THESES OF DOCTORAL (PhD) DISSERTATION

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UPPER RESPIRATORY TRACT FUNCTIONAL DISORDERS IN SPORT AND PLEASURE HORSES

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DOI: 10.54598/001570

Kaposvár

2021

ANTECEDENTS OF THE DISSERTATION

Poor performance may be related to a number of different origins in horses, including the upper and lower respiratory tract, musculoskeletal problems, cardiovascular disorders, nervous system and gastrointestinal tract diseases. Respiratory problems are the most common cause, after musculoskeletal problems, of poor performance in horses.

Horses – contrary to human beings – are obligate nasal breathers, they cannot switch from nasal to oro-nasal breathing. They can only decrease the air resistance by contracting the musculature of the upper respiratory tract (URT).

During inspiration, when the diaphragm contracts, negative pressure develops in the upper and the lower respiratory tract. The nasal passages, followed by the larynx, produce the greatest resistance to airflow within the upper airway. Airflow volume is substantially increased during exercise, resulting from the increased negative pressure. The nasopharynx and the larynx have a tendency to collapse, since neither of them has a bony/stiff structure, and active muscle work is needed to prevent this.

A resting endoscopic examination is useful in identifying URT structural obstructive conditions and may also give information regarding the nasopharyngeal and laryngeal function.

To establish a definitive diagnosis of URT obstruction, exercising videoendoscopy is regarded as the 'gold standard' method. Until recently, dynamic endoscopy has only been performed on high-speed treadmill endoscopy (HSTE). The advent of another type of dynamic endoscopy now referred to as "overground endoscopy" has allowed horses to be examined during exercise under normal training conditions and in their normal environment. The pioneering of OE proved very successful since treadmill exercise does not always allow reproduction of the exact conditions of dynamic obstructions of the equine URT. It provides real-time visualization of the URT and recorded video-endoscopy for

post-test reviewing. It also provides a safe and effective system for imaging the equine URT during ridden exercise at speed. An argument against OE is that normal racing conditions may not be appropriately replicated. Strenuous exercise tests may be more easily performed on a treadmill than by performing multiple exercise intervals in the field. The extent of strenuous exercise that the horse is put under in HSTE is under the control of the veterinarian and not the rider, as most riders tend to pull the horse up when they hear the abnormal respiratory noise. With HSTE the horse can be brought up to maximum exercise, until the abnormality is apparent or the horse shows signs of fatigue. This benefit of HSTE would be most useful in the examination of the URT of racehorses presenting with poor performance and/or abnormal respiratory noise, since the racing discipline demands that the racehorses compete at maximal speeds, much more so than any non-racing sport horse.

The arguments against HSTE includes the cost of the equipment, the limited availability of high-speed treadmills, the safety risk to the horses and the handlers and also the time taken to train both the handlers and the horses to be examined and also that HSTE fails to recreate normal exercise conditions with the influence of the rider being present. This is of particular importance in non-racing sport horses, as some of the manoeuvres or movements required of these sport horses could be contributing factors in the development of dynamic airway instability. Head flexion is a contributing factor to an increase in URT resistance, therefore head flexion during ridden exercise could be important in the induction of an abnormal respiratory sound at exercise. It was concluded that in cases of upper airway dynamic obstruction, rider intervention during ridden exercise can contribute to increasing laryngeal and/or pharyngeal instability in sport horses and therefore OE should be the preferred method for evaluation of the upper airways in sport horses since these changes would not usually be seen with HSTE.

THE OBJECTIVES OF THE THESIS

Describing the results of URT diagnostic evaluation with OGE in non-racing sport horses and pleasure horses. (Study 1)

Investigate our hypothesis that asthmatic diseases might be an underlying cause of dorsal displacement of the soft palate in horses. We also aimed to investigate the clinical manifestation of DDSP correlated to equine asthma syndrome. (Study 2)

Evaluate the upper airway mechanics during OGE examination in Colombian Criollo horses showing abnormal respiratory sound and poor performance during exercise. Furthermore, our goal was to investigate the hypothesis that the characteristics of the special walking gait and the intensively poll flexed neck may play an important role in the mechanics and the function of the URT of the Criollo horses. (Study 3)

METHODOLOGICAL SUMMARY

The present dissertation is based on three studies. The **1.** and **2. study** was carried out in Hungary (MTA-SZIE Large Animal Clinical Research Group) and the **3. study** was performed in Colombia (Universidad CES, Medellin).

General examinations

The studies were restricted to sport show and pleasure horses referred for respiratory examination with a history of poor performance and/or abnormal respiratory noise.

After recording the medical history and performing a physical examination, horses underwent a resting and overground endoscopy performed with Dynamic Respiratory Scope saddle pad version (Optomed, DR v3, France, Les Ulis) or Tele-View Dynamic Equine Exercise Endoscope (TV-506 Articulating Model). The scope was passed from one of the nostrils; in order to handle the horse a nose twitch was applied and no sedatives were used. Resting endoscopic examinations were done immediately prior to the exercising tests. The morphology and function of the larynx and pharynx were evaluated, and occlusion test was performed to induce URT obstruction.

OGE was done in an outdoor arena. The horses were ridden by their usual riders, who were instructed to exercise the horses as usual during OGE, according to the level and discipline of the horses. The riders were asked to ride the horses with loose reins and also in poll flexion at each gate. Horses were ridden until fatigue or until it became evident which URT functional disease was causing the poor performance or abnormal respiratory sound.

When the history, the physical examination and the clinical signs revealed a significant LRT obstruction, bronchoalveolar lavage (BAL) was performed. The importance of BAL fluid cytology was established in light of the history, clinical examination, and endoscopic findings in each case. When inflammation with infectious origin was suspected, culturing of the tracheal lavage (TL) was performed.

Based upon the history and clinical signs of the horses, the tracheobronchial mucus score, the BAL cytology we have divided the horses into groups of mild/moderate or severe equine asthma. All horses with positive culturing of TL were excluded from the study.

Additional examinations and information

In the **study 1,** blood samples were collected in order to measure plasma lactate levels. Blood samples were taken from the jugular vein, at the times indicated in Table 1, into vacuum collecting tubes containing sodium fluoride. All samples were carried to the laboratory in a 4 °C cooling bag within 4 hours after sampling, where the tubes were immediately centrifuged (10 min, 4000 g). After separation, plasma lactate levels were analyzed (Olympus AU /640, Japan, Tokyo).

Table 1: Times of blood sample collections

Blood samples	Timing
0	At rest in the box, prior to the exercise
1	During exercise, after the warm-up session,
2	During exercise, after the most intensive workload
3	At rest in the box, 1 h after work

In **Study 2**, based upon the history and clinical signs of the horses, the tracheobronchial mucus score, the BAL cytology we have divided the horses into groups of mild/moderate or severe equine asthma. We have also taken into account whether the horses developed DDSP during resting endoscopy examinations. Based on these considerations the following groups were established (Table 2).

Table 2: The groups of the horses in Study 2

Groups	Disorders
Group 1	Severe equine asthma
Group 2	Severe equine asthma and DDSP
Group 3	Mild and moderate equine asthma
Group 4	Mild and moderate equine asthma and DDSP

DDSP = Dorsal Displacement of the soft palate

In Study 2, besides the clinical examinations described earlier, additionally an atropine test was performed. After administration 0.02 mg/kg bwt atropine intravenously, we performed a second OGE and evaluated if any significant change was present. This examination aimed to evaluate the upper respiratory tract after decreasing lower airway obstruction, which in turn decreased the negative pressure.

In **study 3**, a special breed, the Colombian paso horses (CPH), also called Colombian Criollo Paso horses were investigated. These horses are found throughout most of Colombia. They have been intensively selected for their gaits since the 1980s. The CPH has been divided into four lineages based on their natural gait (Colombian Paso Fino, Colombian Trocha, Colombian Trocha-gallop, and Colombian Trot-gallop). All horses registered in the studbook, Federación Colombiana de Asociaciones Equinas—Fedequinas, are allowed to participate in competitions in Colombia.

All CPHs perform a so-called walking gait, with at least one limb in stance phase (no aerial phase) with high stride frequency, moreover an important feature of the CPH gait is the extensive collection and the high poll flexion of the neck (Nicodemus et al., 2003). This makes the gait even more exhausting to perform and may also be an important factor in the mechanics of the upper airways.

Statistical analysis

Fisher's exact test was used to search for the likeliness of the coincidence of URT–LRT obstructions. Sample averages of the lactate level intervals were calculated with a one-sample *t*-test at 0.95 confidence levels. Paired sample *t*-test was used to compare the averages (**Study 1**).

Binomial tests (Clopper-Pearson method) with $P \le 0.05$ significance were used to establish estimated intervals of the measured frequencies of DDSP occurring in the studied groups (**Study 2**).

A database was created using Microsoft Excel, and statistical analyses and graphs were performed using the SciPy (1.4.1) library of the Python programming language. Frequency of coexistence of different dynamic URT disorders was investigated. For analysis of qualitative variables, frequency tables were evaluated with proportions for each one of the groups (in accordance with the disorders detected). The odds ratio was used as a measure of the association level with a confidence interval of 95%. For all statistical tests a $P \le 0.05$ was used to determine statistical significance. One-tailed Fisher exact tests were used to check for positive contingency between each pair of functional disorders (**Study 3**).

RESULTS

Study 1

Altogether 19 horses – 9 males, 10 mares – were examined, ranging in age from 4 to 21 years (10.6 ± 4.7). Dorsal displacement of the soft palate (DDSP) was diagnosed in 8/19 horses, which might have developed secondary to URT or lower respiratory tract inflammation or obstruction. None of the DDSP was an isolated finding. Intermittent DDSP was also detected at rest in 4 cases. Recurrent laryngeal neuropathy (RLN) was diagnosed in 15/19 horses, 11 of which were complex cases with other types of URT obstructions. Severe pharyngeal collapse, suspected already at rest, was visible in two cases during exercise. In contrast, pharyngeal collapse diagnosed with nasal occlusion at rest in two cases was fully compensated under the rider. Plasma lactate levels significantly decreased during exercise (table 3).

Table 3. The average of plasma lactate levels

0. Resting (prior to exercise)	$0.93 \pm 0.21 \text{ mmol/l}$
1. During work (after warm up)	$0.48 \pm 0.08 \text{ mmol/l}$
2. During work (after intensive workload)	$0.54 \pm 0.19 \text{ mmol/l}$
3. Resting (post-exercise)	$0.75 \pm 0.2 \text{ mmol/l}$

Study 2

57 horses - 32 males, 25 mares - were examined, ranging in age from 4 to $21 \ (11.3 \pm 3.2)$ years. All horses were examined in the exacerbation phase of the asthmatic disease. Bronchoalveolar cytology and tracheal lavage bacteriology were performed in all cases. It was observed that more than $60\% \ (18/22 \text{ horses}, 59.7 - 94.8\%, \text{ binomial test}$, $95\% \ \text{CI}$) of horses with mild or moderate equine asthma and more than $79\% \ (29/31 \text{ horses}, 78.6\% - 99.2\%, \text{ binomial test}$, $95\% \ \text{CI}$) of horses with severe equine asthma are presented with DDSP during resting endoscopy examination. During the exercising endoscopy, DDSP was detected in all cases of severe equine asthma. In the 2 horses, where the atropine test was performed, we found that the administration of atropine did not lead to any improvement of the upper respiratory obstruction (DDSP). During OE all horses were coughing intensively, simultaneously with the displacement (71.5% - 100.00%, binomial test at $95\% \ \text{CI}$), no other typical abnormal respiratory sounds were detected during DDSP.

Study 3

A total of 40 Colombian Paso horses (CPH) – 16 males, 24 mares – were examined, ranging from 2 to 11,5 years (median 4 or mean 3,48 years) of age. All horses were examined during resting and overground endoscopy. Arytenoid cartilage collapse was observed in 35 out of 40 cases during exercise. Among these, dynamic laryngeal collapse (DLC) was the most significant finding, but ventro-medial luxation of the apex of the corniculate process of the arytenoid (VLAC) and recurrent laryngeal neuropathy was also observed. Dynamic pharyngeal collapse was significantly associated with vocal fold collapse, nasopharyngeal collapse (NPC), medial collapse of the margins of the epiglottis (MCME) and MCME was associated with NPC. Dorsal displacement of the soft palate (DDSP) was only detected in 4 out of 40 cases.

DISCUSSION

Reports of dynamic airway obstructions are primarily concerned with racehorse populations, while reports on such obstructions in sport horses and pleasure horses are scarce. Furthermore, the dynamic URT of Colombian Criollo horse, which is a show/sport horse with characteristic walking gaits, has never been investigated in literature before. A new possible etiology of DDSP was described during the evaluation of the Hungarian horse populations. Equine asthma commonly resulted in DDSP in pleasure horses. We suggest that DDSP is not exclusively associated with the increasing negative pressure driven by the lower respiratory tract obstruction but rather tailored to the combination of the bronchospasm and inflammation of airway mucosa. In these cases, we suggest a primary treatment of the lower airways. Despite the intensive work load of Criollo horses DDSP is relatively uncommon. We propose that the excessive poll flexion

during the gaits of CPH could rather impede the manifestation of DDSP of extrinsic cause.

Among ACC-s, RLN was our most frequent finding, while DLC was only found in one single case among the Hungarian sport and pleasure horse population. On the contrary, in the Colombian Criollo horses DLC was found to be the most common ACC. This latter finding is unusual and DLC is typical to only a few special breeds worldwide. We suggest that the extensive poll flexion, which is an integral part of the CPH's gait and the relatively small laryngeal lumen of these horses are the most important predisposing factors of DLC in our caseload of CPH. Another rare laryngeal dysfunction VLAC was over-represented in our study of CPH.

Although the most important ACC is DLC in CPH-s other possible backgrounds of ACC-s (like RLN or arytenoid dysplasia) should be excluded in each case. We suggest that laryngeal ultrasonography should be routinely performed in CPH horses with DLC. Especially in cases where the bilateral collapse of arytenoids is asymmetric and/or in cases where RDPA is co-appearing.

We suggest that the use/discipline of the horse should be taken into account, when evaluating configuration problems of the URT; for example, in racehorses a caudally and ventrally positioned larynx is disadvantageous, while in sport horses and show horses the rostral position of the larynx can lead to URT obstruction during poll flexion.

It is important to treat the upper and lower respiratory tracts as a single unit, since LRT disorders can often cause URT functional disease (e.g. Equine asthma – DDSP), while URT obstructions could be a factor in lower respiratory problems (RLN – EIPH).

NEW SCIENTIFIC RESULTS

Dorsal Displacement of the soft palate

- 1. Equine asthma commonly co-appears with dorsal displacement of the soft palate.
- 2. The origin of dorsal displacement of the soft palate is typically accompanied by lower airway obstruction and/or airway inflammation in sport and pleasure horses.
- 3. In cases where DDSP is co-appearing with equine asthma, the treatment should primarily focus on the lower airways.

The Colombian Criollo Paso horses

- 4. Among the Colombian Criollo horses the most significant functional problem is the dynamic laryngeal collapse.
- 5. Ventrorostral displacement of the dorsal laryngeal mucosa, which is a rare disease of the upper airways, should be distinguished from rostral displacement of the palatopharyngeal arch in the Colombian Criollo horses.
- 6. Ventrolateral luxation of the arytenoid cartilage was over-represented in Colombian Criollo horses compared with other studies.
- 7. Despite the intensive workload of Colombian Criollo horses DDSP is relatively uncommon.

SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE THESIS

Peer-reviewed papers published in foreign scientific journals

<u>Joó, K.</u>, Szenci, O., Bohak, Z., Povazsai, A., Kutasi, O., 2015. Evaluation of Overground Endoscopy Findings in Sport and Pleasure Horses. Journal of Equine Veterinary Science 35, 756–762.

<u>Joó, K.</u>, Povazsai, A., Bohak, Z., Szenci, O., Kutasi, O., 2021. Asthmatic disease as an underlying cause of dorsal displacement of the soft palate in horses. Journal of Equine Veterinary Science 96,103308.

<u>Joó, K.</u>, Duque, D., Vasquez, T., Parra, L., 2021. Evaluation of overground endoscopy findings in Colombian criollo paso horses. Journal of Equine Veterinary Science. Journal of Equine Veterinary Science 96, 103374.

Peer-reviewed papers published in Hungarian scientific journals

<u>Joó, K.,</u> Nyerges-Bohák, Zs., Szenci, O., Kutasi, O., 2014. Endoscopic examination of dynamic upper respiratory tract disorders in horses. Literature review. Hungarian Veterinary Journal 323-334.

Joó, K., Németh, G., Bohák, Z., Tóth Luca, A., Szenci, O., Kutasi, O., 2019. Significance and possible aetiologies of arytenoid cartilage collapse in horses Literature review Hungarian Veterinary Journal 451-462

Abstracts at international scientific conferences

Joó, K., Kovács, M., Szenci, O., Bohak, Z., Povazsai, A., Kutasi, O., Asthmatic diseases as an underlying cause of dorsal displacement of the soft palate in horses. World Equine Airway Symposium, 13-15. July 2017. Copenhagen, Denmark

Joó, K., Bohak Zs., Povazsai, A., Szenci, O., Kutasi, O., Difficulties in the evaluation of overground endoscopy findings in sport horses. 14th WEVA Congress, 8-10 October 2015. Guadalajara, Mexico

<u>Joó, K.</u>, Szenci, O., Bohak, Z., Povazsai, A., Kutasi, O., The complex nature of dynamic upper airway obstructions, Young WEVA, 2nd International Vetcamp, 8-14. August 2014. Saarlouis, Germany

Abstracts at Hungarian scientific conferences

Joó, K., Kovács, M., Szenci, O., Bohak, Zs., Povazsai, A., Kutasi, O., Overground endoszkópos vizsgálatok értelmezési nehézségei sport lovak esetében, MTA Állatorvostudományok Bizottsága, Akadémiai beszámoló, 26-29. January 2015. Budapest, Hungary

<u>Joó, K.</u>, Sportélettan és teljesítmény - Alsó és felső légutak problémái. 24th Congress of the Hungarian Association of Equine Practitioners, 2-3. December 2016. Telki, Hungary

Joó, K., Kovács, M., Szenci, O., Bohak, Zs., Povazsai, A., Kutasi, O., A kannaporc kollapszus lehetséges eredetei lovakban, MTA Állatorvostudományok Bizottsága, Akadémiai beszámoló, 23-26. January 2017. Budapest, Hungary

Joó, K., Sport- és hobbilovak felső légúti funkciózavarai, Innovatív tudományos műhelyek hazai agrár felsőoktatásban, 27. November, 2017. Debrecen, Hungary