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Analysis of Tracheal Secretion in Healthy Horses Undergoing a Vaquejada Simulation Test

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Abstract

This study aimed to evaluate the occurrence of EIPH in vaquejada horses via endoscopy and tracheal lavage and citology. We used eight Quarter-mile horses that usually perform at vaquejadas. Clinical examination was carried out before the race. After the race, endoscopy and tracheal lavage with 20 ml of saline via the service channel of the endoscope were performed. The samples were sent to the laboratory for processing and confection of the slides. Of the eight horses (four pull and four helper horses), six had hemosiderophages in their tracheal aspirates, confirming the existence of pulmonary hemorrhage after exercise. No difference was observed between the analyzed parameters between the two groups of vaquejada horses (P > 0.05). Tracheal lavage and endoscopy can be used for evaluating this important disease in animal athletes.

Keywords

EIPH, Horses, Sport, Endoscopy

1. Introduction

Respiratory diseases have been identified as one of the main causes of interruption in the training and competition of pure-blooded racehorses [1]. Inflammatory airway disease (IAD) and exercise-induced pulmonary hemorrhage (EIPH) are two common issues that affect the lower airways of racehorses, resulting in a poor performance [2].

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Inflammatory airway disease (IAD) is associated with a low performance and accumulation of mucus and inflammatory cells in the airways. Horses with IAD often have an increased relative neutrophil count in their bronchoalveolar lavage (BAL), and less frequently, the relative count of eosinophils and/or mast cells can be increased. The etiopathogenesis of IAD is unknown, but may involve individual factors and/or acquired immune responses to several factors, including breathable components, such as dust, microorganisms, harmful gases, and air conditioning [3].

The prevalence for IAD in racehorses has been reported as being between 11.2% and 50% [4]-[6]. This disease is prejudicial to athletic performance [7] [8] and is an important cause of losses in the equine industry [9]. Diagnosis is usually obtained by endoscopy of the trachea, where increased production of mucus and pus is observed, and cytology from the tracheal wash or bronchoalveolar lavage (BAL) of the lower respiratory tract [10] [11].

Exercise-induced pulmonary hemorrhage (EIPH) is a prevalent syndrome in horses, with repeated episodes of hemoptysis after exaggerated exercise [12]. Despite its high prevalence (43.8% - 75.4%), little is known about the predisposing factors that lead to EIPH [13]-[15]. Part of the difficulty in determining its pathophysiology and treatment is lack of precision regarding diagnostic methods.

Epistaxis alone has a low specificity and sensibility for detection of EIPH in horses. Endoscopy of the tracheobronchial airways currently remains the most accepted method for detecting EIPH 30 to 90 minutes after exercise. Nevertheless, it is still difficult to obtain a true positive [16]. Cytology of the tracheal lavage (TL) is considered more specific than only an endoscopy exam, being described as a tool for diagnosing pulmonary disease [17]. However, cytology becomes fundamental because it allows the observation of cells that are characteristic of a hemorrhagic process, such as hemosiderophages [16] [18] [19].

Our objective was to analyze the tracheal secretion from healthy horses submitted to a vaquejada simulation test via endoscopy and tracheal lavage and subsequent cytology.

2. Material and Methods

This study has the approval of the Ethics Committee of the UFRPE under protocol number 23082.004734/2013. Eight Quarter mile horses were selected (four pull and four helper horses), two females and six males, with ages between 3 and 18 years (mean 10.5 years). A physical examination, which included heart and respiratory rates, temperature, capillary refill time, intestinal motility, and lung auscultation, was performed in all horses before the test. Mean velocity was obtained by dividing the distance traveled by the mean time.

Simulation of the vaquejada was comprised of five runs with the pull horses and the helper horses with the use of a bull. In the conventional competition, there are three runs before the horse can go to the following level of the course. As most horses pass this first phase, we decided on five runs so that the number of runs would not be over or underestimated.

For endoscopy, the horses were contained with the help of a twitch. The endoscope that was used was an OLYMPUS CF Type EL (9 mm external diameter, 2.8 mm working channel, and 103 cm length). Endoscopy was performed 30 to 60 minutes after exercise, where the respiratory tract was evaluated from the nasal meatus to the trachea for the presence of secretion (color, quantity, and localization), hyperemia of the mucosa, and changes to the respiratory tract observable via endoscopy. Secretions observed on endoscopy were attributed scores from 1 to 5 [20].

For the tracheal lavage, the endoscope, with a catheter for transendoscopic sample collection (MILA® Delivery Catheter) in its working channel, was introduced via the ventral nasal meatus towards the trachea. The catheter was positioned in the distal portion of the trachea, anterior to the carina, in accordance with a previously described technique [21]. For the TL, 20 ml of sterile saline were instilled with the use of sterile plastic syringes (Injex®) and immediately aspirated. The samples were considered adequate when suspended particles or mucus filaments were observed. The lavage was refrigerated in the same syringe used for collection until processing.

Aliquots of the lavage (20 mL) were centrifuged at 110 g for 5 min in a RDE® MC-16 centrifuge. The supernatant was discarded and the sediment of cells used to prepare slides using a linear smear technique [22]. The slides were fixed in methanol for 5 minutes and stained using the May-Grumwald-Giemsa method.

For differential cell analysis, 100 cells were counted using the 1000 X objective lens [23], differentiating the various cell types (epithelial cells, macrophages, lymphocytes, neutrophils, eosinophils, and hemosiderophages).

Descriptive statistics was initially used for analysis of the studied parameter, followed by the T-test to com-

pare the means between the two groups of vaquejada horses (pull horses and helper horses). Results are shown in mean +/− mean standard error. The software used for calculations was SigamaStat®.

3. Results

Mean differential cell count from the tracheal aspirate showed 45.375 ± 8.255 epithelial cells; 0 caliciform cells; 25 ± 9.59 neutrophils; 4.25 ± 1.013 lymphocytes; 0.25 ± 0.164 eosinophils; 11.5 ± 5.510 foamy macrophages; 5.875 ± 2.020 hemosiderophages, and 0.87 ± 0.743 giant cells (**Table 1**).

Observations: mean values in the same row followed by different letters indicate that $P < 0.05$ using T-test. HR: Heart rate; RR: Respiratory rate; LTr: Recovered tracheal lavage; HSF: Hemosiderophages; ME: Foamy macrophages; GC: Giant cells.

Endoscopy, performed from 30 to 60 minutes after the test, revealed tracheal secretion in five of the eight horses. The secretions visible on endoscopy were classified according to the score from 1 - 5 [20]. Horses 1, 2, and 8 had secretion with grade 1 coloration and grade 2 quantity. Horse number 3 had secretion grade 1, coloration grade 1. Horse 6 had secretion grade 1 and grade 3 coloration. All were located on the second third of the trachea. Results were organized into a table (**Table 2**). In horses 1 through 5, the trachea and carina were hyperactive, the mucosa hyperemic, but none had bleeding.

Regarding the changes of the upper airways of the respiratory tract, the presence of epiglottic chondritis was observed in horse number 4, lymphoid follicular hyperplasia in horse 5, dorsal dislocation of the soft palate in horse number 6, and laryngeal hemiplegia in horse 8.

Sedation was not necessary for clinical exam or endoscopy. All horses were considered clinically normal and had no complaints of reduced performance. According to the physical exam performed before the test, heart rate was 46.5 ± 4.732 beats per minute and 33.75 ± 3.369 respirations per minute. During the test, the horses had a mean velocity of 40.11 Km/h, and no animal was observed to have epistaxis or nasal mucus secretion on external examination. The amount of saline aspirated for the tracheal lavage was 18.875 ± 0.639. There were no differences between pull and helper horses for the analyzed parameters ($P > 0.05$).

Table 1. Mean values for the studied parameters in pull and helper horses undergoing vaquejada test, municipality of Itabaina-PB.

Parameter	Types of Horses	
	Pull horses (4)	Helper horses (4)
HR	42.800 ± 4.363 bpm	52.667 ± 10.729 bpm
RR	30.800 ± 3.929 mrpm	38.667 ± 5.925 mrpm
Velocity	32.66 ± 0.237 Km/h	38.57 ± 0.230 Km/h
LTr	19.600 ± 0.400 ml	17.667 ± 1.453 ml
% Epithelial Cells	38.800 ± 10.293	56.333 ± 13.667
Caliciform Cells	0	0
Neutrophils	37.200 ± 12.575	4.667 ± 0.667
Lymphocytes	4.600 ± 1.400	3.667 ± 1.667
Eosinophils	0.400 ± 0.245	0
Total	12.400 ± 3.326	31.00 ±13.577
Macro HSF	4.00 ± 2.811	9.00 ± 2.082
Macro ME	7.800 ± 3.367	17.667 ± 14.667
Macro GC	0.200 ± 0.200	2.00 ± 2.00

Observations: mean values in the same row followed by different letters indicate that $P < 0.05$ using T-test. HR: Heart rate; RR: Respiratory rate; LTr: Recovered tracheal lavage; HSF: Hemosiderophages; ME: Foamy macrophages; GC: Giant cells.

Table 2. Endoscopy findings and changes observed in the tracheal aspirate of vaquejada horses, Itabaiana-PB.

Horse	Upper Airways				Lower Airways			
	HFL	Epiglottis	DDPM	Laryngeal hemiplegia	Tracheal Secretion	Trachea	Carina	Pulmonary bleeding
01	-	-	-	-	1-2-2/3	HR 4	HE	-
02	-	-	-	-	1-2-2/3	HR 4	HE	-
03	-	-	-	-	1-1-2/3	HR 4	HE	-
04	-	Chondritis	-	-	-	HR 4	HE	-
05	Grade II	-	-	-	-	HR 4	HE	-
06	-	-	X	-	1-3-2/3	-	-	-
07	-	-	-	-	-	-	-	-
08	-		-	Grade III	1-2-2/3	-	-	-

HR: Hyperreactive, reddish coloration; HE: Hyperemic.

4. Discussion

History, clinical exam, endoscopy of the respiratory tract, and cytology of the tracheal lavage are valuable tools for investigating respiratory disease [24]. In the current study, we observed that, among these, cytology of the tracheal lavage is the most effective method for diagnosis, for there were no changes on physical exam, clinical history, or even endoscopy that would justify the cytology profile observed in these animals.

The tracheal lavage (TL) can be obtained during endoscopy of the airways, during which the structures of the respiratory tract as well as quantity of mucus can be evaluated. The quantity of mucus is directly related to inflammation of the airways and decreased performance in athlete horses [25]. The presence of mucus in the lower airways means a neutrophilic inflammation was probably involved; however, the absence of mucus does not exclude pulmonary inflammation, which may have been secondary to the accumulation of cells in the lower airways [26]. In this study, TL was easily performed, and 20 ml of saline was infused, of which a mean of 18.875 ± 0.639 ml were recovered. We were also able to observe mucus in five of the eight horses, who also showed an increase in neutrophils, suggesting IAD.

It is important that cytology of the airways be a part of the routine clinical examination in equines, since respiratory disorders have a significant role in the health and performance of horses of all ages and participating in various sports [27].

Also, IAD and EIPH can be hard to identify clinically and can cause an important deficit to athletic performance by exacerbating the hypoxia induced by exercise [28]. The diagnosis, severity, and prognosis, as well as prevalence of presentations such as IAD and EIPH depend on cytology so that incomplete or wrong diagnosis are not made [11] [16] [29].

According to previously published reference values for cell types [2] found in the tracheal lavage, we observed that pull horses had an elevated mean quantity of neutrophils, eosinophils, and hemosiderophages, proving that though the horses were apparently asymptomatic, their cytology results were compatible with IAD and EIPH. Helper horses also had an increase in hemosiderophages, confirming the presence of EIPH.

Racehorses would probably also have some degree of pulmonary hemorhage during exercise, however, only 1% have epistaxis, while 50% are positive on endoscopy, and 100% are positive on BAL (bronchoalveolar lavage) [30]. Our study confirms the findings of a previous study, since none of the eight examined animals were positive via endoscopy and six had hemosiderophages, which confirms some degree of post-exercise bleeding [30]. Of the six horses that showed hemosiderophages, four are considered positive for hemorrhage because the percentage observed was higher than 5%. Of these, three were helper horses and one was a pull horse.

In a similar manner, hemosiderophages were observed in the tracheal lavage of 86% of racehorses [31]. The high rate in which hemosiderophages were seen on cytology was already expected, since all equines submitted to exaggerated exercise suffer some kind of pulmonary hemorrhage [21].

Considering also that the hemorrhage is commonly located is the caudal lung lobe, it is likely that the TL presents a more diluted material than the BAL when we consider the presence of hemosiderophages [32]. Therefore, it is possible that in the present study, the presence of EIPH is being underestimated because of the use of TL instead of BAL.

The percentage of neutrophils in the airways of healthy horses should be <20% of the total for the TL [25] [33]. In this study, all four pull horses (4, 5, 7, 8) had an increase in the percentage of neutrophils and eosinophils, and one of these had lymphoid follicular hyperplasia (animal 5), reinforcing the presence of a viral infection. Horse 6 had a large number of macrophages and giant cells, probably due to the presence of coccoid bacilli, which would lead these cells to divide rapidly to fight off the infection. Their increase is an important finding in the inflammatory process of the lungs [34] and at the same time represents a type Th2 immune response [35] since giant cells are not frequently seen in the tracheal lavage [25].

Although endoscopy is an easily performed exam, it is still little used routinely. In a previous study with 100 horses, 22 underwent endoscopy, of which 20 had a tracheal sample collected for cytology [24]. Endoscopy is the only method capable of identifying functional disorders of the upper respiratory tract [36] [37].

Endoscopy of 510 equines after exercise showed that 58.21% of the horses had obstructive diseases of the upper respiratory tract, with 17.80% having more than one obstructive condition [38].

On our endoscopy exams, we were able to observe functional changes to the upper respiratory tract, such as laryngeal hemiplegia, epiglottic chondritis, and dorsal dislocation of the soft palate. Furthermore, the exam was of fundamental importance because it allowed us to visualize the tracheal mucosa and to localize and quantify existing secretions.

Endoscopy can also help in estimating the severity of the EIPH through the quantity of blood present in the airways, since epistaxis is only seen in 0.25 to 13% of thoroughbred racehorses with EIPH [39]. However, in our study, we did not observe any pulmonary hemorrhage via endoscopy, which highlights the importance of performing a cytology of the tracheal lavage, without which there would be false negatives for EIPH.

In this study, clinical evaluation did not show relevant changes, since all horses were healthy and clinically normal. It has been established that normal heart rate and respiratory rate prior to exercise do not exclude the possibility of horses having some degree of pulmonary bleeding [40].

As in other studies, chemical restraint was unnecessary during endoscopy, and only a twitch was sufficient [41].

5. Conclusions

Endoscopy and cytology of the tracheal lavage are fundamental tools for the diagnosis of respiratory diseases, since endoscopy permits the identification of functional disorders and cytology, and the visualization of the predominant cell type, allowing an identification of the existing process as EIPH or IAD.

Larger studies are required in order to determine the occurrence of exercise-induced pulmonary hemorrhage and inflammatory airway disease in horses used in vaquejada, seeing as this is a sport that has differences when compared with other modalities, which may lead to different diseases.

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