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EQUNE ASTHMA: OVERVIEW OF NOVEL DIAGNOSTIC METHODS

Respiratory disease in horses is one of the most important problems which veterinarians, owners and breeders have to manage. One of the most frequent if not dominant condition is known as equine asthma syndrome (EAS) or severe equine asthma (SEA). Before 2016 there were plenty of terms that referred to the same disease such as IAD (Inflammatory Airway Disease), RAO (Recurrent Airways Obstruction) SP-RAO (Summer Pasture Associated RAO) or COPD (Chronic Obstructive Pulmonary Disease). Recent research has proven that mechanism of these syndromes is similar enough to consider it as one disease that belongs to the group of noncontagious diseases of the respiratory track. Asthma as a heterogeneous group of conditions induced by IgE-mediated reactions and type III hypersensitivity that can affect 10 - 30 % of horses. However, depending on the population and geographical region, some studies have found that EA can occur in 60-100% of adult horses. The clinical symptoms include dyspnea, coughing and loss of performance, caused mainly by the bronchoconstriction and the compromised gas exchange. The widespread occurrence and ambiguous diagnosis, combined with progressive nature of equine asthma, determines seriousness of the problem. Therefore, new therapies and diagnostic methods are urgently needed.

Based on gathered data, the aim of this paper was to investigate early identification of EA using the newest techniques. Although severely asthmatic horses often show visible signs of disease, mild or moderate form of EA sometimes occurs subclinically. Also, patients during clinical examination might present a temporary remission which makes preliminary scoring difficult and often results in false negative diagnosis. The most often used diagnostic procedure includes cytological analysis of tracheal wash (TW) and bronchoalveolar lavage fluid (BALF). TW method in horses was first

described in 1980. Tracheal wash is considered to be a good representation of the whole lung because secretions from the affected lung areas is collected in the trachea. If aspiration is performed by a transtracheal method, samples can be collected almost sterile. However, even if endoscope is preferred, TW still can be used in cases which an infectious disease is suspected. Advantages of this type of examination in clinical practice is potential avoidance of sedation and local anesthesia that are often required during BALF. However, BALF is considered being more sensitive technique for detecting lower airway inflammation because cell population in the trachea is not representative for the cell population in the lower airways. Those two methods are based on cutoff values for mostly three types of cells: neutrophils, eosinophils and metachromatic cells. Due to limited guidelines, references and variation in sampling techniques new cutoff values have been recently proposed for BALF- >10% neutrophils, >5% mast cells, or >5% eosinophils. BALF is still consider being the gold standard of diagnostics of EA, although its huge disadvantage is the lack of a significant association between lower airway inflammation detected with cytology and the degree of pulmonary dysfunction in EA. Therefore, other methods, potentially enabling an early recognition of the condition, have gained interest in equine medicine and research. The clinical severity of the disease seems to be correlated with the amount of smooth muscle in a horse's airways, especially more distally within the bronchial tree. Severe asthmatic horses have 300% more peripheral smooth muscle than age-matched healthy controls. A technique that allows imaging transversal scans of the bronchial wall (endobronchial ultrasound - EBUS) is using a miniature radial ultrasound probe inserted through the working channel of a videoendoscope. Moreover, through the assessment of large airway smooth muscle remodelling EBUS provides the means for predicting future histological alterations occuring in the peripheral airways. Another method validated for the non-invasive assessment of remodeling and inflammation in the central airways is endobronchial biopsy. Biopsies are easy to obtain, and when processed for standard histology, they allow the assessment of all airway tissues, including smooth muscle. However, they do not permit a precise quantification of the deepest bronchial structures. They are also limited to the bronchial bifurcations of the most proximal airways and, in the contrary to EBUS, cannot be repeated over time at the same site which limits their usefulness to monitor disease progression or treatment response. However, histology differentiates the bronchial structures more precisely than EBUS, providing histological-grade detail, and does not require expensive equipment and technical expertise. Another new approach in diagnosing EA is the investigation of exhaled breath condensate (EBC). This non-invasive sampling method has been studied widely in human patients with asthma as EBC H2O2 concentration and pH appear to be correlated with the presence and severity of the disease. In a study on equine cases, there were no significant differences in EBC pH or H2O2 between the healthy and affected horses. However, a trend for a reduced pH in horses with EA was observed. Another study described a good correlation between the presence and severity of the disease and H2O2 concentration in EBC in horses with EA. As the knowledge of the usefulness of measuring these parameters in the investigation of EA in horses is still limited, more research is needed. As opposed to human medicine, no relationship between equine asthma severity and airway acidification has been documented. If a similar correlation exists in horses, it could help better understand the pathogenesis

of EA. Investigation of EBC also allows to evaluate the metabolic profile of bio-fluids of respiratory origin. In EBC of horses affected by EA the concentration of methanol, trimethylamine and acetone is significantly higher than in healthy horses, while the concentration of formate, lactate and acetate is much lower. Possibly, these metabolites can become biomarkers for the diagnosis and treatment of EA. As EA is associated with increased allergen-specific IgE against a range of environmental proteins, new methods enabling IgE profiling in EA-affected horses are gaining interest. A strong correlation between BALF proteins and sera specific IgE profiles was discovered. The development of a comprehensive microarray platform allowed effective detection of allergen-specific equine IgE in serum, revealing a lot of novel pollen, bacteria, mould and arthropod proteins significant in the aetiology of EA. Antigen arrays could potentially serve as an aid in the early diagnosis of EA, allowing to introduce accurate allergen-avoidance regimes or allergen-specific immunotherapy before the occurrence of severe respiratory signs, which may help slow down the progression of EA or even prevent the occurrence of its acute form. However, more loci are likely to contribute to the development of EA . These include genes contributing to chronic inflammation and remodeling in the peripheral lung tissue, related to PPP3CB/NFAT, RhoA, and LTB4/GPR44 signaling pathways. It is also important that susceptibility and resistance to other allergies, certain bacterial lower airway infections and parasites are dependent on specific genetic background. These factors may be potentially relevant in EA, especially in its milder forms in younger horses, for which no specific genetic risk factors are reported. Due to EA's pathogenetic complexity it is very unlikely that single-gene tests will be diagnostically useful. However, a genetic profiling panel taking into account several genetic factors could enable an early identification of EA. Ideally, this would be combined with an assessment of environmental risk factors. Such assessment is already used in predicting the risk of asthma in humans.

These methods are the result of advances in understanding of the dynamics of a severe equine asthma. Fast, highly efficient methods based on genetics or pH measurement in exhaled air can potentially greatly reduce the need for currently used tests. However, still much research in this field is needed in order to estimate the clinical relevance of these techniques. Using not only cytology but also biopsy and specific imaging techniques, let veterinarians more precisely estimate the severity of a certain case and therefore adjust the treatment to an individual patient. Future development of techniques like metabolomic studies on EBC and serological testing may help better understand the complex pathogenesis of EA and improve not only the methods of an early diagnosis, but also the treatment of the disease. As a lot of genes associated with this disease have been discovered, developing an optimal genetic testing panel may soon enable an early identification through a simple blood test. The main advantage of introducing non-invasive diagnostic methods on a larger scale would be their faster and easier sampling procedure that does not have to be executed by qualified personnel. Nevertheless, more invasive methods like endoscopic biopsies will most likely still remain a valuable source of additional information about the condition of the patient and the disease progression. Possibly in the near future a golden standard other than BALF will be introduced.

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