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**Science-in-brief: Equine asthma diagnosis: Beyond bronchoalveolar lavage cytology**

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3 **Equine asthma diagnosis: beyond bronchoalveolar lavage cytology**

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27

28 **Abstract**

29 The diagnosis of equine asthma is currently based on the presence of clinical signs indicative of a  
30 lower airway disease combined with abnormal bronchoalveolar lavage fluid (BALF) cytology  
31 results. However, the type and degree of lower airway inflammation is not correlated with the  
32 severity of the clinical signs, and it does not prognosticate on the progression of the disease. We  
33 summarize here the results of recent work published in non-veterinary journals or presented at  
34 veterinary congresses reporting on the structural alterations (remodeling) occurring in the equine  
35 asthmatic airways and their relationship with lung function and inflammation. Using archived  
36 tissues, endobronchial and thoracoscopic peripheral lung biopsies, remodeling was found to occur  
37 in both central and peripheral airways. The observed changes included increased in the smooth  
38 muscle mass, fibrosis, and deposition of elastic fibers, which were correlated with the lung function.  
39 Endobronchial biopsies and endobronchial ultrasound are new methods that have been validated for  
40 the non-invasive assessment of remodeling and inflammation in the central airways of clinical  
41 equine cases, which reflect peripheral airway lesions. The future implementation of these methods  
42 could change the clinical approach to equine asthma in favor of an early recognition of the  
43 condition for its prevention, and hopefully, for the development of targeted therapies to prevent, or  
44 even reverse, established tissue remodeling and inflammation.

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47 It has long been known that the domestication of horses led to the development of chronic non-  
48 infectious small airway diseases. The terminology used to describe these conditions has evolved in  
49 parallel with findings related to their clinical, anatomical, histologic, or functional features. Heaves,  
50 equine emphysema, chronic bronchiolitis, equine chronic obstructive pulmonary disease (COPD),  
51 summer pasture associated obstructive pulmonary disease (SPAOPD), lower airway diseases,  
52 recurrent airway obstruction (RAO), and inflammatory airway disease (IAD) have been used either  
53 interchangeably or to describe specific manifestations or characteristic of the disease. As new  
54 features (functional, anatomical, and pathobiological) of these conditions are emerging, the term  
55 «equine asthma» has been proposed [1], given its many similarities with human asthma [2].

56 Driven by the incurable nature of the condition in some horses, and the progression of clinical signs  
57 often leading to premature retirement or even euthanasia in its severe form, our research group has  
58 focused its attention on the alterations (remodeling) affecting the lower airways and their response  
59 to current therapies. We will summarize here recent findings that may change the paradigm  
60 dictating the clinical approach to equine asthma in favor of an early recognition of the condition for  
61 its prevention, and hopefully, the development of targeted therapies to prevent, or even reverse,  
62 established tissue remodeling. Although BAL cytology remains the cornerstone of the diagnosis of  
63 equine asthma, novel diagnostic methods have been developed to assess the remodeling affecting  
64 the equine airways and to eventually identify horses predisposed to progress to the severe  
65 phenotype.

66

### 67 *Equine asthma: definitions and diagnostic challenges*

68 Based on the severity of clinical signs, equine asthma may be divided into three main phenotypes,  
69 namely mild, moderate, and severe. Mild equine asthma comprises horses with lower airway  
70 inflammation identified by bronchoalveolar lavage fluid (BALF) cytology or abnormal lung

71 function, presenting clinical signs of airway diseases that may be limited to a decreased  
72 performance. The clinical signs of horses with moderate equine asthma are more obvious and  
73 usually include coughs and possibly tachypnea at rest. These conditions were previously known as  
74 IAD. Severe equine asthma (heaves, RAO, SPAOPD, equine emphysema and COPD) comprises all  
75 cases characterized by recurrent episodes of increased respiratory effort at rest (labored breathing),  
76 which is at least partially reversible with therapy [3].

77 The clinical diagnosis of equine asthma should ideally be based on the documentation of lower  
78 airway obstruction; however, the lack of sensitive and portable lung function tests currently  
79 prevents this approach in clinical practice. For these reasons, the diagnosis currently relies on  
80 history, clinical signs, and in the presence of lower airway inflammation as detected by BALF  
81 cytology. The rationale behind this approach comes from the groundbreaking work of Dr. Viel who  
82 reported that the neutrophilia in BALF cytology correlated with the severity of the pulmonary  
83 lesions [4]. Another descriptive study also speculated on a possible correlation between the small  
84 airway lesions and the clinical severity [5]. However, the clinical significance of BALF  
85 inflammation deserves further investigations as the cut-off normal values for the different cell types  
86 in BALF have recently been questioned, and little is known on the relationship between the degree  
87 and type of BALF inflammation, the severity of disease, and the response to therapy.

88

89 ***What is the clinical significance of bronchoalveolar lavage inflammation in equine asthma?***

90 There is no data in the literature clearly linking the degree and type of bronchoalveolar  
91 inflammation to specific pulmonary lesions in asthmatic horses. That is, how should a clinician  
92 interpret an increase in neutrophil vs. mast cell or eosinophil differential cell count in equine  
93 asthma? What is the clinical implication of a mild vs. a moderate increase in either of these cell  
94 populations? Normal cut-off values for inflammatory cell percentages in BALF have been

95 established, but they provide practitioners no clues concerning the severity of the pulmonary lesions  
96 associated with the disease or to the prognosis in terms of progression and reversibility of the  
97 disease. Lastly, several studies have shown that BALF neutrophilia does not normalize after  
98 effective treatment with inhaled or systemic corticosteroids when horses are kept in the offending  
99 environment [6; 7]. These findings add further complexity to our ability to interpret the clinical  
100 meaning of BALF inflammation in everyday practice, when horses are under different management  
101 conditions, and receiving different asthma medications.

102 In this perspective, our group has recently studied in well-characterized mild and severely asthmatic  
103 horses the relationship between the severity of specific pulmonary lesions and BALF neutrophilia  
104 [8]. The results show that in mild equine asthma, luminal neutrophilia (>5% in BALF) is associated  
105 with the presence of acute (neutrophilic) bronchiolitis. However, no association was found with the  
106 structural alterations present in the small airways and in the pulmonary parenchyma. Conversely, in  
107 severe equine asthma, two different cytological phenotypes were observed: a classical neutrophilic  
108 phenotype (>20%) and a paucigranulocytic phenotype, characterized by a moderate (5-20%) or by a  
109 lack of increase in neutrophils at BALF cytology. Unexpectedly, in neutrophilic severe equine  
110 asthma, the peripheral airway lesions were milder than those observed in paucigranulocytic cases.  
111 Paucigranulocytemia was also associated with peripheral mucostasis, which was not detected in  
112 horses with the neutrophilic phenotype. The presence of mucostasis could explain the reduced  
113 BALF neutrophilia in this group, as mucus plugs could prevent the most peripheral bronchioles and  
114 alveoli to be reached and sampled during the BAL procedure. However, if this was the case, a  
115 decrease in macrophages rather than neutrophil differential cell count would have been expected  
116 due to the typically more distal location of these cells into the bronchial tree. Overall, these findings  
117 suggest a complex role for airway neutrophils in equine asthma pathophysiology, and the possibility  
118 that pulmonary neutrophils may sustain phenotypic switching as recently hypothesized in equine  
119 and human asthma [9; 10].

120

121 ***What's beyond bronchoalveolar lavage cytology?***

122 Due to the absence of a significant association between lower airway inflammation detected with  
123 BALF cytology and the degree pulmonary dysfunction in equine asthma, the study of structural  
124 airway alterations (remodeling) affecting both the small and the large airways has recently gained  
125 interest in equine respiratory medicine and research.

126 Early studies have described milder histological lesions in the central airways of asthmatic horses  
127 when compared to those observed in the peripheral airways [4; 11]. This was recently confirmed by  
128 our group using histomorphometric methods. On average, severe asthmatic horses have 300% more  
129 (i.e. 3 fold increase) airway smooth muscle in their peripheral airways when compared to those of  
130 age-matched healthy controls, while only a 50% increase is observed in central airways [12; 13].  
131 This is important, as recent findings from a cohort of severely asthmatic horses (n=12) suggested  
132 that the clinical severity of the disease is correlated with the amount of peripheral smooth muscle  
133 [14]. The peripheral airway myocytes of severely asthmatic horses are also biochemically and  
134 functionally different from those isolated more cranially in the bronchial tree and trachea. Indeed, a  
135 specific myosine isoform ((+)insert), which is known to promote a faster contraction of the smooth  
136 muscle, is overexpressed in peripheral airways of asthmatic horses [15; 16].The changes are not  
137 limited to the smooth muscle layer, as there is also a deposition of collagen in the peripheral airway  
138 wall in asthmatic horses that is positively correlated with pulmonary resistance [17]. Also, the  
139 positive association between elastic fiber content and pulmonary elastance observed in controls is  
140 lost in asthmatic animals. Collectively, these findings indicate that there is a strong structure and  
141 function relationship in equine asthma, and that the remodeling of the airways represents a novel  
142 therapeutic target.

143 Although the changes in the peripheral airways are more severe, the assessment of large airway  
144 smooth muscle remodeling by means of endobronchial ultrasound (EBUS) predicts the histological  
145 alterations occurring more distally within the bronchial tree [14]. EBUS is an imaging technique  
146 producing transversal scans of the bronchial wall using a miniature radial ultrasound probe inserted  
147 through the working channel of a videoendoscope. Also, when the histological alterations of the  
148 central airways are assessed using a newly developed score, a correlation with the severity of  
149 airway obstruction measured with the impulse oscillometry technique is observed [18]. This score  
150 evaluates the inflammation and remodeling affecting all the structures comprised within the  
151 bronchial wall. These two techniques (endobronchial biopsy and EBUS) have their own advantages  
152 and limitations [12; 18; 19]. Endobronchial biopsies are easy to obtain, and when processed for  
153 standard histology, they allow the assessment of airway epithelium, lamina propria, and smooth  
154 muscle. However, they do not permit a precise quantification of the deepest bronchial structures  
155 (smooth muscle, cartilage, and peribronchial tissues) [19]. Moreover, they are limited to the  
156 bronchial bifurcations (carinas) of the most proximal airways (up to 15 bronchial generations) and  
157 cannot be repeated over time at the same site, which limits their usefulness to monitor disease  
158 progression or treatment response. On the other hand, EBUS, a non-invasive imaging method  
159 performed during bronchoscopy that also evaluates the central airways, allows the cross-sectional  
160 study of the bronchial wall at all levels of the bronchial tree, and it can be repeated over time on the  
161 same airways. However, it differentiates the several bronchial structures less precisely than  
162 histology, it does not provide histological-grade detail, and it currently requires expensive  
163 equipment and technical expertise [12].

164 Using these techniques, we have also studied how peripheral and central remodeling and  
165 inflammation respond to treatment in severely asthmatic horses. While inhaled corticosteroid  
166 monotherapy cannot reverse BALF neutrophilia unless concomitant antigen avoidance strategies are  
167 adopted, it is able to partially reverse peripheral smooth muscle remodeling after only 3 months of  
168 treatment [14]. Interestingly, a decrease in the fast contracting (+)isoform of myosine was observed



169 after 3 months of inhaled corticosteroid monotherapy [15]. Also, a correlation was found between  
170 the maximal velocity of shortening of peripheral airway smooth muscle of asthmatic horses and the  
171 time elapsed since the end of their corticosteroid treatment [16]. The effects of corticotherapy on the  
172 peripheral airways are not potentiated by the administration of  $\beta_2$ -agonist bronchodilators, at least in  
173 the first 3 months of treatment, while it better controls the airway neutrophilia and accelerates the  
174 reversal of collagen deposition in central airways [14]. Treatment duration must be prolonged up to  
175 12 months in order to significantly reduce peripheral collagen deposition, however [6]. Based on  
176 these results, the reversal of BALF neutrophilia observed in the horses treated with a combination  
177 of inhaled corticosteroids and  $\beta_2$ -agonist bronchodilators was not associated with any specific  
178 parameter of central or peripheral remodeling and inflammation, again indicating the limited  
179 usefulness of BALF cytology when assessing response to therapy.

180

### 181 *Future directions*

182 Recent evidence suggests that although equine asthma is a disorder mainly affecting to the  
183 peripheral airways, alterations are present at all levels of the bronchial tree. Studying remodeling  
184 and inflammation occurring in the central and peripheral airways of horses with asthma of different  
185 severity could reveal important diagnostic or prognostic information. Indeed, there are currently no  
186 means to predict horses with mild asthma that would progress towards the severe form of the  
187 disease. Identifying early remodeling or inflammatory markers may fulfill this goal. In the field of  
188 equine respiratory research, an effort should be made to develop minimally invasive techniques for  
189 the study of both central and peripheral airway structures and function. Our results suggest that  
190 central airway remodeling could reflect structural changes occurring in the peripheral airways, at  
191 least for the smooth muscle compartment [14], but more studies are needed to confirm this theory  
192 and to investigate whether this is true for the remaining structures that make up the bronchial wall.

193 Finally, the existence of an open equine respiratory tissue bank ([www.brte.ca](http://www.brte.ca)) represents an  
194 invaluable tool to further foster research collaboration in this field. Through this virtual platform, it  
195 is possible to gather information and have access to tissues harvested from well characterized  
196 asthmatic or healthy horses originating from different specialized centers all around the world. The  
197 routine collection of endobronchial biopsies in clinical practice after a BAL is performed would  
198 also accelerate the discovery of prognostic factors for the progression of the disease using real-life  
199 cases.

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