

## Cytology profile and age influence in the equine bronchoalveolar lavage in healthy and asymptomatic inflammatory airway disease

Perfil citológico e influência da idade no lavado broncoalveolar de equinos saudios e com doença inflamatória de vias aéreas assintomática

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### RESUMO

Doenças inflamatórias pulmonares são observadas na espécie equina desde sua domesticação. Dentre as enfermidades de maior importância do trato respiratório equino, estão os distúrbios não infecciosos de vias aéreas posteriores. Este trabalho teve por objetivo determinar o perfil citológico e o efeito da idade na avaliação do lavado broncoalveolar (LBA) de equinos saudios e com Doença Inflamatória das Vias Aéreas (DIVA) assintomática. Foram utilizados 32 equinos adultos, aparentemente saudios, de ambos os sexos (20 machos e 12 fêmeas), com faixa etária média semelhante, sendo 15 animais do grupo controle e 17 animais do grupo doente assintomático. Os grupos foram estabelecidos segundo os critérios de GERBER et al. (2004) para acúmulo de muco traqueal após a realização de exames físicos diretos e endoscopia traqueal. Foi realizado o LBA e o percentual dos valores médios obtidos na análise citológica dos cavalos saudios e com DIVA assintomática foram, respectivamente: 56,9% e 48,0% de macrófagos, 37,2% e 40,0% de linfócitos, 3,3% e 7,1% de neutrófilos, 1,7% e 1,4% de mastócitos, 0,3% e 0,7% de eosinófilos; e 0,5% e 0,4% de células epiteliais. Concluiu-se que a DIVA nos animais integrantes do grupo doente assintomático caracterizou-se por um discreto infiltrado de neutrófilos e que a idade não influencia na avaliação citológica do LBA.

**Palavras-chave:** equino, doença inflamatória das vias aéreas, citologia broncoalveolar.

### ABSTRACT

Pulmonary inflammatory diseases have been observed in equine since their domestication. Non-infectious disease processes of the lower airways are amongst the most

important diseases of the equine respiratory tract. This study aims to determine the cytology profile and effect of age on the evaluation of equine bronchoalveolar lavage (BAL) obtained from healthy animals and from horses with asymptomatic inflammatory airway disease (IAD). Thirty two horses, including 20 males and 12 females, without clinical signs of pulmonary disease and with age varying from 4 to 21 years, were assigned to two groups: 15 animals in the control group and 17 in the asymptomatic group. Groups were divided according to physical examination and mucous accumulation observed during tracheal endoscopy. The BAL results from healthy and asymptomatic horses were, respectively: 56.9% and 48.0% macrophages, 37.2% and 40.0% lymphocytes, 3.3% and 7.1% neutrophils, 1.7% and 1.4% mast cells, 0.3% and 0.7% eosinophils; 0.5% and 0.4% epithelial cells. When horses in the two groups were analyzed according to their age range, no differences were found. In conclusion, IAD in the asymptomatic group was significantly different from the control group. The asymptomatic horses presented a discrete infiltrate of neutrophils, which does not influence cytological evaluation of BAL.

**Key words:** horse; inflammatory airway disease; bronchoalveolar cytology.

### INTRODUCTION

Respiratory diseases are the most common source of economic losses in the world equestrian industry (HODGSON & HODGSON, 2002) and inflammatory airway disease (IAD) is one of the most important pulmonary afflictions in sport horses. With

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the exception of decreased performance, the majority of horses presenting IAD demonstrate only mild clinical signs, very often only detected under maximum physical effort (LESSA et al., 2008).

This condition is still not completely understood, especially due to the lack of agreement among researchers concerning cytology patterns obtained from bronchoalveolar samples from healthy and IAD positive horses. The main reasons for the lack of agreement are the variety of techniques used to perform the bronchoalveolar lavage (BAL) (WINDER et al., 1989; VRINS et al., 1991; LAPOINTE et al., 1994), the presence of asymptomatic disease the degree of exposure to contaminants and environment allergens (MAIR et al., 1987), and differences in age of the subjects (VIEL & HEWSON, 2003). This study has the objective to determine the cytology profile and the effect of age on the evaluation of BAL samples obtained from healthy and asymptomatic IAD horses.

## MATERIAL AND METHODS

The samples were taken from 32 adult horses of mixed breeds, belonging to Brazilian Army (Regimento de Cavalaria de Guardas Andrade Neves), in Rio de Janeiro, Brazil. Twenty males and 12 females with ages ranging from four to 21 years utilized in moderate intensity sport activity were recruited to participate in the study. The horses were apparently healthy, had no disease history or medical treatment during the 30 days preceding data collection. Blood sample were collected from the horses and analyzed for WBC and plasma fibrinogen and values were within normality according to published parameters (GRONDIN & DEWITT, 2010).

The horses were kept in semi-confinement, in stall boxes of 9m<sup>2</sup>, with cement floor and no bedding. Feeding included 5 to 6kg animal<sup>-1</sup> day<sup>-1</sup> of commercial horse concentrate with 12% total protein, four kg of Coast-Cross grass hay/animal/day and water *ad libitum*. The animals were groomed daily and turned out for voluntary exercise in a paddock 4 to 6 times a week during 6 hours a day. The subjects were dewormed every three months and vaccinated against influenza, tetanus, eastern and western encephalomyelitis, rabies, equine adenitis and leptospirosis.

Normal horses were selected after being clinically evaluated and reference values were considered normal for vital functions as described by HOUSTON & RADOSTITS (2002) and normal for pulmonary percussion and auscultation according to MCGORUM et al. (2002).

Only one endoscopic examination was conducted on each experiment animal in the mornings,

with an Olympus CF 10L colonoscope advanced from the nasal cavity up to the tracheal carina. Both nasal cavities were examined. The trachea was considered normal when presented intact, with shiny mucous membranes of pale color and a sharp carina (DIXON, 1997). The semi-quantitative evaluation of the tracheal mucous was conducted using a score from 0 to 5, according to GERBER et al. (2004).

Animals were classified as normal or asymptomatic based on the results from endoscopic examinations, and further sub classified according to age group into mature (older than seven years old) and younger (<seven year old) according AINSWORTH (2010). Overall there were 15 animals in the control group (normal physical examination and endoscopic scores of 0 or 1), subdivided into young control (nine horses) and mature control (six horses). The asymptomatic group consisted of 17 animals (normal physical examination but endoscopic evidence of disease demonstrated by scores 2 to 5), and was subdivided into young asymptomatic (nine horses) and mature asymptomatic (eight horses).

Those horses were mechanically restrained and sedated with intravenous 10% xylazine chloridrate (SEDAZINE<sup>®</sup>) at 0.5 to 1.1mg kg<sup>-1</sup> before BAL collection. An equine silicone BAL catheter (BIVONA<sup>®</sup>) was introduced up to the bronchi while topical anesthesia of the respiratory tract was achieved by 0.5% lidocaine (LIDOVET<sup>®</sup>). A BAL sample was obtained by infusing 250ml of warm (37°C) 0.9% saline according to the technique described by HOFFMAN & VIEL (1997).

Samples were considered adequate if at least 40% of the infused liquid was recovered (ROBINSON, 2001) and when surfactant and turbidity were observed. Liquid collected was transferred to glass vials and kept refrigerated at 4-6°C until processing, within a maximum of four hours from sampling.

Slides from the samples were obtained by cytocentrifugation (CYTOPRO 7620, WESCOR<sup>®</sup>) at 110g for five minutes, supernatant was fixated with metallic alcohol and stained with May-Grunwald, Giemsa (Merck<sup>®</sup>). Readings were conducted on an optic microscope with magnification 1000x (immersion) by differential counting of 500 cells.

Differences between groups and the effect of age were analyzed by ANOVA (SAS Institute Inc., Cary, NC) and significance level set at 5%.

## RESULTS AND DISCUSSION

The mean percentage of differential nucleated cell count in each of the groups is presented in tables 1 and 2. BAL cytology profile of the control

Table 1 – Percentage of the differential cell count present in equine BAL from control and asymptomatic groups. Rio de Janeiro, May to December 2006.

Group	Macrophages	Lymphocytes	Neutrophils	Mast cells	Eosinophils	Epithelial Cells
Control (n=15)	56.9 ± 14.8 <sup>a</sup>	37.2 ± 15.4 <sup>a</sup>	3.3 ± 1.5 <sup>a</sup>	1.7 ± 1.2 <sup>a</sup>	0.3 ± 0.4 <sup>a</sup>	0.5 ± 1.2 <sup>a</sup>
Asymptomatic (n=17)	48.0 ± 14.0 <sup>a</sup>	40.0 ± 12.2 <sup>a</sup>	7.1 ± 3.8 <sup>b</sup>	1.4 ± 1.1 <sup>a</sup>	0.7 ± 1.3 <sup>a</sup>	0.4 ± 0.9 <sup>a</sup>

Different letters in the same column indicate significant differences (ANOVA; P<0.05).

group (n=15) was characterized by the prevalence of alveolar macrophages and lymphocytes, followed by neutrophils, mast cells, eosinophils and a small percentage of epithelial cells (Table 1). These results are similar to the ones described by COUËTIL & DENICOLA (1999), HOFFMAN (1999; 2008), LESSA (2003) and in a recent IAD *Workshop* (ROBINSON, 2003) where volumes between 200 and 500ml were used in the collections of BAL.

IAD in the asymptomatic group was characterized by the presence of a discrete neutrophil infiltrate, without significant changes in other cell types (Table 1). This type of infiltrate has been described, sometimes together with increased numbers of mast cells and eosinophils (MOORE et al., 1995; BAIN, 1997; VIEL, 1997; LESSA, 2003; LESSA et al., 2005; ROBINSON, 2003). CHRISTLEY & RUSH (2007) characterize IAD as an inflammatory process with a neutrophilic infiltrate, and less commonly with eosinophilic and mast cell infiltrates.

Age did not significantly influence the differential nucleated cell count results on the BAL of the healthy controls when young and mature animals were compared (Table 2). This finding differ from what has been previously described by HOFFMAN (1999) and VIEL & HEWSON (2003), who demonstrated that healthy animals present a physiologic increase in

neutrophil numbers with age. A similar situation was observed in the asymptomatic group, with no significant influence of age on the BAL results between asymptomatic young and mature horses (Table 2).

A small infused volume during collection of the BAL sample might result in an increase in neutrophil numbers due to relative high concentration of cells per volume retrieved (SWEENEY et al., 1992). However this factor is unlikely to have influenced the results displayed here, since the infused volume was fixed at 250ml for both groups and the retrieved volume was a minimum of 40% of the total infused, according to guidelines from the International Workshop on Equine Chronic Airway Disease (ROBINSON, 2001).

Even though the volume of 250ml is considered small by SWEENEY et al. (1992) and HOFFMAN (1999), only the percentage of neutrophils was significantly different between control and asymptomatic horses. If the difference observed had been caused by a lower dilution, all cell types would have been equally affected. Therefore we can conclude that the difference in neutrophil count between these two groups is related to the diseased pulmonary status of the asymptomatic animals.

The results from this work concur with previous information stating that the cytology profile, especially the neutrophil percentage, differs between healthy horses and the ones with asymptomatic IAD.

Table 2 - Percentage of the differential cell count present in equine BAL from control (cont) and asymptomatic (asym) groups, subdivided by age group, young (y) and mature (M). Rio de Janeiro, May to December 2006.

Group		Macrophages	Lymphocytes	Neutrophils	Mast cells	Eosinophils	Epithelial Cells
Cont	Y(n=9)	54.2 ± 13.7 <sup>a</sup>	40.0 ± 14.4 <sup>a</sup>	3.4 ± 1.4 <sup>a</sup>	1.7 ± 1.3 <sup>a</sup>	0.3 ± 0.3 <sup>a</sup>	0.1 ± 0.3 <sup>a</sup>
	M(n=6)	58.3 ± 13.3 <sup>a</sup>	35.3 ± 14.0 <sup>a</sup>	3.0 ± 1.8 <sup>a</sup>	2.2 ± 0.9 <sup>a</sup>	0.2 ± 0.4 <sup>a</sup>	1.0 ± 1.8 <sup>a</sup>
Asym	Y(n=9)	54.8 ± 12.2 <sup>a</sup>	34.7 ± 12.1 <sup>a</sup>	8.1 ± 3.4 <sup>a</sup>	1.3 ± 1.0 <sup>a</sup>	0.4 ± 1.0 <sup>a</sup>	0.6 ± 1.3 <sup>a</sup>
	M(n=8)	40.8 ± 11.7 <sup>a</sup>	46.0 ± 10.0 <sup>a</sup>	6.0 ± 4.2 <sup>a</sup>	1.4 ± 1.3 <sup>a</sup>	1.2 ± 1.5 <sup>a</sup>	0.2 ± 0.3 <sup>a</sup>

Different letter in the same column indicate significant differences (ANOVA; P<0.05).

## CONCLUSION

In conclusion, IAD in asymptomatic horses is characterized by a neutrophil infiltrate and age does not influence cytological evaluation of BAL.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Protocol number CEUA/126 UFF.

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