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Cytology Results of Bronchoalveolar Lavage Fluid in Horses with Recurrent Airway Obstruction

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Abstract. Bronchoalveolar lavage is essential in evaluating airway's status and response to treatment in horses with recurrent airway obstruction. In this study we investigated the changes of bronchoalveolar lavage (BAL) fluid in horses with recurrent airway obstruction undergoing treatment with corticosteroids. Cytologic examination revealed decreased neutrophils percentage in BAL fluid obtained from horses with recurrent airway obstruction at 21 days of treatment after receiving dexamethasone, compared with initial results before treatment. Cytologic examination of BAL fluid provided important data regarding the local inflammatory status of the airways before and after treatment.

Key words: neutrophil, inflammation, airway, cytology, dexamethasone

INTRODUCTION

Recurrent airway obstruction (RAO) is a disease characterized by airway inflammation, bronchoconstriction and chronic obstruction of the airways [8]. Local airway inflammation, for both upper and lower airways, and pulmonary inflammation, is assessed by performing special diagnostic techniques like endoscopy, transtracheal aspiration (TTA), bronchoalveolar lavage (BAL) or pulmonary function testing [5, 6, 7]. The neutrophilic inflammation of the lower airways and the pathological changes of lungs in RAO affected horses, correlates with the results offered by cytological examination of BAL fluid [8]. These techniques are highly necessary because early diagnosis of the disease folowed by a rigurous treatment with corticosteroids, the use of bronchodilators and environmental management [1], will help the horse to have a satisfying sport performance [8].

MATERIAL AND METHOD

This study was conducted on 8 horses with recurrent airway obstruction (RAO).

The horses, males and females were of different breeds, or cross bred, with ages between 7 and 16 years old. In all cases history was taken, horses were clinicaly examinated and in some of them a hiperventilation plastic bag was used or underwent mild physical exercise to intensify respiratory sounds.

All horses were investigated by bronchoalveolar lavage on day 1, folowed by treatment with dexamethasone, and another bronchoalveolar lavage at the end of treatment, on day 21.

Bronchoalveolar lavage blind technique was performed in the standing horse using a special silicone BAL catheter, under mild sedation. Detomidine and butorphanol was used to

sedate the horses, and a twich was aplied on the horse's upper lipp to minimise movement of the horse.

The sample was collected using 300-500 ml of sterile saline solution. After collection, it was macroscopically examinated to evaluate the color, transparency and the presence of flocculent debris.

Floculent samples were filtrated through two layers of gauze to remove excess mucus strands and other debris. The samples were processed by centrifugation for 5 minutes at 1,000 RPM. For cytological interpretation direct smears were prepared from the sediment, after the supernatant was removed, and stained using Dia Quick Panoptic (DQP) or Diff Quick stains. From each sample about 3-5 smears were prepared and stained. Cytological interpretation was based on the percentage of each cellular population from the BAL sample. This was determined by differential cell counting of 300 consecutive cells in order to obtain an accurate representation of the cell types present, under oil immersion (1,000 X) in order to assure the specific morphology of each cell [4].

The treatment was performed by daily intravenous administration of 0.1 mg dexamethasone /kg body weight for the first 7 days, than the dose was reduced to 0.05 mg/kg body weight for the next 7 days, and the last week of treatment the horses received only 4 intravenous injection at 48 hours intervals of 0.05 mg/kg body weight. During the 21 days of treatment all horses received soaked hay, and were stabled on a dust free bedding.

Results were analyzed for normality of distribution using the Shapiro–Wilk normality test. The majority of data sets were found to be normally distributed. Mean comparison was done with the Anova; a confidence level of 95% ($P<0.05$) was considered significant. The R free software package was used for all the statistical analyses (R Development Core Team, 2010).

RESULTS AND DISCUSIONS

Initial clinical examination of all RAO affected horses revealed increased respiratory efforts, cough, abnormal respiratory sounds, weezing and crackels due to increased mucus quantities within airways. History revealed that the horses had access to moldy straw during the last 3 months prior to sampling and in some of them the simptoms became evident when the concentration of pollen in the environment was higher that usual in the spring. At 7 days after receiving dexamethasone, clinical signs improved differently in horses with RAO and at the end of treatment, all horses showed no clinical signs of RAO, but still some of them revealed a mild respiratory discomfort when physicaly exercised.

The volumes of BAL fluid samples obtained was between 30 to 80 ml per sample. Macroscopic examination of the BAL fluid, revealed various amounts of mucus and floculent material in samples from RAO affected horses before treatment. At the end of treatment the samples contained a clear or mild turbit fluid in RAO treated horses. Some samples had a mild pink color of the fluid retreived at the end of the lavage, and after the centrifugation, a small quantity of erythrocytes was noticed in the sediment.

Dia Quick Panoptic (DQP) and Diff Quick staining allowed a quick evaluation of the cellular population in the sample, being a fast technique which makes it very useful in a clinician's work (a total of 70 seconds).

Total nucleated cell number in the BAL fluid was not evaluated because according to Hewson and Viel (2002), only an estimated cell count can be obtained if saline solution is infused to facilitate collection of the sample, since this dilutes the cell concentration in the sample.

Table 1.

Results (mean ± SD) of cytological examination of BAL fluid obtained from RAO affected horses before treatment (RAO, day 1) and after treatment (RAO, day 21). *** Value differs extremely significant (P < 0,01) from initial results

Variable / Group	Neutro-phils (%)	Macro-phages (%)	Limpho-cytes (%)	Eosino-phils (%)	Mast cells (%)	Epithelial cells (%)
RAO, day 1	77.38±3.85	13.13±1.73	5.88±0.83	1.75±1.16	1.50±1.07	0.38±0.52
RAO, day 21	35.13±3.04 ***	26.88±2.36 ***	29.38±2.50 ***	0.75±0.71	1.750.71	6.13±1.13 ***

The higher percentage of neutrophils (77.38 ± 3.85 %) in samples from horses with RAO before treatment compared with 5 – 30 % in healthy horses, according to data provided by Hewson and Viel (2002), reveals a local inflammation, and confirms the inflammatory features of this disease. The value of neutrophils percentage found at the end of treatment (35.13±3.04) is close to values found in healthy horses according to data provided by Hewson and Viel (2002), and the difference is extremely significant (P<0.001) compared to initial results found on day 1.

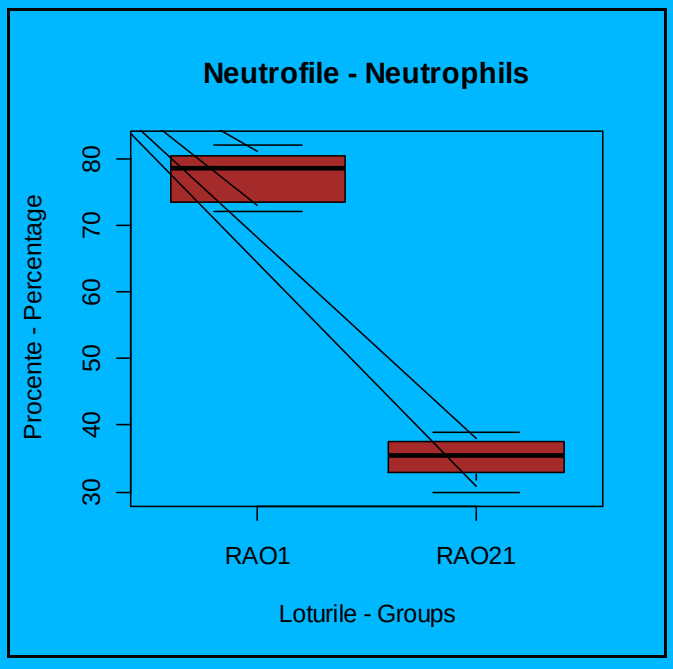

Figure 1. Neutrophils percentage in BAL fluid from horses on day 1 before treatment (RAO1) is extremely significantly higher than at the end of treatment, on day 21 (RAO21).

The percentage of macrophages was extremely significantly lower (P<0.001) after treatment. Cytology revealed in some horses the presence of macrophages containing erytrocytes and also in other horses fungal spores, hyphae or polen. The presence of fungal spores and hyphae in the macrophage's cytoplasm does not indicate that the pacients had fungal pneumonia. This was correlated with moldy straw exposure prior to sampling.

The presence of erytrocytes in BAL fluid observed in some horses was correlated with possible bleeding associated with the procedure, especially when movement of the head and tube induced lesions of the small airways mucosa. Erytrophagocytosis is not patognomonic for effort induced pulmonary hemorrage, and can be seen if fresh blood was present in the airways. Also if the sample contains erytrocytes secondary to bleeding associated with the procedure, viabile macrophages from the BAL fluid can engulf the cells if the slides are not prepared and fixed immediately after the sampling [3].

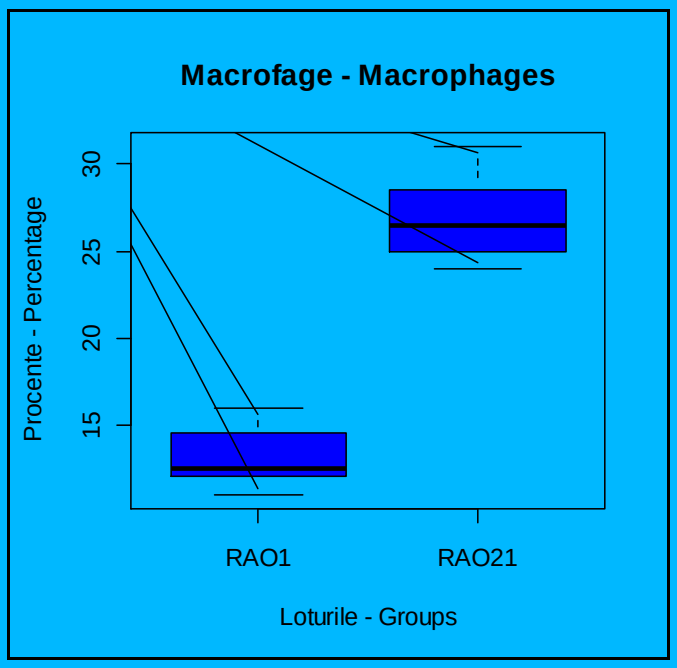

Figure 2. Macrophages percentage in BAL fluid from horses on day 1 before treatment (RAO1) is extremely significantly lower than at the end of treatment, on day 21 (RAO21)

Lymphocytes were found in a higher percentage after treatment compared with initial results, and the difference is extremely significant ($P<0.001$).

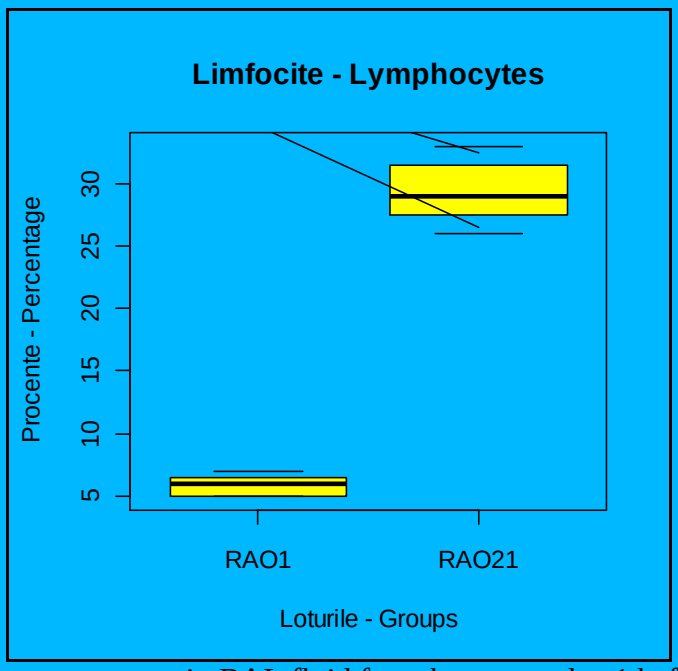

Figure 3. Lymphocytes percentage in BAL fluid from horses on day 1 before treatment (RAO1) is extremely significantly lower than at the end of treatment, on day 21 (RAO21).

No significant difference was found regarding the percentage of eosinophils and mast cells between the results before and after treatment. The low percentage of eosinophils in BAL samples from horses with RAO confirms the fact that this disease is not always associated with a specific allergy after the onset of the disease, though in some horses, macrophages containing polen were noticed.

There was an extremely significant difference ($P<0.001$) between epithelial cells percentage from initial results before treatment and epithelial cells percentage after treatment, though it doesn't have any clinical importance.

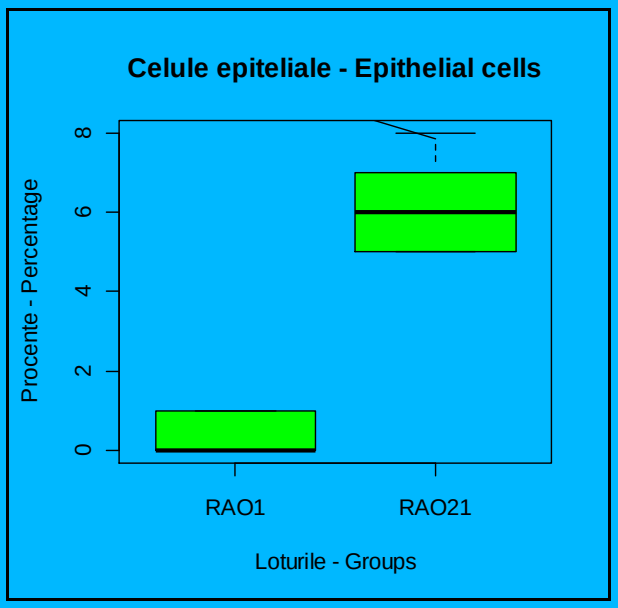

Figure 4. Epithelial cells percentage in BAL fluid from horses on day 1 before treatment (RAO1) is significantly lower than at the end of treatment, on day 21 (RAO21).

CONCLUSIONS

- The increased neutrophils percentage in BAL fluid from RAO horses confirms a local inflammatory process.
- Dexamethasone reduces local inflammation of the airways, wich is proved by the value of the neutrophils percentage in BAL fluid at the end of treatment.
- The presence of fungal spores and hiphae fagocytosed by macrophages does not indicate a mycotic airway disorder, but offers valuable data regarding the horse's management and stable microclimate.

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