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THE EFFECT OF DRY SALINE AEROSOL THERAPY ON ARTERIAL BLOOD GAS PARAMETERS IN HORSES WITH MILD EQUINE ASTHMA

EFECTUL TERAPIEI CU AEROSOLI SALINI USCAȚI ASUPRA PARAMETRILOR GAZELOR ARTERIALE SANGUINE LA CAII CU FORME UȘOARE DE ASTM ECVIN

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ABSTRACT

The purpose of this study was to test the effect of dry saline aerosol on blood gas changes in horses diagnosed with equine asthma and healthy horses. We hypothesized that the positive effect of dry saline aerosol therapy as a mucolytic would lead to changes in arterial blood gases due to improved gas exchange. The analysis of the effect on respiratory efficiency and blood oxygenation was done by monitoring and interpreting PaO_2 (arterial partial oxygen pressure), $PaCO_2$ (arterial partial CO_2 pressure), and SaO_2 (arterial oxygen saturation of haemoglobin). The study was performed on 44 horses (21 males and 23 females). The horsed were divided into two groups: Group 1 - 23 patients diagnosed with mild equine asthma and Group 2 - 21 healthy patients without evident respiratory symptomatology. All patients received the same treatment protocol for seven days, 60 minutes a day, with blood samples taken on the first and last day of the-rapy. Group 1 showed statistically significant improvement in clinical status and arterial blood gasometry ($p < .05$) after seven days of therapy. The results of Group 2 were in the normal range, statistical improved values were detected for PaO_2 and $PaCO_2$. The study shows promising results; using the SaltMed dry aerosol generator can improve the clinical status of horses with mild equine asthma signs.

Keywords: SaltMed, dry aerosols, asthma, halotherapy, horses

REZUMAT

Scopul acestui studiu a fost de a testa efectul aerosolilor salini uscați asupra modificărilor gazelor sanguine la caii diagnosticați cu astm ecvin comparativ cu caii sănătoși. Ipoteza noastră a fost că efectul pozitiv al terapiei cu aerosoli cu soluție salină uscată ca mucolitic ar duce la modificări ale gazelor sanguine arteriale datorită îmbunătățirii schimbului de gaze. Analiza efectului asupra eficienței respiratorii și a oxigenării sângelui a fost realizată prin monitorizarea și interpretarea valorilor PaO_2 (presiune arterială parțială a oxigenului), $PaCO_2$ (presiune arterială parțială CO_2) și SaO_2 (saturația arterială de oxigen). Studiul a fost efectuat pe 44 de cai (21 masculi și 23 femele). Caii au fost împărțiți în două grupe: Grupul 1 - 23 de pacienți diagnosticați cu astm bronșic ecvin formă ușoară și Grupul 2 - 21 de pacienți sănătoși, fără simptomatologie respiratorie evidentă. Toți pacienții au primit același protocol de tratament timp de șapte zile, 60 de minute pe zi. Prelevarea probelor de sânge a fost făcută în prima și ultima zi de terapie. Grupul 1 a arătat o îmbunătățire semnificativă statistic a stării clinice și a valorilor gazelor sângelui arterial ($p<0,05$) după șapte zile de terapie. Rezultatele Grupului 2 au fost în intervalul normal, au fost detectate valori semnificative statistic pentru PaO_2 și $PaCO_2$. Studiul arată rezultate promițătoare; utilizarea aparatului generatorului de aerosoli uscați SaltMed poate îmbunătăți starea clinică a cailor cu semne de astm ecvin.

Cuvinte cheie: SaltMed, aerosoli uscați, astm, haloterapie, cai

Halotherapy (gr. halos = salt) is a technique that uses aerosolized microparticles of salt (sodium chloride), provided (4, 25) in a man-made environment to treat respiratory diseases. It is used as an alternative to speleotherapy (gr. speleos = cave), a special form of climatotheraphy that uses certain conditions found in salt-mines to treat the respiratory and skin-related conditions (4, 9, 25). The Equine Asthma Syndrome (E.A.S) is a term that describes a variety of signs ranging from mild (Inflammatory airways disease – I.A.D) to severe (Recurrent Airways Obstruction – R.A.O. and Summer Pasture-Associate R.A.O.) and has been introduced in 2016 by the ACVIM Consensus Statement (13) as a new understanding of I.A.D. describing non-infectious, inflammatory, recurrent (chronic), and reversible disorders of adult horses. The E.A.S. phenotypes are classified as mild/moderate asthma (I.A.D.) characterized clinically by nonspecific respiratory signs like chronic intermittent coughing and decreased

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performance, and severe asthma (RAO/SPARAO) characterized by airflow obstruction, associated with cough and chronic emphysema, mucus hypersecretion, and airway hyperreactivity (6, 12). Mild/moderate E.A.S. pathogenesis is not entirely known as many etiological agents, mainly non-infectious ones, are involved in the pathogenesis of the disease (13). Horses housed in unacceptable conditions are exposed to a high amount of aerosolized particles, gases, and allergens in a cumulative manner (20). Several studies identify these as causative in the aetiology of IAD. (16, 28, 37). The contributions of the environmental and stable condition in I.A.D. aetiology is partially understood, compared with the numerous studies that support the role of hay and bedding allergens and endotoxins in severe E.A.S. (R.A.O.) aetiology (13, 20). The most common allergens, involved in severe equine asthma, found in fodder include dust, moulds, or pollen 911, 19, 29).

In cases of I.A.D., bronchoalveolar lavage fluid (BALF) shows neutrophilia, this appears to be associated with coughing symptoms (5), occasionally other types of cells can be present in BALF like mast cells or eosinophils (7, 24). Horses with asthma are usually adults, afebrile, have repeated episodes of increased respiratory effort at rest, and generally respond to corticosteroid treatment (8, 26, 27).

There are many similarities between human asthma, feline asthma (35), and equine asthma (22). Human asthma is a chronic inflammatory condition whose main clinical trait is a variable and (at least partially) reversible respiratory obstruction (32). Another important feature of human asthma, first described in the early 20s (18), is airway remodelling. This terminology describes a set of changes in the airways' structural cells and tissues (29), whose impact on clinical asthma presentation is largely unknown (34).

Medical treatment of E.A.S. is focused on decreasing lung inflammation (13). Halotherapy is a known drug-free treatment method of nonspecific inflammatory pulmonary diseases, bronchial hyperactivity, and asthma in humans (3, 10). Considering the similarities between E.A.S. and asthma in humans, the use of halotherapy could prove useful in decreasing or even treating the signs of mild asthma in horses.

The purpose of this study was to test the effect of dry saline aerosol on blood gas changes in horses diagnosed with equine asthma and healthy horses. We hypothesized that the positive effect of dry saline aerosol therapy as a mucolytic would lead to changes in arterial blood gases due to improved gas exchange.

MATERIALS AND METHODS

We used the SaltMed device (manufactured by TechnoBionic S.R.L. Buzău, Romania). The generation of micro-particles is performed through a cartridge with a special structure containing strainers and dry salt. The dry salt is stirred through a ballooning motion. The particles' erosion through a flow of air generated by a pump leads to the small-sized aerosolized particles (between 1 and 5 μ) sent to the horse's nostrils where they are inhaled and delivered to the distal airways. For the analysis of arterial blood gases, the automatic analyser SIEMENS RAPID Point® 500 (Siemens) was used, which can process a sample in a maximum of 60 seconds. The test samples were represented by arterial blood, collected from the carotid artery in order to obtain a general overview of the oxygenation status, in 2 ml heparinized syringes, analysed within a maximum of 10 minutes after collection.

The number of horses examined was N = 67 horses, 32 males, 35 females, 29 diagnosed with mild equine asthma, and 38 healthy patients. The mean age of the patients was 9.67 4.39 years.

The diagnosis of mild asthma syndrome was made based on several criteria: the history of the patients, clinical signs, evaluation of the respiration and lung functionality using the Improved clinically Detectable Equine Asthma Scoring System (IDEASS) (Fig. 1) (7), as well as evaluation of blood gas changes. The horses were diagnosed with mild asthma or were considered clinically healthy based on the data obtained.

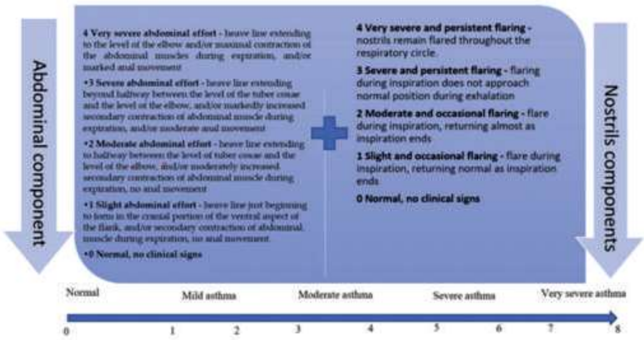

Fig. 1. Improved clinically Detectable Equine Asthma Scoring System (IDEASS) (7)

All the patients initially taken into the study were tested to evaluate if they can tolerate the mask needed for dry saline aerosol therapy. The custom mask (Fig. 2) adapted for horse dry saline aerosol administration was used for 20 minutes, and the patient's behaviour was monitored. Horses that presented restlessness, excessive movement of the head and evident discomfort after the mask was applied were excluded from the study. Because the mask was not tolerated, several horses (11 males and 12 females) were excluded, of which four were patients diagnosed with mild equine asthma, and 19 were healthy individuals. The final number of horses included in the study was N = 44 horses, 21 males and 23 females.

The horses were divided into two groups: G_1 = 23 patients (10 females and 13 males) with a diagnosis of mild equine asthma, and G_2 = 21, control group, healthy patients (13 females and eight males), without clinical signs of E.A.S.

Fig. 2. Mask used for halotherapy in horse

Work protocol

All the patients diagnosed with equine asthma were examined at the Equine Clinic of the Faculty of Veterinary Medicine of Cluj–Napoca. The clinically healthy group was examined either at the Equine Clinic or in the field. The mean age and the standard deviation of the individuals in G_1 were 7.13 2.9 years with a median of 7.00 years, 56.6% male and 43.4% female. The mean age and standard deviation of the individuals in G_2 were 7.42 2.5 years and a median of 8.00 years; there were 38.1% males and 61.9% females.

Both groups (G_1 and G_2) were subjected to the same protocol that consisted of three stages. Stage I – each patient was clinically examined, and arterial blood was sampled. An initial analysis of the blood gases (PaO_2, $PaCO_2$, SaO_2) and pH was evaluated for each sample. Stage II – SaltMed device was used on each patient for seven days, 60 minutes each day, split into two episodes of 30 minutes in the morning and evening. Due to the small size of dried salt particles, the SaltMed device emits approximately 3 mg/24 h. After each utilization, the mask was cleaned using 70% ethyl alcohol. Between SaltMed device utilization, the horses from both groups were housed in stables in similar conditions, ensuring clean bedding and proper ventilation of the stable, and were fed fresh hay. Stage III – After seven days of dry saline aerosol therapy, arterial blood was sampled from each patient again, and a final blood gas analysis (PaO_2, $PaCO_2$, SaO_2) and pH were performed for each sample. No other treatment or medication was administered to the patients.

For the analysis of arterial blood gases, we used the SIEMENS RAPID Point 500 (Siemens Manufactured) analyser, the device can process a sample in maximum of 60 seconds. The arterial blood was sampled in 2. ml sterile heparin syringes containing 80 U.I. heparin. The blood was sampled from the common carotid artery and analysed within 10 minutes after collection.

Statistical analysis

The statistical analysis was performed using SPSS Statistics (by I.B.M.). The data from both groups were evaluated for normal distribution using Skewness and Kurtosis z-value and Saphiro-Wilk's normality test (1). The z values of Skewness and Kurtosis for G_1 and G_2 are outside of the accepted interval -1.96 – 1.96. The p values of Saphiro-Wilk's test for pH, PaO_2, $PaCO_2$, SaO_2 for both groups are lesser than 0.05, the null hypothesis being rejected, the data from both groups were not normally distributed (30).

To evaluate if the values of pH, PaO_2, $PaCO_2$, SaO_2 present statistical significance after SaltMed therapy in G_1 and G_2, non-parametric Mann-Whitney U test was used. The test compared intra-group values from before and after SaltMed therapy, the values of pH, PaO_2, $PaCO_2$, SaO_2 are reported as mean and standard deviation, and the significance of test was reported at $p<0.05$. The values of IDEASS score for group G_1 show non-normal distribution, with a Saphiro-Wilk's normality test $p < 0.05$. The comparison of IDEASS score from before and after SaltMed therapy in group G_1 was performed using non-parametric Mann-Whitney U test, the results were reported as mean and standard deviation, and the test significance was reported at $p < 0.05$. Values of IDEASS score of groups G_2 were not significant, no statistical comparison was necessary.

RESULTS AND DISCUSSIONS

Group G_1 results

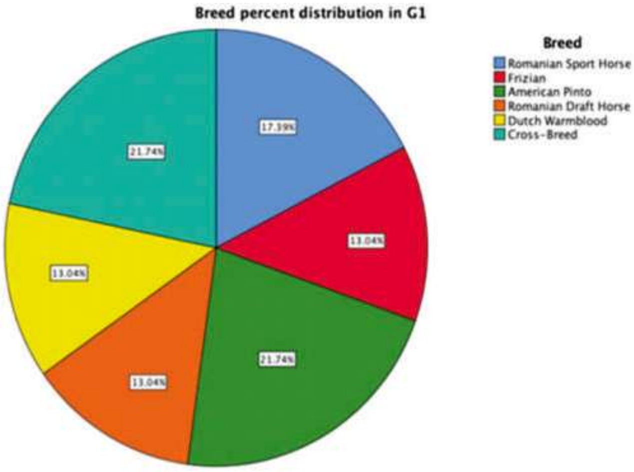

Fig. 3. Breed percent distribution in G_1

Table 1

Mean and standard deviation of the G1 group gas parameters

Characteristic $G_1 = 23$	Before SaltMed Therapy**	After SaltMed Therapy**	Mann-Whitney U test*
PaO_2 89-115 mmHg***	77.05 ± 2.31	85.24 ± 2.32	p = 0.001
$PaCO_2$ 37-49 mmHg***	46.48 ± 1.76	42.54 ± 1.66	
SaO_2 95-100%***	96.33 ± 0.72	98.62 ± 0.53	
pH 7.364 – 7.444***	7.37 ± 0.01	7.44 ± 0.02	

* The Mann-Whitney U test compares values before and after SaltMed therapy in G_1. Test significance was established at $p<0.05$
** Mean and standard deviation
*** Normal values for blood gas parameters (28)

The mean and standard deviation of the age for the individuals in G_1 was 7.13 2.9 years with a median of 7.00 years; there were 56.6% male and 43.4% female. The G_1 breed distribution is presented in Fig. 3.

The mean value and standard deviation of IDEASS score before halotherapy in G_1 were 2.78 0.42, after seven days of halotherapy the mean value and standard deviation were 1.49 0.51, and the statistical evaluation show's significant change (p=0.001) of IDEASS score after halotherapy compared with the values recorded before.

The statistic evaluation of G_1 data was made between values of pH, PaO_2, $PaCO_2$, and SaO_2 taken before starting SaltMed therapy and compared with the values obtained after seven days of halotherapy.

The measured values of data for the G_1 – group of horses, diagnosed with mild equine asthma are presented in Table 1.

The data obtained for G_1 was analysed and compared with the reference values. There is an increase in oxygenation parameters (PaO_2 and SOa_2) after Salt Med halotherapy. Compared to the reference values for PaO_2 (89 - 115 mmHg) and for SaO_2 (95 – 100%) (28), the PaO_2 and SaO_2 before SaltMed usage indicate low values at rest as a result of the disturbance of haematosis, with deficient gas exchange and possible airways inflammation impaired ventilation-perfusion. Arterial blood gas parameters in G_1, correlated with the clinical signs manifested by mild expiratory dyspnoea, coughing episodes, and decreased tolerance to exercise which were used to diagnose mild EA.S.

After the seven days treatment period with dry saline aerosols, a statistically significant improvement of these parameters can be observed in G_1, respectively an increase of the PaO_2 from 77.05±2.31 mmHg to 85.24±2.32 mmHg (Z=-5.812, p=0.001).

The oxygen saturation increased after dry salt aerosol administration from 96.33± 0.72% to 98.62± 0.53% (Z=-5.630, p=0.001). The values of PCO_2 show a statistically significance decrease (Z=-5.00, p=0.001) after dry saline aerosol from 46.48±1.76 to 41.82±1.66 mmHg.

The IDEASS score shows statistically significant changes in values before and after halotherapy (Z = -5.54, p=0.001), presenting an improvement after seven days of treatment. The mean and standard deviation of IDEASS value of G_1 decreased from 2.78 ± 0.42 to 1.48 ± 0.51 after seven days of SaltMed halotherapy. Dry saline aerosol therapy shows improved oxygen saturation, increasing the lung index of gas exchange efficiency. An improvement in clinical respiratory signs followed the improvement in the blood oxygenation.

Group G_2 results

The mean age of the individuals in G_2 was 7.42 2.5 years with a median of 8.00 years; there were 38.1% males and 61.9% females. The breed distribution of G_2 is presented in Fig. 4.

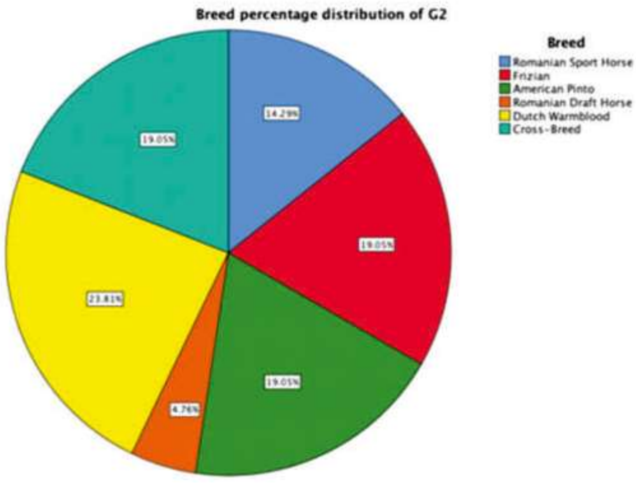

Fig. 4. Breed percentage distribution of G_2 regarding the G_2 control group, the blood gas values remained similar before and after halotherapy (Table 2)

The comparison of blood gas values from before and after seven days of treatment using SaltMed device shows statistical significance for PaO_2 and $PaCO_2$. The measured values are within the normal range, showing a mild increase in values of PaO_2 from 97.47± 1.73 mmHg to 98.49±1.04 mmHg (Z=-2.06,

Table 2

Mean and standard deviation of the G_2 group gas parameters

Characteristic $G_2 = 21$	Before SaltMed Therapy**	After SaltMed Therapy**	Mann-Whitney U test*
PaO_2 89-115 mmHg***	97.47 ± 1.73	98.49 ± 1.04	p = 0.039
$PaCO_2$ 37-49 mmHg***	43.69 + 0.76	43.18 + 0.54	p = 0.019
SaO_2 95-100%***	99.22 ± 0.58	99.35 ± 0.33	p = 0.89
pH 7.364 – 7.444***	7.40 + 0.03	7.41 + 0.03	p = 0.39

* The Mann-Whitney U test compares values before and after SaltMed therapy in G_2. Test significance was established at p<0.05
** Mean and standard deviation
*** Normal values for blood gas parameters 1

p=0.039), and decrease of $PaCO_2$ from 43.69±0.76 mmHg to 43.18±0.54 mmHg (Z=-2.34, p=0.019). The changes could indicate an improvement in the alveolar-arterial gradient and an improvement in the gas exchange index. The IDEASS score for the G_2 group was 0, no changes were seen in the score value after seven days of SaltMed therapy.

SaltMed dry saline aerosol therapy in the control group lead to minimal changes in the blood gas parameters that could indicate an increase in the alveolar-arterial gradient and better blood oxygenation.

Halotherapy is already recognized in human medicine, either in the form of nebulization of dry salt aerosols or exposure to a saline environment (4, 25). Through the mucolytic and antiseptic properties of the nebulized salt, masked halotherapy is a natural anti-congestive and antibiotic and a stimulator of pulmonary vascularization(3, 10, 31), which, at least in theory, should have positive effects on mild equine asthma as well as human asthma. The salt particles that stimulate the respiratory cilia movement also positively impact the sputum of mucus, changing its appearance and consistency and acting as mucolytic. The dry saline aerosol effect was observed instantly by the accumulation of a large quantity of serous and liquid mucus on the mask rather than the discharge before halotherapy (more viscous, difficult to remove mucus, which remained attached to the airways).

Saline inhalation determines a quicker improvement of signs of acute respiratory tract disorders. This device is used in human medicine as adjunctive therapy. It generates dry micro-particles of Sodium Chloride that improve upper and lower respiratory signs (31). The micronized sodium chloride (1-5 μm) is easily transported in the upper and lower respiratory tract, where it dissolves in the soluble part of the mucus layer that covers the respiratory epithelium. The local osmotic effect will attract the interstitial tissue's water to the respiratory tract lumen, decrease the inflammatory edema, and increase the mucus quantity (36). Through this mechanism, the mucus becomes more fluid and is easily eliminated through coughing. Through this simple mechanism, sodium chloride (NaCl) has a beneficial effect on the respiratory tract, improving many signs of the disorders of the respiratory tract (2, 23, 33). Inhaled sodium chloride in the respiratory tract has positive effects as an anti-inflammatory, and local antiseptic and also an ability to liquefy mucus and eliminate secretions (31). These effects lead to an improvement in the respiratory act and the exchange of alveolar-capillary gases.

The blood gas parameters in G_1 show a statistically significant improvement (p<0.05) after SaltMed dry saline aerosol therapy. This correlated with symptomatology improvement indicates a direct effect of the saline aerosol therapy on patients diagnosed with mild equine asthma syndrome. Thus, the respiratory clearance and gas exchange's efficiency was improved, and the respiratory effort was reduced. In the control group, saline aerosol therapy could indicate the increased gas exchange and blood oxygenation.

SaltMed dry saline aerosol therapy in horses is a promising adjunct in the treatment and management of equine patients with mild asthma syndrome. Although the possibility of total remission of disease-induced changes is possible in the case of mild asthma, palliative treatment is extremely important for maintaining a quality of life as close as possible to that of a healthy animal. Current therapies used such as corticosteroids, bronchodilators, and mucolytics have proven effective in all forms of equine asthma syndrome, dry saline aerosol therapy could be added as non-pharmacological therapy, with beneficial effects in improving patients' clinical condition. The use of dry aerosol therapy requires an understanding of technical and methodological aspects. The aerosol mask used in dry aerosol therapy must consider the horses' anatomy and physiologic features. The aerosol therapy mask application requires adaptation to the horse's exclusive nasal breathing. The mask used was custom-made, we have not used a commercial one. Masks for human use are not indicated, as the application on the horse's nose is improper for correct aerosolization. A proper aerosol device for horses should be comfortable, robust, have a mask well adapted to the equine face, be easy to transport and use in the field, be easy to wash and sterilize, and be cheap to maintain (15). Arterial oxygen pressure is influenced by the pressure of ambient air, oxygen, and inspired carbon dioxide, the total oxygen consumption of the body, carbon dioxide produced, and the rate of ventilation and alveolar infusion. Since the alveolar surface available for O_2 diffusion depends on the surface of open alveoli, in mild E.A.S., interleukin-mediated oedema of bronchiolar mucosa may occur, followed by mucus hypersecretion, as well as bronchospasm. The bronchospasm results from chronic inflammation and metabolites of arachidonic acid that stimulate cholinergic nerve endings of the bronchial smooth muscle. That makes gas exchange at the pulmonary level deficient (21).

Oxygen saturation is directly related to the partial pressure of oxygen and represents the degree of erythrocyte hemoglobin saturation with oxygen. Lower values of SaO_2 result from insufficient oxygenation due to the reduced gas exchange and reveal the existence of respiratory pathology. Oxygen saturation and partial oxygen pressure are directly proportional, and when the value of one increase, so will the other, reflecting improved oxygenation. The exception to the rule is when oxygen saturation and other factors are affected, which should be considered when interpreting the results. The solubility of oxygen is dependent on its partial pressure but also on its solubility as a gas.

The majority of oxygen in the body is that of haemoglobin, calculated by PaO_2 - partial pressure of arterial oxygen, through which the availability of oxygen for the body's vital organs can be evaluated (17).

If we also consider $PaCO_2$ - the partial pressure of arterial carbon dioxide, we can follow the alveolar-arterial gradient - an index of the lung's gas exchange efficiency. Oxygen saturation can remain somewhat normal even within extremely low values (below 70 mmHg) of PaO_2 as found in asthma patients. Thus, patients will have an extremely low oxygen supply, which implies, clinically, a relatively normal patient at rest but extremely intolerant to exertion. This phenomenon occurs due to the ability of haemoglobin to remain saturated even if blood oxygen is low (13).

Another aspect worth mentioning in this context is the blood pH. Most patients with mild asthma will have mild acid-base imbalances due to chronic alveolar inflammation and inefficient gas exchange: CO_2 will be increased or at the upper limit. This phenomenon occurs either due to the accumulation of mucus, which acts as an insulating layer at the alveolar level and interferes with the lungs' ability to eliminate carbon dioxide or due to bronchospasm, which practically blocks alveolar ventilation. Both pathophysiological mechanisms are characteristic of obstructive respiratory diseases such as equine asthma (13, 14). The subjects managed to maintain carbon dioxide at normal, but upper limit levels. During dry salt therapy sessions using the SaltMed device, no side effects were observed in G_1 and G_2 patients. The study lacks information regarding the atmospheric conditions' effect on the SaltMed dry salt cartridge since no data was recorded regarding the atmospheric humidity and pressure. The salt capsule in the SaltMed device can suffer from interaction with the environment, like changes in the atmospheric pressure and air humidity, which can affect the therapy session's outcome.

The SaltMed device could prove effective in treating signs in patients diagnosed with mild equine asthma syndrome both due to the ease of use of the device but also due to its concept. For optimal usage, the device must provide the necessary salt micro-particles that can be efficiently attracted into the inspiratory torrent. The external conditions must be favourable by ensuring a dry environment. We consider that the equipment we used has, in general, a good construction. However, due to the atmospheric humidity and the hygroscopic properties of the salt, it is necessary to carry out a comparative study focused on the particle size of the salt and the use of air pumps that use atmospheric air, variable in humidity, as well as with pumps that use air or oxygen sources, with controlled humidity and at the lowest possible level.

CONCLUSIONS

The results of this study showed statistical improvement in blood gas parameters after using halotherapy in horses with mild equine asthma, improvement in the oxygenation was shown also in the control group. The limitation of the study is the missing correlation between the clinical signs and the BALF cytology. This study opens the possibility for further investigation regarding the influence of halotherapy on the BALF cytology aspects. Another limitation that can influence the quality of dry saline aerosol therapy is the impossibility of evaluating the quality of the dimension of micro dry salt particles produced. Future studies that focus on the effect of humidity of the atmospheric air on the dry salt device's ability to generate particles of 1-5 microns or creating a system that delivers air with low humidity are recommended for dry salt therapy success. Although future studies on larger horse populations take into consideration more strict criteria of inclusion and housing a valuable asset in the management of horses with mild E.A.S.

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REFERENCES

1. *Aguilera-Tejero E., Estepa J.C., López I., Mayer-Valor R., Rodríguez M.,* (1998), Arterial blood gases and acid-base balance in healthy young and aged horses. Equine Veterinary Journal, 30(4): 352-354
2. *Anderson S.D., Spring J., Moore B., Rodwell L.T., Spalding N., Gonda I., Chan K., Walsh A., Clark A.R.,* (1997), The effect of inhaling a dry powder of sodium chloride on the airways of asthmatic subjects. European Respiratory Journal, 10(11):2465-2473
3. *Bar-Yoseph R., Kugelman N., Livnat G., Gur M., Hakim F., Nir V., Bentur L.,* (2017), Halotherapy as asthma treatment in children: A randomized, controlled, prospective pilot study: Effect of Halotherapy in Asthmatic Children. Pediatr Pulmonol, 52 (5):580-587
4. *Beamon S.P., Falkenbach A., Fainburg G., Linde K.,* (2001), Speleotherapy for asthma. Cochrane Database of Systematic Reviews, 2:CD001741
5. *Bedenice D., Mazan M.R., Hoffman A.M.,* (2008), Association between Cough and Cytology of Bronchoalveolar Lavage Fluid and Pulmonary Function in Horses Diagnosed with Inflammatory Airway Disease. Journal of Veterinary Internal Medicine, 22 (4):1022-1028
6. *Bullone M., Lavoie J-P.,* (2020), The equine asthma model of airway remodeling: from a veterinary to a human perspective. Cell Tissue Res, 380(2): 223-236
7. *Calzetta L., Rogliani P., Page C., Roncada P., Pistocchini E., Soggiu A., Piras C., Urbani A., Matera M.G.,* (2018), Clinical effect of corticosteroids in asthma-affected horses: A quantitative synthesis. Equine Vet J, 50(5):594-601
8. *Calzetta L., Roncada P., Di Cave D., Bonizzi L., Urbani A., Pistocchini E., Rogliani P., Matera M.G.,* (2017), Pharmacological treatments in asthma-affected horses: A pair-wise and network meta-analysis. Equine Vet J, 49(6):710-717
9. *Chervinskaya A.V., Zilber N.A.,* (1995), Halotheraphy for treatment of respiratory diseases. J Aerosol Med, 8(3):121-132
10. *Cherviskaya A.V., Zilber N.A.,* (1995), Halotherapy for treatment of respiratory diseases. Journal of Aerosol Medicine, 8(3):221-232
11. *Clements J.M., Pirie R.S.,* (2007), Respirable dust concentrations in equine stables. Part 1: Validation of equipment and effect of various management systems. Research in Veterinary Science, 83(2): 256-262
12. *Couetil L., Cardwell J.M., Leguillette R., Mazan M., Richard E.,Bienzle D.,Bullone M.,Gerber V., Ivester K., Lavoie J.P., Martin J., Moran G., Niedźwiedź A., Pusterla N., Swiderski C.,* (2020), Equine Asthma: Current Understanding and Future Directions. Front Vet Sci, 7:450
13. *Couëtil L.L., Cardwell J.M., Gerber V., Lavoie J-P., Léguillette R., Richard E.A.,* (2016), Inflammatory Airway Disease of Horses - Revised Consensus Statement. J Vet Intern Med, 30(2):503-515
14. *Durando M.M., Martin B.B., Hammer E.J., Langsam S.P., Birks E.K.,* (2010), Dynamic upper airway changes and arterial blood gas parameters during treadmill exercise. Equine Veterinary Journal, 34(S34):408-412
15. *Duvivier D.H., Votion D., Vandenput S., Lekeux P.,* (1997), Aerosol therapy in the equine species. The Veterinary Journal, 154(3):189-202
16. *Gerber V., Robinson N.E., Luethi S., Marti E., Wampfler B., Straub R.,* (2010), Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. Equine Veterinary Journal, 35(5):491-495
17. *Gy C., Leclere M., Vargas A., Grimes C., Lavoie J.,* (2019), Investigation of blood biomarkers for the diagnosis of mild to moderate asthma in horses. J Vet Intern Med, 33(4):1789-1795
18. *Huber H.L., Koessler K.K.,* (1922), The pathology of bronchial asthma. Arch Intern Med, 30:689-760
19. *Ivester K.M., Couëtil L.L., Moore G.E., Zimmerman N.J., Raskin R.E.,* (2014), Environmental Exposures and Airway Inflammation in Young Thoroughbred Horses. J Vet Intern Med, 28(3):918-924
20. *Ivester K.M., Couëtil L.L., Zimmerman N.J.,* (2014), Investigating the Link between Particulate Exposure and Airway Inflammation in the Horse. J Vet Intern Med, 28(6):1653-1665
21. *Johansen T., Johansen P., Dahl R.,* (2014), Blood gas tensions in adult asthma: a systematic review and meta-regression analysis. Journal of Asthma, 51(9):974-981
22. *Kutasi O., Balogh N., Lajos Z., Nagy K., Szenci O.,* (2011), Diagnostic Approaches for the Assessment of Equine Chronic Pulmonary Disorders. Journal of Equine Veterinary Science, 31(7):400-410
23. *Laube B.L., Swift D.L., Wagner H.N., Norman P.S., Adams G.K.,* (1986), The effect of bronchial obstruction on central airway deposition of a saline aerosol in patients with asthma. Am Rev Respir Dis, 133(5):740-743
24. *Lavoie J.P., Cesarini C., Lavoie-Lamoureux A., Moran K., Lutz S., Picandet V., Jean D., Marcoux M.,* (2011), Bronchoalveolar Lavage Fluid Cytology

and Cytokine Messenger Ribonucleic Acid Expression of Racehorses with Exercise Intolerance and Lower Airway Inflammation: Cytokine Expression in IAD. Journal of Veterinary Internal Medicine, 25(2):322-329

25. *Lăzărescu H., Simionca I., Hoteteu M., Mirescu L.*, (2014), Speleotherapy - modern bio-medical perspectives. J Med Life, 7(Spec 2):76-79
26. *Leclere M., Lavoie-Lamoureux A., Gélinas-Lymburner É., David F., Martin J.G., Lavoie J-P.,* (2011), Effect of Antigenic Exposure on Airway Smooth Muscle Remodeling in an Equine Model of Chronic Asthma. Am J Respir Cell Mol Biol, 45(1):181-187
27. *Leclere M., Lavoie-Lamoureux A., Lavoie J-P.,* (2011), Heaves, an asthma-like disease of horses: Heaves, an asthma-like disease of horses. Respirology, 16(7):1027-1046
28. *McGorum B.C., Ellison J., Cullen R.T.,* (1998), Total and respirable airborne dust endotoxin concentrations in three equine management systems. Equine Veterinary Journal, 30(5):430-434
29. *Millerick-May M.L., Karmaus W., Derksen F.J., Berthold B., Holcombe S.J., Robinson N.E.,* (2011), Particle mapping in stables at an American Thoroughbred racetrack: Particle mapping in stables at an American Thoroughbred racetrack. Equine Veterinary Journal, 43(5):599-607
30. *Mishra P., Pandey C., Singh U., Gupta A., Sahu C., Keshri A.,* (2019), Descriptive statistics and normality tests for statistical data. Ann Card Anaesth, 22(1):67
31. *Opriţa B., Pandrea C., Dinu B., Aignătoaie B.,* (2010), SALTMED - The Therapy with Sodium Chloride Dry Aerosols. Therapeutics, Pharmacology and Clinical Toxicology, 14(3):201-204
32. *Papi A., Brightling C., Pedersen S.E., Reddel H.K.,* (2018), Asthma. The Lancet, 391(1012):783-800
33. *Phipps P.R., Gonda I., Anderson S.D., Bailey D.,* (1994), Bautovich G. Regional deposition of saline aerosols of different tonicities in normal and asthmatic subjects. Eur Respir J, 7(8):1474-1482
34. *Prakash Y.S., Halayko A.J., Gosens R., Panettieri R.A., Camoretti-Mercado B., Penn R.B.,* (2017), An Official American Thoracic Society Research Statement: Current Challenges Facing Research and Therapeutic Advances in Airway Remodeling. Am J Respir Crit Care Med, 195(2):e4–19
35. *Santoro D., Marsella R.,* (2014), Animal Models of Allergic Diseases.Veterinary Sciences,1(3):192-212
36. *Wark P., McDonald V.M.,* (2018), Nebulised hypertonic saline for cystic fibrosis. Cochrane Data base of Systematic Reviews, 9:CD001506
37. *Whittaker A.G., Hughes K.J., Parkin T.D.H., Love S.,* (2009), Concentrations of dust and endotoxin in equine stabling. Veterinary Record, 165(10): 293-295.