NOTA PRÉVIA

Comparison between linear smear concentration and cytocentrifugation techniques to evaluate equine bronchoalveolar lavage cytology

Comparação entre as técnicas de esfregaço linear e citocentrifugação para avaliação citológica do lavado broncoalveolar equino

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Abstract

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. Cytocentrifugation is the choice cytological preparation technique, but demands specific and costly equipment. Therefore, the present paper intends to verify the applicability of the linear smear technique to evaluate BAL samples. For this, BAL samples of 30 equines were used and the cytological preparations were done by cytocentrifugation and linear smear techniques. All glass microscope slides were fixed and stained with Giemsa for the differential cell count. Regarding the effect of the preparation technique on differential counts, no significant difference in any cell type was found. The linear smear is a reliable alternative and can be recommended as a substitution to cytocentrifugation.

Keywords: Horse. Bronchoalveolar lavage. Cytocentrifugation. Linear smear.

Resumo

O lavado broncoalveolar (LBA) é um método sensível para diagnosticar doenças do trato respiratório posterior e vem sendo utilizado por diversos pesquisadores. A citocentrifugação, técnica de escolha para processar amostras citológicas de LBA, exige equipamentos específicos e caros. Por isso, este trabalho verificou a aplicabilidade da técnica de esfregaço linear para avaliação citológica do LBA. Foram utilizadas amostras de LBA de 30 equinos adultos. As preparações citológicas foram realizadas tanto por citocentrifugação quanto por esfregaço linear. Todas as lâminas foram fixadas e coradas com Giemsa para realização da contagem celular diferencial. Não foram encontradas alterações morfológicas significativas e nem diferenças estatísticas entre nenhum dos tipos celulares processados pelos dois métodos, o que permite afirmar que o método de esfregaço linear é uma alternativa segura para avaliação morfológica celular do LBA de equinos, podendo ser utilizado no lugar da citocentrifugação quando esta não estiver disponível.

Palavras-chave: Cavalo. Lavado broncoalveolar. Citocentrifugação. Esfregaço linear.

Diseases involving the respiratory system of horses are among the most commonly found by clinicians and the efficient treatment depends on an adequate diagnosis[1]. The bronchoalveolar lavage (BAL) is a simple, safe and low-invasive technique, as well as, considered a sensitive method in the diagnosis of diseases of the distal portion of the lower respiratory tract[2,3,4,5].

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The BAL´s preparation method for cytological examination can significantly affect the results of cellular count. Methods such as cytocentrifugation, membrane filtration and centrifugation onto a cover slip are used in human medicine[6]. For equines, many papers report the preparation of microscope slides by cytocentrifugation[4,5,7,8] or by smears of the sediment obtained in conventional centrifugation[7,8].

Cytocentrifugation is a widely used method[6], indicated due to the low cellularity of the recovered fluid and the need of cellular concentration. Despite the higher morphological quality of the analyzed cells[7], this method requires specific and expensive equipments; therefore it is not easily accessible for most field veterinarians.

This report is concerned with the application of more accessible techniques of cytological preparation that also allow a precise diagnosis, and its effect on the differential counting of cell types present in bronchoalveolar cytology. In order to compare the linear smear concentration technique to cytocentrifugation, cell morphology and differential counts were evaluated in both methods. The samples were taken from 30 healthy adult horses (13 males and 17 females) of mixed breeds, aged 11-22 years, with an average weight of 460kg. All animals were stabled in rural areas of Rio de Janeiro State and were 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 of body weight of 10% Xylazine hydrochloride (Sedazine®) intravenously and restrained with the use of twitches. BAL samples were obtained with an infusion of 500mL of sterile 0.9% saline heated at 37ºC and divided into two aliquots of 250mL 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, both samples were pooled and maintained under refrigeration up to six hours, until the end of sample processing[8].

Aliquots of 200µL of cellular suspension of BAL were submitted to cytocentrifugation (CYTOPRO 7620, WESCOR®) at 110*g* during five minutes. The linear smear was done with 10mL aliquots of cellular suspension from the same samples of BAL, centrifuged in conventional equipment (bench centrifuge RDE® model MC-16) using the same gravitational acceleration. The obtained sediment was re-suspended with 50µL of equine serum and the glass microscope slides were prepared according to Cowell and Tyler[9].

In order to evaluate the effects of different BAL processing techniques, glass microscope slides of the recovered samples submitted to the cytocentrifugation method were compared to those prepared by the linear smear technique. All microscope slides were fixed and stained by the Romanowsky method (Giemsa – Merck®). A differential count was performed on 500 nucleated cells under optical microscopes (Olympus® CX 40).

The statistical analysis of the effects of different preparation techniques in the counting of cell types was achieved using the non-parametric test of Kruskall-Wallis, with a 5% significance level. This work was approved by the Ethics Committee with the protocol number 00106/09 CEPA/ UFF.

The effect of the preparation technique of the glass microscope slide in differential counts is shown in table 1. Although Hoffman[8] affirms that macrophages, mast cells and eosinophils are more prevalent in differential counts when using the cytocentrifugation method versus the smear technique, there were no significant differences between these techniques for any cell type in this report.

Cytological characteristics observed in the linear smear were less preserved than in the cytocentrifugation method, as previously observed[7,8], but BAL cells prepared by the linear smear technique yielded well preserved cell morphology, as observed by Thomp-

son et al.[6]. Though the cytocentrifugation technique is the method of choice[6,7,8], the linear smear is a reliable alternative for cytological analyses of equine BALs and can be recommended as a substitution to cytocentrifugation when the latter cannot be applied routinely.

Table 1 - Differential cytological count (%) of BAL, of all 30 animals, according to glass microscope slide preparation techniques (CC and LS) - Rio de Janeiro - 2009

Cell Type	CC (X ± *s*)	LS (X ± *s*)	p	CV
Macrophages	51.40 ± 14.73	51.01 ± 19.13	0.8534	33%
Lymphocytes	40.83 ± 13.24	42.72 ± 18.97	0.9941	39%
Neutrophils	4.71 ± 4.14	2.93 ± 2.42	0.0508	91%
Eosinophils	1.73 ± 4.23	2.20 ± 6.04	0.4735	263%
Mast cells	0.88 ± 1.26	0.77 ± 0.98	0.8213	136%
Epithelial cells	0.45 ± 1.24	0.37 ± 0.96	0.5718	268%

CC – cytocentrifugation; LS – linear smear; average (X), standard deviation (*s*), probability level of the Kruskall-Wallis test (p), coefficient of variation (CV)

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