

Effects of single and duplicate infusions of 250mL of saline solution in the cytological evaluation of bronchoalveolar lavage in equines*

Vanessa Viscardi¹, Aline del Carmen Garcia Lopes², Joana de Castro Faria Beling², Gisela Vasconcelos Gioia³, Rodolpho de Almeida Torres Filho⁴, Daniel Augusto Barroso Lessa⁵ e Nayro Xavier de Alencar⁶⁺

ABSTRACT. Viscardi V., Lopes A.C.G., Beling J.C.F., Gioia G.V., Torres Filhos R.A., Lessa D.A.B. & Alencar N.X. **Effects of single and duplicate infusions of 250mL of saline solution in the cytological evaluation of bronchoalveolar lavage in equines.** [Efeito da infusão única e em duplicata de 250mL de solução salina na avaliação citológica do lavado broncoalveolar em equinos.] *Revista Brasileira de Medicina Veterinária*, 37(1):33-35, 2015. Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminense, Rua Vital Brazil Filho 64, Niterói, RJ 24230-340, Brazil. E-mail: nayro@vm.uff.br

The bronchoalveolar lavage (BAL) is a sensitive method to diagnose diseases of the distal portion of the lower respiratory tract and has been broadly used by numerous researchers. Different volumes of infusions are employed to obtain BALs in horses, which can influence the cytology. Therein, the present paper intends to determine the effects of a single and a duplicate infusion of 250 mL of saline solution in BAL cell type count. BAL samples of 30 equines obtained with the infusion of saline solution divided into two aliquots of 250mL (A1 and A2) were used. The cytological preparations were done by cytocentrifugation and all glass microscope slides were fixed and stained with Giemsa for the differential cell count. As for the effect of different infusion volumes (A1 and A2) on the cell type count, there were significant differences only among neutrophil percentages ($p=0,0236$), that was lower in the latter samples (A2). Although the infusion of 250mL (A1) allows the obtaining of a representative sample of cell population in the lower respiratory tract, it is not appropriate to compare these results to currently accepted reference values, making it necessary to establish reference values for this volume.

KEY WORDS. Horse, cytology, bronchoalveolar lavage, infusion volume.

RESUMO. O lavado broncoalveolar (LBA) é um método sensível de diagnóstico das enfermidades da porção distal do trato respiratório posterior e tem sido amplamente utilizado por diversos pes-

quisadores. Diferentes volumes de infusão são aplicados para obter o LBA de cavalos, o que pode interferir na contagem citologia diferencial. Dentro deste contexto, este trabalho estudou o efeito da in-

* Received on December 21, 2012.

Accepted for publication on February 28, 2014.

¹ Tenente PM Veterinária, MSc. Esquadrão Escola de Cavalaria, Av. Marechal Fontenele 2906, Jardim Sulacap, Rio de Janeiro, RJ 21740-360, Brazil. E-mail: vanessaviscardi@yahoo.com.br

² Médicas veterinárias autônomas. Rua Fernando Monteiro Lindenberg, casa 13. Vila Velha, ES 29101-100. Emails: aline_garcia@yahoo.com.br; joana_beling@yahoo.com.br

³ Graduanda em Medicina Veterinária, Universidade Federal Fluminense (UFF), Rua Vital Brazil Filho, 64, Niterói, RJ 24230-340, Brazil. E-mail: gisela.gioia@hotmail.com

⁴ Professor, DSc. Faculdade de Veterinária, UFF, Rua Vital Brazil Filho, 64, Niterói, RJ 24.230-340. E-mail: rattf@vm.uff.br

⁵ Professor Associado I, DSc Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, UFF, Rua Vital Brazil Filho 64, Niterói, RJ 24230-340. E-mail: lessadab@vm.uff.br

⁶ Professor Associado III, Dsc. Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, UFF, Rua Vital Brazil Filho 64, Niterói, RJ 24230-340. + Author fou correspondence, E-mail: nayro@vm.uff.br

fusão única e em duplicata de 250mL de solução salina na citologia do LBA de equinos. Amostras de LBA de 30 equinos adultos foram obtidas com a infusão de solução salina, dividida em duas alíquotas de 250mL (A1 e A2). As preparações citológicas foram feitas por citocentrifugação e fixadas e coradas com Giemsa para a realização da contagem diferencial dos tipos celulares. Foi observada diferença estatisticamente significativa ($p=0,0236$) em relação aos neutrófilos, cuja percentagem foi menor nas amostras A2. Apesar da infusão única de 250mL (A1) possibilitar a obtenção de amostras representativas da população celular do trato respiratório posterior, não é apropriado comparar estes resultados com aqueles comumente utilizados como valores de referência, sendo necessário estabelecer novos valores de referência para este volume de infusão.

PALAVRAS-CHAVE. Cavalos, citologia broncoalveolar, volume de infusão.

INTRODUCTION

The respiratory system has great influence on the health and optimum performance of equines in sports and leisure as well as in terms of reproduction (Rossdale et al. 1985, Bailey et al. 1999). Diseases involving the respiratory system of horses are among the most commonly found by clinicians and the efficient treatment depends on an adequate diagnosis (Roy & Lavoie 2003).

The bronchoalveolar lavage (BAL), originally developed by Finley et al. (1976) in human medicine and adapted by Viel (1983) for horses, allows the analysis of cell types and proteins from small airways. Nowadays, this procedure, besides being considered a simple, safe and low-invasive technique, is also a sensitive method in the diagnosis of various horse diseases of the distal portion of the lower respiratory tract (Lessa et al. 2005, Couëttil et al. 2007, Hoffman 2008, Jorge 2011, Lessa et al. 2011).

Literature describes numerous techniques to perform the collection and processing of BAL samples. Amid the factors that can interfere in the interpretation of these are the different volumes of infused saline solution for the sample collection (Sweeney et al. 1992, Couëttil et al. 2001, Pickles et al. 2002, Hoffman 2008). For this reason, it is necessary to evaluate the effect of varying infusion volumes on the results of BAL cytological evaluations.

Ergo, the present research has the objective of evaluating the effects of a single and a duplicate infusion of 250mL of saline solution in the cytological evaluation of bronchoalveolar lavage in equines.

MATERIALS AND METHODS

This work was submitted to and approved by an ethical committee under the protocol number 0068/08 of the Comitê de Ética em Pesquisa Animal/UFF.

The BAL samples were obtained from 30 adult healthy horses of mixed breeds, regularly dewormed and vaccinated, maintained in similar sanitary-hygienic and feed managements. Physical examination, which included thoracic percussion and auscultation, was normal in all horses.

The horses were sedated using 0.5 -1.1mg/mL/kg body weight 10% Xylazine hydrochloride (Sedazine[®]) intravenously and restrained with the use of twitches. BAL samples were taken after two infusions of 250mL of sterile 0.9% saline heated at 37°C via BAL catheter 300 (Bronchoalveolar Lavage Catheter, Surgivet[®]). After each infusion, aspiration was performed manually, considering that the minimum recovered volume was 40% of the infused volume. Afterwards, the samples were maintained under refrigeration up to six hours until the end of sample processing (Hoffman 2008). The cytology of the samples obtained after the first infusion of 250mL (A1) were compared with the cytology of samples obtained after mixing A1 with the second infusion of 250mL (A2).

Aliquots of 200µL of cellular suspension of each sample (A1 and A2) of BAL were submitted to cytocentrifugation (Cytopro 7620, Wescor[®]) at 110g during five minutes. All microscope slides were fixed and stained by the Romanowsky method (Giemsa - Merck[®]). Microscopic fields were selected randomly to perform the differential count on 500 nucleated cells that included neutrophils, eosinophils, mast cells, macrophages, lymphocytes and epithelial cells. The cytological evaluation was performed by a unique examiner under optical microscopes (Olympus[®] CX 40) with the 100x oil immersion objective.

The mean and standard deviation of the samples A1 and A2 were obtained and their effects were evaluated by a non-parametric Kruskal-Wallis test with a 5% level of significance

RESULTS AND DISCUSSION

The effects of the single (A1) and the duplicate (A2) infusions of 250mL on the counting of cell types is shown in Table 1. There were significant differences only between the percentages of neutrophils ($p=0.0236$), that was lower in A2. This result indicates that methodological factors of BAL collection affect some cell percentages. Sweeney et al. (1992) also observed a higher percentage of neutrophils and a lower percentage of mast cell in samples obtained with 50 mL infusions when compared to those with 350mL.

In this research, volumes of 250mL were used due to the available commercial forms of saline solution vials. Though there are significant differen-

Table 1. Differential BAL cytological count (%) of healthy horses (n=30), after single (A1) and duplicate (A2) infusion of 250mL. Rio de Janeiro, Brazil.

Cell Type	A1($\bar{x} \pm s$)	A2($\bar{x} \pm s$)	P
Macrophages	46.91 \pm 15.01	46.81 \pm 14.76	0.9234
Lymphocytes	43.01 \pm 15.63	45.56 \pm 14.45	0.4508
Neutrophils	5.03 \pm 4.15	3.46 \pm 4.06	0.0236*
Eosinophils	2.80 \pm 7.95	1.94 \pm 4.53	0.3344
Mast cell	1.65 \pm 1.46	1.79 \pm 2.00	0.6672
Epithelial cells	0.61 \pm 2.18	0.45 \pm 1.29	0.5764

A1 = single infusion of 250mL; A2 = after mixing with the second infusion of 250mL; Average (\bar{x}), standard deviation (s), probability level of the Kruskal-Wallis test (p). *Significant difference between cell types.

ces between neutrophil counts, the average percentages are compatible with internationally accepted values (<5%) for healthy animals (Robinson 2003). On the other hand, although they are within normal rates, this can lead to misinterpretations, especially for animals with limit values, being inappropriate to use a single infusion volume of 250mL (A1) with the reference values described in literature.

CONCLUSION

It is necessary to take into account the infused volume when performing an equine BAL cytological evaluation. Although the infusion of 250mL (A1) allows sampling of the cellular population of the lower respiratory tract, it is inappropriate to compare its results with the currently accepted reference values, making it necessary to establish specific reference values for this volume.

Acknowledgements. The authors are grateful for the assistance received from Bravet[®], BD[®] and Fort-Dodge[®] to accomplish this experiment.

REFERENCES

- Bailey C.J., Reid S.W.J., Hodgson D.R. & Rose R.J. Impact of injuries and disease on a cohort of two- and three-year-old thoroughbreds in training. *Vet. Rec.*, 145:487-493, 1999.
- Couëtill L.L., Rosenthal F.S., Denicola D.B. & Chilcoat C.D. Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. *Am. J. Vet. Res.*, 62:538-546, 2001.
- Couëtill L.L., Hoffman A.M., Hodgson J., Buechner-Maxwell V., Viel L., Wood J.L.N. & Lavoie J.P. Inflammatory Airway Disease of Horses. *J. Vet. Int. Med.*, 21:356-361, 2007.
- Finley T.N., Swenson E.W., Curran W.S., Huber G.L. & Ladman A.J. Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. *Annals Int. Med.*, 66:651-658, 1967.
- Hoffman A.M. Bronchoalveolar lavage: sampling technique and guidelines for cytologic preparation and interpretation. *Vet. Clin. - Eq. Prac.*, 24:423-425, 2008.
- Jorge M.L.L.A. *Atividade da Fosfatase Alcalina (EC 3.1.3.1) no Fluido Epitelial Pulmonar de Equinos da Polícia Militar do Estado do Rio de Janeiro*. Dissertação (Mestrado em Clínica e Reprodução Animal), Universidade Federal Fluminense, Niterói/RJ, Brasil, 2011.
- Lessa D.A.B., Machado C.H., Duarte C.S., Wachholz L., Lima J.R.P.A. & Fernandes W.R. Enfermidades do trato respiratório posterior em equinos de equitação no Rio de Janeiro: prevalência e aspectos clínico-laboratoriais. *Rev. Bras. Ciên. Vet.*, 12:1-2, 2005.
- Lessa D.A.B., Jorge M.L.L.A., Viana E.B. & Fernandes W.R. Análise do líquido broncoalveolar de equinos portadores de doença inflamatória das vias aéreas. *Braz. J. Vet. Res. Anim. Sci.*, 48:123-130, 2011.
- Pickles K., Pirie R.S., Rhind S., Dixon P.M. & McGorum B.C. Cytological analysis of equine bronchoalveolar lavage fluid. Part 1: comparison of sequential and pooled aliquots. *Eq. Vet. J.*, 34:288-291, 2002.
- Robinson N.E. Inflammatory airway disease: defining the syndrome. Conclusions of the Havemeyer Workshop. *Eq. Vet. Educ.*, 15:61-63, 2003.
- Rossdale P.D., Hopes R., Wingfield Digby N.J. & Offord K. Epidemiological study of wastage among racehorses 1982 and 1983. *Vet. Rec.*, 116:66-69, 1985.
- Roy M.F. & Lavoie J.P. Tools for the diagnosis of equine respiratory disorders. *The Vet. Clin. - Eq. Prac.*, 19:1-17, 2003.
- Sweeney C.R., Humber K.A. & Roby K.A. Cytologic findings of tracheobronchial aspirates from 66 Thoroughbred racehorses. *Am. J. Vet. Res.*, 53:1172-1175, 1992.
- Viel L. *Structural - functional correlations of the lung in horses with small airway disease*. PhD dissertation, University of Guelph. Guelph, Canada, 1983.