no further fluid was obtained. The amount of recovered fluid and surfactant were recorded, as well as the presence and quality of cough during procedure. Recovered fluid was expressed as volume in milliliter and then recalculated to percentage. The amount of recovered surfactant has been graded by a plus (+) or minus (–) scale: "–" no surfactant, "+" low amount of surfactant, "++" medium amount of surfactant, and "+++" large amount of surfactant. The presence and quality of cough was graded by a similar plus (+) or minus (–) scale: "–" no cough, "+" mild cough, "++" medium cough, and "+++" severe cough.

The BALF for each individual horse was pooled in a sterile specimen cup, placed on ice, and processed within 2-4 hours after collection. To carry out the cytologic examination of the BALF, a 10 mL aliquot was centrifuged at 300*g* for 10 minutes using a centrifuge (Beckman Coulter Allegra x-22; Beckman Coulter Inc., Brea, California), and the smear of the sediment was stained with Wright's stain. A 400-leukocyte differential count was performed; epithelial cells were not included in the differential count.[18] More than 25% of neutrophils in a differential cell count were required to cytologically define asthma in horses.[19]

2.4 | Statistical analysis

Data have been presented as mean and SD. For statistical assessment, results of the amount of surfactant and the presence and quality of cough (plus (+) or minus (–) scale) have been transformed to natural numbers. Analysis by the Shapiro test for normality indicated that the data were normally distributed, including also data after conversion (both $P > 0.05$), and the F test to compare variances indicated that the data had equal variances ($P > 0.05$). The unpaired *t*-test was performed

TABLE 1 Modified clinical staging of equine asthma, according to Tilley et al[17]

Variable	0	1	2	3	4	5
Clinical assessment[a]						
Cough score	None	Coughs at specific times of day (feeding/exercising/making beds)	Frequent cough with periods of no coughing	Very frequent cough		
Nostril flare	None	Flares during inspiration (returns to normal at end inspiration)	Flares on inspiration and exhalation (slight movement can still be seen)	Flares on inspiration and expiration (no movement can be seen)		
Abdominal lift	None	Slight flattening of ventral flank	Obvious abdominal flattening and "heave line" extending no more than halfway between cubital joint and tuber coxae	Obvious abdominal lift and "heave line" extending beyond halfway between cubital joint and tuber coxae		
Airway endoscopy[b]						
Mucus accumulation	None, clean	Little, multiple-small blobs	Moderate, larger blobs	Marked, confluent or stream-forming	Large, pool-forming	Extreme, profuse amounts
Mucus color	None, clean	Colorless	White	Thick white	Yellow	Thick yellow
Mucus localization and stickiness	None, clean	1/2 ventral	2/3 lateral	3/4 dorsal	Threading	Threading
Mucus apparent viscosity	None, clean	Very fluid	Fluid	Intermediate	Viscous	Very viscous

Final asthma stage: stage 0, no asthma (total score = 0); stage 1, mild asthma (1 ≤ total score < 2); stage 2, moderate asthma (3 ≤ total score ≤ 4); stage 3, severe asthma (5 ≤ total score = 6).
[a] Final clinical score (CS): 0 (CS final score < 2), 1 (2 ≤ CS final score ≤ 4), 2 (5 ≤ CS final score ≤ 6), 3 (7 ≤ CS final score ≤ 9).
[b] Final airway endoscopy score: 0 (ES final score < 8.5), 1 (8.5 ≤ ES final score ≤ 12), 2 (12 < ES final score ≤ 16), 3 (ES final score > 16).

to evaluate the results. All tests were performed using R for Windows (version 3.2.1). P <0.05 was considered statistically significant.

3 | RESULTS

The results of the BALF analysis, clinical assessment, and endoscopy of the lower airways are shown in Table 2. A t test did not reveal any differences among components of clinical examination such as cough score, nostril flares, and abdominal lift, but overall, there was a statistical difference between the groups.

Endoscopic examination of lower airways according to modified Tilley et al scale did not reveal any difference among assessed variables such as mucus accumulation, mucus color, and mucus apparent viscosity, except mucus localization and stickiness.[17] Final endoscopy score (ES) was not significant when comparing both groups.

Final asthma stage, which is a combination of the final result of clinical examination, and final ES did not differ between the groups.

The results of quality of cough during BAL, the amount of surfactant in BALF, and the amount of fluid recovered expressed as a percentage are shown in Table 2.

BALF 400-cell leukocyte differential count did not reveal any differences between the groups in respect to mean percentage of neutrophils, lymphocytes, macrophages, eosinophils, and mast cells.

Quality of cough during BAL and amount of surfactant in BALF did not differ between the groups. The average fluid recovery in the horses treated with salbutamol was 52.29% ± 14.65%, compared with 37.54% ± 13.09% for the group of horses not treated with salbutamol (P = 0.013).

4 | DISCUSSION

In our study, single pretreatment with salbutamol was effective in increasing recovery of BALF in horses with severe equine asthma. Presumable positive effect of salbutamol results from its bronchodilatatory effect. Although salbutamol did not reveal any differences in the amount of surfactant and quality of cough during procedure, the results suggest its usefulness in diagnostic approach to horse with asthma. Except higher amount of BALF, salbutamol leads to lower vacuum level being applied during procedure, because of easier recovery.

The presumptive disease of the horses in our study was severe equine asthma. The diagnosis of severe equine asthma was confirmed through history, clinical examination, endoscopy, and cytological evaluation of BALF. Based on history and clinical examination, our study population did not have other diseases associated with the confirmed diagnosis.

It has earlier been observed that there is a negative correlation between the degree of bronchial collapse and the recovery of fluid,[8,9] and therefore, we could assume that administration of a bronchodilatator would increase the fluid recovery. Clinicians have hypothesized this theory, but to our knowledge, there has not been a study confirming it.

In horses with severe equine asthma, the smooth muscles of the bronchi are hyperreactive and collapse more readily during BALF aspiration than in healthy horses.[9] Horses with severe inflammation in BALF cytology have lower BALF volume recovery than those with mild, moderate, or no inflammation.[10] This supports the theory that inflammation contributes to the degree of collapse and therefore the BALF recovery. The proportion of neutrophils in BALF is an indicator of airway inflammation, and there is positive correlation between the percentage of neutrophils in BALF and bronchial collapse.[9] Except final

TABLE 2 Results of BALF cytology, clinical assessment, endoscopic evaluation, quality of cough during BAL, amount of surfactant in BALF, and amount of fluid recovered in horses pretreated and not pretreated with salbutamol

	Not treated		Treated		
	Mean	SD	Mean	SD	*P* value
Clinical assessment					
Body temperature (°C)	37.4	0.37	37.4	0.45	0.34
Heart rate (bpm)	38	5.65	39.75	4.0	0.46
Respiratory rate (breaths/min)	26.5	6.39	26.45	9.37	0.87
Cough score	2.18	0.83	2.16	0.83	0.94
Nostril flare score	2.25	0.57	2.08	0.79	0.52
Abdominal lift score	2.37	0.71	1.66	0.77	0.12
Final clinical score[a]	2.68	0.6	2.08	0.51	0.009
Airway endoscopy					
Mucus accumulation	3.68	1.13	3.5	1.08	0.66
Mucus color	3.5	0.96	3.5	0.79	0.57
Mucus localization and stickiness[a]	3.68	0.94	2.58	0.9	0.004
Mucus apparent viscosity	3.18	1.16	2.91	0.9	0.51
Final endoscopy score	1.87	0.88	1.75	0.86	0.71
Final asthma stage	2.56	0.72	2.1	0.57	0.13
BALF 400-cell leukocyte differential count					
% neutrophils	61.85	12.25	66.1	15.53	0.42
% lymphocytes	16.09	9.8	13.79	10.28	0.55
% macrophages	21.46	9.3	19.33	9.8	0.56
% eosinophils	0.34	0.38	0.56	0.73	0.31
% mast cells	0.23	0.43	0.16	0.32	0.65
Bronchoalveolar lavage					
Quality of cough during BAL	1.5	0.63	1.41	0.66	0.73
Amount of surfactant in BALF	1.18	0.75	1.5	0.67	0.26
Amount of fluid recovered (%)[a]	37.54	13.09	52.29	14.65	0.013

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid.
Values are expressed as mean and SD.
[a] Differences statistically significant ($P < 0.05$).

clinical score (CS) and mucus localization and stickiness observed during endoscopy, there were no differences in other variables between the groups, including proportion of neutrophils. We assume, in the light of the lack of differences between BALF differential count, that higher fluid recovery is the result of pretreatment with salbutamol.

Salbutamol stimulates the β-receptors in the smooth musculature, causing relaxation and dilatation of the bronchi.[13] Based on our study, we can suspect that the positive relationship of salbutamol with BALF recovery is because of this bronchodilatatory effect. This reduces the chance of bronchial collapse and increases the total capacity of peripheral bronchi when fluid is aspirated. It also relieves coughing during the procedure and therefore decreases the chance that the BAL catheter is moved during the BAL. Generally, the procedure becomes safer, predictable, and controlled when administering salbutamol before BAL.

This is reflected in the results obtained in our study, in which there are clear differences within the groups. In the group of horses not treated with salbutamol, there is a bigger difference between individual horses, the poorest fluid recovery being 10.67%, and the highest recovery being 50%. Whereas in horses treated with salbutamol before BAL, the poorest fluid recovery was 37.14% and the highest recovery was 68%. This shows that salbutamol gives more consistent volume returns. Statistical analysis reveals that there is a significant difference between the groups, with the group receiving a single pretreatment of salbutamol having a higher BALF recovery percentage. On the background of the history and our examinations, we observed that the horses were in different stages of severe equine asthma. This is another factor adding to the high variability between individual horses and between the groups.

As mentioned previously, horses were allocated to the groups randomly, and we should expect comparable results of clinical examination and other ancillary tests. Except final CS and mucus localization and stickiness observed during endoscopy, other assessed parameters were comparable. Particularly, when analyzing BALF differential count, there were no differences between the groups. However, fluid recovery was different between the groups because of salbutamol effect, and some variations might occur in BALF differential count, depending on fluid volume and technique used.[20] However, there were no differences between sequential and pooled BALF aliquots, and therefore, all aliquots can be considered to represent the cytology of the lavaged lung segment.[21] At this stage, we are not able to state if the groups used in our study were comparable, as would have required the use of additional tests.

Except differences in the amount of fluid recovered, there was no other positive effect of salbutamol on BAL procedure. Quality of cough during procedure was similar, as well as amount of surfactant.

However, poor BALF recovery often leads to higher vacuum level being applied during procedure.[9] High negative pressure leads to moderate bronchi collapse, even in mild affected cases, negatively affect the welfare of the animal. Moreover, high vacuum can lead to cell damage and getting unclear results.[5]

Our study had limitations. Because horses were in different stages of the disease, it would be helpful to perform blood gas analysis and measurement of intrathoracic pressure before BAL to have a homogenous group. This was not possible because of economic reasons and availability of equipment. These examinations would have been useful to evaluate the severity of bronchoconstriction and categorizing the stage of equine asthma. Performing blood gas analysis and measurement of intrathoracic pressure would have given a more objective view to assess the severity of the disease and how salbutamol affects the bronchi in different stages of the disease.

We conclude that clinicians should consider pretreatment with salbutamol before performing BAL procedures in horses with severe equine asthma, in order to aid in fluid recovery for cytological evaluation.

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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

In accordance with the Experiments on Animals Act from January 15, 2015 (*Journal of Laws of the Republic of Poland*, 2015, item. 266), the study does not require the approval of the Ethics Committee. All procedures were performed during the study with the owner consent.

HUMAN ETHICS APPROVAL DECLARATION

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

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