

PhD THESIS

Morphofunctional aspects of saline aerosol therapy in equine asthma syndrome

(SUMMARY OF THE DOCTORAL THESIS)

PhD student **Laura Pienar (Condor)**

Scientific coordinator **Prof. univ. dr. Ionel Papuc**



INTRODUCTION

In recent years, in our country, as in other European countries, the horse has changed its status from a burden and traction animal to a pet, racing, leisure and therapy. The equine species is recognized for its outstanding performance in work, sports and leisure. Achieving these performances is based on the proper functioning of all systems and organs that support the performance of physical effort. Respiratory function is a major factor in achieving these performances and at the same time a factor that can limit the capacity for effort. Horses have a marked predisposition to some respiratory diseases due to either airway damage or morpho-functional alteration of the alveolo-capillary tissue. Respiratory disorders in horses are pathological entities with diverse etiology and various clinical and anatomopathological aspects, because the respiratory system is directly related to the external environment, the air, but also to the internal environment, the blood.

The American College of Veterinary Medicine has reclassified the pathology of the respiratory tract in horses and proposed that inflammatory airway disease (IAD) and recurrent airway obstruction (RAO) be renamed equine asthma (COUËTIL AND COLAB., 2016). Equine asthma is a non-infectious airway syndrome that results from an allergic reaction to various inhaled particles. The most common allergens are found in hay, straw and dust in inadequately ventilated stables. Inhaled allergens produce an allergic reaction that narrows and obstructs small airways. This obstruction has three main causes: mucus buildup, bronchoconstriction, inflammation and thinning of the airway walls (GALEN ASHLEY, 2017).

Considering the common aspects of asthma encountered in humans and the two forms of respiratory pathology IAD and RAO, encountered in horses, it can be concluded that the adoption of the term equine asthma is appropriate. Equine asthma is associated with inadequate microclimate conditions, dry fodder and is seasonal in nature. This disease is caused by an allergic reaction to various aero-allergens, and there are many similarities between this syndrome and human asthma (KUTASI ORSOLYA, 2016). Recent studies on this pathology have clearly shown that aeroallergens in the dust inside the stable play a major role in triggering clinical manifestations (IVESTER AND COLAB., 2018, OLAVE AND COLAB., 2021, MONKI AND COLAB., 2021).

At present, a variety of inhalation techniques are available in the therapy of respiratory diseases in horses, some being specially designed for this species, and others being taken over and adapted from human medicine (PIRIE AND MCGORUM, 2017). Most used are metered dose inhalers, mechanical nebulizers, and inhalers powered by the patient's respiratory flow.

New therapeutic approaches have always been a challenge in medicine, so the use of a device that generates dry saline aerosol particles in the treatment of certain respiratory diseases in horses can be considered as a new therapeutic method that will diversify the means of interventions.

The choice of the research topic was based on all these listed arguments to which is added the well-known fact that a certain function of the body has a well-defined anatomical correspondence. Knowing this, the clinician will corroborate the clinical data with those of complementary investigation to confirm or complete the diagnosis, data that will have a direct impact on the prognosis and on the therapeutic conduct.

STRUCTURE OF THE WORK

The doctoral thesis consists of two parts: part I - *The current state of knowledge* and part II - *Personal contribution*. It comprises 10 chapters, 3 chapters are devoted to bibliographic substantiation research and 6 chapters to personal research.

Chapter 1 of the thesis describes in detail the anatomical and physiological elements of the respiratory system in horses.

Chapter 2 presents the examination and semiology of the respiratory tract in horses and the methods of diagnosis in equine asthma syndrome. The respiratory examination is a difficult stage because it involves the use of a varied examination methodology, due to the semiological complexity of this system, and the disorders at this level which are manifested through an extremely diverse symptomatology (PAPUC, 2017). From the vast pathology of the respiratory system encountered in the horse species, we will describe only the asthma syndrome because this syndrome can benefit from the complementary therapy with saline aerosols. Clinical examination in asthma syndrome highlights the following symptoms: chronic, strong cough, mucous or mucopurulent discharge, tachypnea and expiratory dyspnea. With careful auscultation near the nostrils, the prolonged whistling exhalation, called wheezing, can be perceived.

In patients with mild asthma, the symptoms are mildly manifested, there is intolerance to exercise, possibly coughing during and immediately after exercise, and in patients with severe asthma, increased respiratory effort at rest is the key symptom, to which is added the intolerance to exertion and accumulation of mucus with frequent cough and cholinergic bronchospasm. All symptoms are reversible or at least strongly attenuated if the environment is ameliorated, especially by avoiding exposure to dust or mold from hay. Aspects of hypertrophy of the abdominal muscles, occur at the time of vicarious emphysema and are irreversible, regardless of the therapeutic or managerial intervention on the disease (COUËTIL AND COLAB., 2016).

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At auscultation, vesicular murmur and moist sub crackling rales with small bubbles are perceived. Increased viscosity of bronchiolar mucus makes it possible to detect wheezing rales. In most patients, the internal temperature has values in the normal range of 37,5-38,2°C (MIRCEAN, 2016). Prolonged inspiration triggers coughing and aggravates the symptoms described. Although the clinical signs are characteristic, a paraclinical examination is also required, a major role being played by tracheobronchial endoscopy and broncho-alveolar lavage.

Chapter 3 is reserved for aerosol therapy. An aerosol can be defined as a gas containing very finely dispersed particles, liquid or solid, in suspension. Most aerosols contain particles of different sizes, with the heterogeneity being described by the diameter of each sphere. The term monodisperse particles is used when the particles are the same size. However, most aerosols contain a wide range of particles of different sizes and are classified as polydisperse particles (AGNEW, 1984).

Inhalation therapy plays an important role in the management of respiratory diseases in horses. This type of therapy is considered an alternative therapy to systemic therapy that provides a higher concentration of locally acting active substance, thus minimizing the side effects of general drug administration. Inhalation of substances allows rapid and high-density storage of drugs in the peripheral airways (BRAIN AND VALBERG, 1979). The main advantage of inhalation therapy is the possibility of delivering the drug to the desired location, i.e. the airway. The administration of systemic drugs remains a common method of treatment, but has the disadvantage that it can affect the whole body, consequently it can have unwanted side effects on other organs. The disadvantage of inhalation therapy is due the factors that influence the particle size and their distribution, including the properties of aerosolized solutions and the method of their generation (VOTION AND COLAB., 1997). Therefore, compared to systemic therapy, inhalatory therapy makes it more difficult to determine the exact dose and working strategy, but there is a general consensus that treatment should be tailored to each case and the drug should be administered until the desired effect is achieved (PIRIE AND MCGORUM, 2017).

Inhalation therapy with dry saline aerosols, also called halotherapy, targets the lower airways. Larger particles will be deposited in the upper airways, following an inertial impact process, and particles smaller than 0.5 μ will most likely be eliminated with the expiratory fraction (DUVIVIER AND COLAB., 1997). Practically, only particles with dimensions between 1-5 μ are truly considered therapeutic aerosols, an aspect valid for all species (CLAY AND COLAB., 1987). The use of equine masks, either those that are adapted for a single nostril or those that cover the entire snout, eliminates the dependence on synchronizing the administration of aerosols with the inspiration process and improves their characteristics. The masks are equipped with valves that

direct the flow of inspiratory air through the inside of the inhaler and the flow of exhaled air through its outside (LAVOIE, 2001).

Chapter 4 is intended for the purpose and objectives of the research. Because the pathology of the respiratory tract is extremely complex and frequently diagnosed in horses, therapy methods need to be improved to provide multiple approaches to ensure therapeutic success.

The aim of this study was to identify and use a new therapeutic method in equine asthma syndrome in horses in the Transylvanian area by testing the SaltMed device for generating dry saline aerosols, tracking their effect on the respiratory system and blood oxygenation. as well as to establish a working protocol that is reliable and can be applied by both the veterinarian and the owner.

The objectives of the paper. In order to achieve the proposed goal, the following objectives were established: identification of the most relevant studies in the field in order to establish a protocol for the implementation of the intervention stages in respiratory diseases in horses; implementation of a new therapeutic method in equine asthma by using the SaltMed device; observation and description of the operation of the SaltMed device in horses and assessment of the effects of saline aerosols on the respiratory system and on arterial blood gases; establishing a decision-making tree regarding the methods of diagnosis in asthma syndrome and determining the triggers of the disease; interpretation of the results obtained for each horse and elaboration of protocols to improve the applied technique, in order to obtain an optimal effect.

Chapter 5 covers the materials and working methods used. The biological material was represented by a total of 62 horses from the Transylvanian area that were examined from a clinical point of view. Of these, 2 groups were formed, 1 group of 26 clinically healthy horses and 1 group of 36 horses diagnosed with various pathologies in the respiratory tract. The horses studied were between 2 and 17 years old, of which 32 were male and 30 were female. The inclusion criteria in the group of horses with respiratory pathologies were represented by recurrent chronic respiratory failure due to an obstructive-inflammatory phenomenon of the terminal bronchi and the alveolar-bronchiolar sphincter; persistent or recurrent bronchial stenosis; permanent air crisis expressed by a symptomatology equivalent to the attempt to compensate the deficient function.

In this type of pathology, the lower respiratory tract is especially affected, and if the animal is subjected to exertion, the bronchial muscles contract, their lumen is significantly reduced, and breathing becomes difficult. If the animal is subjected to exertion, breathing becomes difficult, suffocation attacks take place, coughing occurs, and inspiratory intercostal circulation can often be seen.

For the treatment of equine asthma, the SaltMed inhaler was used, which distributes dry NaCl micro-particles, being an adjuvant therapy device in the treatment

of various upper and lower respiratory tract diseases. The SIEMENS RAPIDPoint® 500 device was used to analyze arterial blood gases, an automatic analyzer that can process a sample in a maximum of 60 seconds, providing several parameters. Working steps were identical for each patient, represented by the following: clinical examination, collection and analysis of the blood sample before therapy, preparation and start of the SaltMed device, application of the mask, shutdown of the device, collection and analysis of the blood sample after therapy.

RESULTS OF THE PERSONAL RESEARCH

Chapter 6 is devoted to Study I entitled *Histopathological Aspects of Respiratory and Lung Pathology in Horses* and it aims to identify and interpret the histopathological changes encountered in the pathology of the respiratory tract in horses and to follow the induced changes and the location of the pathological process. The histopathological examination was performed according to the classical principles and methods of sampling, transport, processing and examination. A wide range of lesions at the bronchiolar, vascular, and alveolar level were found in the cases studied.

Chapter 7 is devoted to Study II entitled *The effect of dry saline aerosol therapy on arterial blood gas parameters in horses with mild to moderate asthma* and it aims to test the effect of dry saline aerosols on blood gases in horses diagnosed with asthma compared to horses. diagnosed clinically healthy. The SaltMed inhaler, manufactured by TechnoBionic SRL Buzău, Romania, was used for dry saline aerosol therapy. All patients diagnosed with equine asthma were examined at the Equine Clinic of the Faculty of Veterinary Medicine Cluj-Napoca. The control group, clinically healthy, were examined at the Equine Clinic or in the field. Statistical analysis of the results was performed using the SPSS Statistics program. The results of this study show a statistical improvement of the parameters of the gases in the arterial blood after the use of halotherapy in horses with mild and medium forms in asthma syndrome and an improvement in oxygenation, aspects also encountered in the control group.

Chapter 8 is dedicated to study III entitled *Diagnosis and therapy of equine asthma in adult horses* and it aims to monitor the therapeutic intervention based on dry saline aerosols in a group of horses diagnosed with equine asthma compared to a group of healthy horses. The biological material used in this study was a total of 17 horses, male and female, of different ages and breeds. The horses studied were of different and mixed breeds. All patients were consulted at the Equine Clinic of the Faculty of Veterinary Medicine, Cluj-Napoca. In terms of service, they were recreational, traction, companion, and sports horses. A number of 4 horses were diagnosed with asthma, mild and moderate forms, 5 horses were diagnosed clinically

healthy, and a number of 8 horses were diagnosed with bronchiolar failure. All horses followed the same diagnostic, treatment, and monitoring protocol.

The therapeutic protocol used in the treatment with dry saline aerosols complied with the requirements of the protocol used in human medicine, its duration being 60 minutes per day for 7 days. The adaptation of aerosol therapy to the patients studied considered the particularities of the species and the type of service, so that all the conditions for a proper inhalation of salt micro-particles were ensured, while at the same time the degree of tolerability of the mask was noted and graded. Centralization of arterial blood gas results revealed an improvement in specific parameters in all patients diagnosed with mild and moderate forms of asthma, the result being a positive one, both clinically and paraclinically. At the end of the treatment period, the results regarding the reliability of the maneuvers by this therapeutic method were good. The results of dry saline aerosol therapy were also confirmed by histopathological examination.

Chapter 9 presents the general conclusions and recommendations. Following the results obtained by monitoring and analyzing the respiratory process in the patients studied, it can be stated that dry saline aerosol therapy is a new and promising method of therapy, which can be applied as interventional therapy to horses diagnosed with mild and moderate asthma, as adjunctive therapy to drug-treated horses or as a preventive measure in situations where feeding and housing conditions are inadequate.

Chapter 10 is devoted to the elements of originality and innovative contributions of the thesis.

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Morphofunctional aspects of saline aerosol therapy in equine asthma syndrome

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