TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2022, vol. 7, No 2(13): 44–49

# "EQUINE ASTHMA" SYNDROME – CLINICAL, ENDOSCOPIC AND BACTERIOLOGIC INVESTIGATIONS IN EIGHT TINKER HORSES

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**ABSTRACT**

The present study included 8 mares of the Tinker breed with clinic of Recurrent airway obstruction (RAO), characterized by difficulty breathing, coughing, rapid fatigue during exercise. No changes in body temperature and heart rate were found. There were no abnormalities in erythrocyte count, erythrocyte indices, hemoglobin, hematocrit, platelets, leukocytes and differential blood count. Tracheal endoscopy revealed an accumulation of increased mucus (grade 3–5), edema of the tracheal bifurcation, and spasm of the main bronchi. The following microorganisms were isolated in tracheal lavage (TW): *Streptococcus equi*, subsp. *zooepidemicus* (2 horses), *Streptococcus pneumoniae* (1 horse), *Pasteurella spp.* (1 horse), *Klebsiella pneumoniae* (1 horse) and *Pseudomonas aeruginosa* (1 horse). Cytological examination of tracheal specimens revealed mainly neutrophil leukocytes. The other cells observed (macrophages and lymphocytes) were in a significantly lower percentage.

**Key words:** horse, asthma, endoscopy, TW, cytology, microbiology.

## Introduction

The term "equine asthma" syndrome is introduced recently to describe the mild Inflammatory airway disease (IAD) and severe Recurrent airway obstruction (RAO) (Leclere et al., 2011; Bullone et al., 2015; Couëtil et al., 2016). Horses with RAO usually demonstrate marked lower airway obstruction associated with frequent non-productive cough, increased respiratory effort at rest and exercise intolerance (Robinson et al., 2003; Couetil et al., 2007; Rettmer et al., 2015). Generally affected horses are over 7 years of age (Couetil and Ward, 2003; Hotchkiss et al., 2007). In comparison, IAD affect horses of all ages and clinical signs include mild and occasional cough, a normal respiratory rate and not so obvious deterioration of the sport condition (Holcombe et al., 2006; Widmeret al., 2009; Koblinger et al., 2011). In most of cases, hematology parameters in horses with IAD are not remarkable (Moore et al., 2004; Riihimaki et al., 2008) and nonspecific and may consist of lower red cell indices (Sanchez et al., 2005; Richard et al., 2010). Excessive amount of tracheobronchial mucus in the lower respiratory tract is a constant endoscopic finding for both pathologies (Gerber et al., 2004; Cardwell et al., 2011). Cytological analysis of TW is a valuable diagnostic method to assess the health status of the lower respiratory tract in horses (Mair et al., 1987; Hoffman, 2008; Rossi et al., 2018). The presence of severe neutrophilia (>25% cells), lymphopenia and a reduced number of alveolar macrophages in tracheal mucus is considered a typical finding for severe RAO (Derksen et al., 1985; Couetil et al., 2001). Recently a new cutoff for neutrophils (>20%) in tracheal fluids was implemented in clinical practice for more accurate diagnosis of airway inflammation (Couëtil and Hawkins, 2013; Rossi et al., 2018). The most commonly isolated microflora in tracheal wash fluid (TWF) consists of the following representatives: *Streptococcus spp.*, *Actinobacillus spp.*, *Chlamydophila spp.*, *Mycoplasma spp* and *Pasteurellaceae* (Laus et al., 2009; Cardwell et al., 2014; Bond et al., 2017; Manguin et al., 2020).

**Materials and methods**

The study was conducted with eight mares of the Tinker breed, showing signs of a respiratory problem, including frequent coughing, increased respiratory rate, shortness of breath and well-defined physical intolerance. The period of the test lasts from May to July 2021. The age of the horses was between 8 and 14 years. Dewormed every 6 months, the last of which was done two months ago. Horses were vaccinated annually against influenza and tetanus. The mares came from the same stable, and the first cases of the disease were observed four years ago. The clinical examination, incl. body temperature, heart rate, respiratory frequency was performed by routine methods. A morphological examination (Exigo hematology analyzer, Sweden) of blood, taken from jugular vein was performed, including the following parameters: red blood cells (Er), erythrocyte indices (MCV, MCH, MCHC), hemoglobin (Hb), hematocrit (Hct), platelets (Plt), white blood cells (Leu) and differential blood count (DBC). Endoscopy of respiratory tract was performed after sedation using xylazine (0.3−0.5 mg/kg, i.v, Xilazine®, Bioveta, Czech Republic) and butorphanol (0.02−0.05 mg/kg, i.v., Butomidor®, Richterpharma, Austria). In some horses, an additional twitch was used to restrain. Sampling was done without prior exercise for at least 15 h. A 300 cm long, 11 mm diameter video endoscope (EICKVIEW 150, Germany) was introduced via the ventral nasal meatus into the trachea. The amount of the mucous accumulation was quantify using the evaluation system, introduced by Couëtil et al. (2016) as Grade 0 = no visible mucus, Grade 1 = single to multiple small blobs of mucus, Grade 2 = larger but nonconfluent blobs, Grade 3 = confluent or stream forming mucus, Grade 4 = pool forming mucus, Grade 5 = profuse amounts of mucus. Then endoscope was settled in to the middle third of cervical trachea where 50 mL of sterile 0.9% saline was injected via a sterile catheter inserted through the working channel of the endoscope. Subsequently the endoscope was advanced to the thoracic inlet where the samples of mucoid material were aspirated via the catheter. Storage in plain tube for bacteriology and EDTA tube for morphology analyze was used. The slides for cell differentiation and percentage distribution were prepared after centrifugation of the samples and subsequent May-Grünwald-Giemsa staining. Differential counts of inflammatory cells was estimated by counting 300 cells from TW slides and expressed as a percentage of total cells (Rossi et al., 2018). All samples for microbiology were centrifuged at 1500 rpm for 15 minutes. Then a culture from each sample was prepared on blood agar with 5% defibrinated sheep blood and on Mac Conkey agar (Hi Media, India). The incubation of the cultures was performed at 37 ºC for 24-48 hours under aerobic conditions. Pure cultures were subjected to further stereoscope identification through the morphological characteristics of their colonies such as shape, periphery, size, pigmentation and hemolysis. Isolated cultures were Gram stained. Catalase and oxidase tests were performed. Samples incriminated as Streptococcus spp. were further differentiated by a set of biochemical tests with the Christie's, Atkins and Munch-Petersen reaction (CAMP) and for the hydrolysis of esculin. Gram-negative isolates were inoculated on TSI (Triple sugar iron) agar, Indole production and motility, Simmons citrate agar and tested for sugar reduction for further identification.

**Results and discussion**

Clinical examination of the horses did not reveal deviation in internal body temperature (37.4±0.4ºC) and heart rate (48±7 beats/min). They manifested a markedly increased respiratory frequency (41±6 /min). The horses showed frequent muffled coughs, difficulty breathing and severe physical intolerance with refusal to exercise at an appropriate level. Previously, Robinson et al.

(2003), Houtsma et al. (2015), Rettmer et al. (2015) and Rossi et al. (2018) reported a similar clinical manifestation in horses affected by recurrent airway obstruction, which gave us reason to accept it as typical of "asthmatic" syndrome.

The results of blood tests showed no significant abnormalities: red blood cells 8.2 ±1.5 T/l, MCV - 40.2±2.5 fl, MCH - 14.4±1,8 pg, MCHC - 339±12 g/l, hemoglobin - 148±12 g/l, hematocrit - 38.5±3.8 %, platelets - 210±24 G/l, white blood cells -8,32±1,8 and differential blood count: Neu - 69±3.4%, Lym – 28.5±2.4%, Mon – 2.7±0.4%, Eos - 4±1.2%, Ba – 0.84±0.2%. These results confirmed the previously reported by Moore et al. (2004) and Riihimaki et al. (2008). Therefore, we assume that hematological examination is not of diagnostic value in horses with asthma-like symptoms and agree with their conclusion that it can be used to differentiate from other pathologies of inflammatory nature, such as bronchitis, bronchopneumonia, pleuropneumonia.

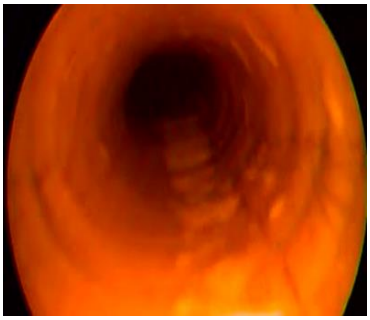

**Figure 1: Grade 3- stream forming mucus**

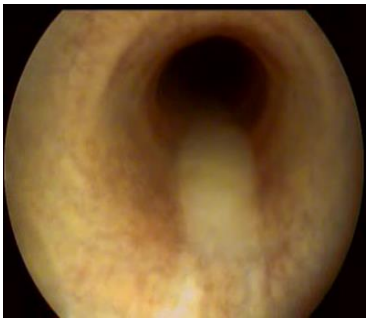

**Figure 2: Grade 4 – pool forming mucus**

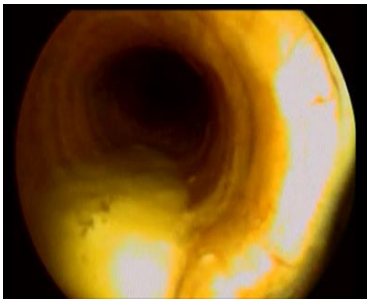

**Figure 3: Grade 5 – profuse amounts of mucus**

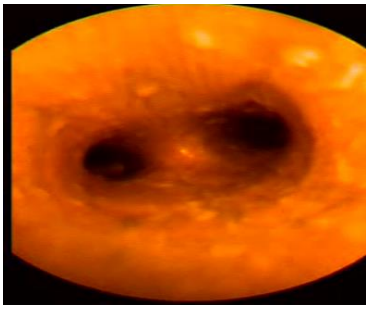

**Figure 4: Severe spasm and edema of tracheal bifurcation**

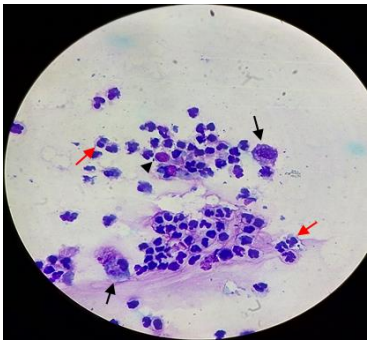

**Figure 5: Macrophages (black arrows), neutrophils (red arrows), Goblet cells (arrowhead)**

Endoscopically, the amount of tracheal mucus was evaluated as grade 3 (two horses), grade 4 (three horses) and grade 5 (one horses) (Fig. 1, 2, 3). In two horses, we observed edema and lack of luster of the tracheal bifurcation. In the same animals, we noted a pronounced spasm of the main bronchi. (Fig. 4). Similar results in severe affected horses were published before (Gerber et al., 2004; Couëtil et al., 2016; Feudo et al. 2021). According to Gerber (2004), increased tracheal mucus (grade 4−5) has a high specificity in horses with RAO, while intermediate amounts (2−3) overlap between healthy and RAO-affected horses. The lack of mucus or a small number of mucus spots in the trachea (grade 0−1) represented a normal finding and clear evidence of no airway obstruction. Based on our current research, we fully support these statements and believe that they can be used in clinical practice for diagnostic purposes in horses affected by „asthma". Cytological examination of TW fluids (Fig. 5) shoved a high percentage of neutrophils (81±7%) and a lower percentage of macrophages (12±4%) and lymphocytes (9±3%). The large number of neutrophils in the mucus indicated inflammatory processes in the lower respiratory tract and strongly suggested RAO. This finding was in accordance with previously data, reported by Malikides al. (2003), Laus et al. (2009) and Rossi et al. (2018). Particular attention should be paid to the fact that some of the tests were performed after high-speed exercise while the samples in our study were obtained at rest, which may have some impact over results. Despite the differences in the design of the tests performed, we consider that a large amount of tracheal secretion combined with the increased percentage of neutrophils in it is a reliable sign of lower airway obstruction. In microbiological examination of tracheal samples were isolated as follows: *Streptococcus equi*, subsp. *zooepidemicus* (2 horses), *Streptococcus pneumoniae* (1 horse), *Pasteurella spp.* (1 horse), *Klebsiella pneumoniae* (1 horse) and *Pseudomonas aeruginosa* (1 horse). No bacterial contamination was detected in two horses. At this stage, we cannot define the role of isolates as a factor in the occurrence or maintenance of „Equine asthma" syndrome. It has been hypothesized that their presence may be a consequence of dysbacteriosis or the result of previous treatment with antibiotics and corticosteroids (Manguin et al., 2020). In our opinion, additional studies are needed to clarify the role of microorganisms in the occurrence of asthma syndrome.

## Conclusion

"Equine asthma" syndrome is a common disorder of the normal function of respiratory tract and combines the following forms of pathology: inflammatory airway disease (IAD) and recurrent airway obstruction (RAO). The diagnosis of the more severe form (RAO) is based on the clinical findings incl. shortness of breath, cough, rapid fatigue and typical characteristic endoscopic detection in the trachea (mucus 3rd - 5th degree). The presence of predominantly neutrophilic cells in the TW can be taken as an indicative characteristic of RAO. Further studies are needed to elucidate the role of microorganisms in the etiology of "asthma" in horses.

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