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COMPARISON OF BRONCHOALVEOLAR LAVAGE AND RESPIRATORY SECRETION CYTOLOGY IN HORSES WITH CLINICALLY DIAGNOSED CHRONIC PULMONARY DISEASE

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SUMMARY

Thirty-nine horses and 3 ponies underwent a thorough respiratory examination and were grouped as follows: healthy (4 horses and 1 pony); mild chronic pulmonary disease (CPD 11 horses); moderate CPD (16 horses and 1 pony); and severe CPD (8 horses and 1 pony). Bronchoalveolar lavage (BAL) fluid collected from all animals and respiratory secretions (RS) obtained from 39 of these animals were evaluated cytologically and the results were compared.

It was concluded that cytological examination of either BAL fluid or RS was useful in diagnosing various equine pulmonary diseases. The only advantage that BAL offered over RS sampling was in cases in which there was no RS available in the trachea. In addition, the severity of the CPD did not always correlate with either RS or BAL cytology.

KEY WORDS: horses – bronchoalveolar lavage – respiratory secretions – cytology – chronic pulmonary disease

VERGLEICH DER ZYTOLOGISCHEN BEFUNDE VON BRONCHOALVEOLÄR LAVAGE-FLÜSSIGKEIT UND VON BRONCHIALSEKRET BEI PFERDEN MIT CHRONISCHEN LUNGENKRANKHEITEN

39 Pferde und 3 Ponies wurden auf Grund des klinischen Lungenbefundes in folgende Gruppen eingeteilt: gesund (4 Pferde, 1 Pony); leichtgradig chronische Lungenkrankheit (11 Pferde); mittelgradig (16 Pferde, 1 Pony); hochgradig (8 Pferde, 1 Pony). Von 39 Tieren wurden bronchoalveoläre Lavage-Flüssigkeit und Bronchialsekret zytologisch untersucht und die Befunde verglichen.

Die Ergebnisse zeigen, dass sich die Zytologie sowohl von der bronchoalveolären Lavage-Flüssigkeit wie auch vom Bronchialsekret für die Diagnose von chronischen Lungenkrankheiten eignet. Die Gewinnung von bronchoalveolärer Lavage-Flüssigkeit ist nur dort sinnvoll, wo kein Bronchialsekret entnommen werden kann. Die zytologischen Befunde korrelierten nicht in allen Fällen mit dem Schweregrad der Erkrankung.

SCHLÜSSELWÖRTER: Pferd – Bronchoalveolär-Lavage – Bronchialsekret – Zytologie – chronische Lungenerkrankung

INTRODUCTION

The cytological examination of transtracheal aspirates, tracheal wash specimens or respiratory secretions (RS) have been generally applauded (*Beech*, 1975; *Nuytten* et al., 1983; *Whitwell* and *Greet,* 1984; *Roszel* et al., 1985; *Mair*, 1987; *Winder* et al., 1989) but also criticized (*Derksen* et al., 1989) as a clinical aid in the diagnosis of equine chronic pulmonary disease. Perhaps the most difficult aspect encountered in the clinical evaluation of the respiratory system is the identification of horses with subclinical pulmonary disease. Conventional pulmonary function tests, which are time consuming, may not identify these horses (*Viel*, 1983) and cytological examination of RS may not consistently aid in a diagnosis of mild chronic small airway disease (*Winder* et al., 1989). Cytological evaluation of bronchoalveolar lavage (BAL) fluid, on the other hand, has been successfully used to assess the severity of equine chronic small airway diesase (*Viel*, 1983).

The purpose of this study was to compare the usefulness of RS and BAL cytology in the clinical diagnosis of equine

chronic pulmonary diesase (CPD) and in the assessment of disease severity.

ANIMALS, MATERIAL AND METHODS

Animals: Thirty-nine horses and three ponies of various breeds and ages (Table 1) were used in this study. All animals were examined thoroughly and were grouped according to their clinical diagnosis (*Grünig* et al., 1988). *Respiratory secretions*: Respiratory secretions were obtained via fiberoptic endoscopy as described (*Grünig* et al., 1988). One healthy horse and two horses with mild CPD did not have any RS in the trachea for sampling. Direct smears of respiratory secretions were stained with May-Grünwald Giemsa and were examined at 40x, 100x and 1000x magnifications. Cell numbers were scored semiquantitatively: 1-cell type not seen; 2-very few cells; 3-widely scattered cells; 4-moderate numbers of cells; 5-moderate to many cells; 6-many cells; 7-massive numbers of cells.

Bronchoalveolar lavage: Bronchoalveolar lavage was performed in all animals as described (*Viel*, 1983). Briefly, the horses were sedated using xylazine/morphine. A flexible fiberoptic endoscope was advanced to the carina. Surface anaesthesia was carried out using approximately 50 to 100 ml of 1% lidocaine. The endsocope was advanced into a caudal bronchus until it could not be moved any further. Then two 250 ml aliquots of pre-warmed (37 °C) 0.9% saline were infused into the lung and aspirated immediately using a vacuum of -7 to -9 mm Hg. The lavage fluid was filtered through a single layer of gauze to remove mucus. The volume of the recovered fluid was measured. One aliquot was set aside for cell differential, total cell count and for determination of cell viability.

For differential cell counts, 10 ml of lavage fluid was centrifuged at 400 g for 10 minutes. The cell pellet was resuspended in 10 ml RPMI 1640 medium (Gibco Ltd, Scotland). Cytocentrifuge (Cytospin Shandon, Instrumentgesellschaft, Zürich) smears were made by spinning 300 to 500 µl of the cell suspension and 50 µl of horse serum at 24 to 36 g for 10 minutes. Two preparations were made for each lung and stained with May-Grünwald Giemsa stain. A differential cell count was made for each lung by counting 400 cells at 1000 x magnification. The mean cell score of both lungs was used for statistical analysis.

Statistical analysis: Chi-square statistics were caculated to test for association between clinical categories and the occurrence of different cell scores (i. e. scores 1 to 3 combined = low versus scores 4 to 7 combined = high) in RS. The error probability was corrected using the Bonferroni-Holm test procedure (*Essl*, 1987). For statistical analysis of BAL specimens the Statview 512+ system (*Feldman* and *Gagnon*, 1986) was used. Means of volume recovered, total cell count, percentage viability and numbers of the different cell types in differential cell counts from BAL fluid were compared by analysis of variance and multiple comparisons using the method of least significant difference. For all analyses, $p < 0.05$ was considered significant.

RESULTS

Based on the results of the clinical respiratory examinations, animals were placed into one of the following groups: healthy (4 horses and 1 pony); mild CPD (11 horses); moderate CPD (16 horses and 1 pony); severe CPD (8 horses and 1 pony). The breeds and mean ages are listed in Table 1.

The mean recovered volume, mean total cell count and mean percentage cell viability of BAL fluid for each group are shown in Table 2. Although there was no significant difference between groups, the percentage of lavage fluid recovered decreased with an increase in disease severity.

Respiratory secretions: The RS cell scores for each group are shown in Table 3. Both on a group and on an individual horse basis, all healthy horses had RS cytology compatible with their diagnosis i. e. a predominance of macrophages and / or

Table 1: Breed, mean age and clinical diagnosis of 39 horses and 3 ponies used in this study.

Clinical diagnosis	Breed	Mean age ±SD (years)
Healthy (5)	Light draft horse (2) European warmblood (2) Icelandic pony (1)	11 ± 5
Mild CPD (11)	European warmblood (8) Standardbred (2) Crossbred (1)	10 ± 3
Moderate CPD (17)	European warmblood (13) Thoroughbred (2) Icelandic pony (1) Light draft horse (1)	11 ± 4
Severe CPD (9)	European warmblood (8) Welsh pony (1)	13 ± 3

CPD: chronic pulmonary disease

Table 2: Mean volume recovered, mean total cell count and mean percentage viability of bronchoalveolar lavage fluid obtained from healthy horses and from horses with chronic pulmonary disease.

Clinical diagnosis (No)	Mean volume instilled (ml)	*Mean volume recovered (ml) ±SEM	*Mean total cell count (x 10^5 ml) ±SEM	*Mean percentage viability ±SEM
Healthy (5)	1000	699 ± 37.95	4.15 ± 0.78	91.2 ± 2.44
Mild CPD (11)	1000	672 ± 83.13	3.26 ± 0.5	83.2 ± 3.15
Moderate CPD (17)	1000	656 ± 37.38	5.56 ± 0.90	89.11 ± 1.53
Severe CPD (9)	1000	531 ± 49.3	5.75 ± 0.78	86.56 ± 2.04

* Within parameters, means were not different ($p < 0.05$).

Table 3: Cell scores of RS obtained from clinically healthy horses and from horses with chronic pulmonary disease (CPD).

Clinical diagnosis	(No)	Neutrophils No. with High	Low	Eosinophils No. with High	Low	Mast cells No. with High	Low	Lymphocytes No. with High	Low	Epithelial cells No. with High	Low	Macrophages No. with High	Low
Healthy	(4)	0	4[a]	0	4	0	4	0	4	3	1	2	2
Mild CPD	(9)	3	6	3	6	2	7	0	9	8	1	4	5
Moderate CPD	(17)	15	2[b]	0	17	1	16	8	9	8	9	12	5
Severe CPD	(9)	9	0[b]	1	8	0	9	3	6	3	6	4	5

Within columns means with different superscripts are different ($p < 0.05$).

Table 4: Mean differential cell counts ± SEM of BAL fluid obtained from clinically healthy horses and from horses with chronic pulmonary disease (CPD).

Clinical diagnosis	(No)	Neutrophils ± SE	Eosinophils ± SE	Mast cells ± SE	Lymphocytes ± SE	Epithelial cells ± SE	Macrophages ± SE
Healthy	(5)	3.4 ± 0.9[a]	0 ± 0	4.5 ± 0.8	27.8 ± 4.8	0.1 ± 0.1	65.2 ± 5.2a
Mild CPD	(11)	4.4 ± 1.2[a]	1.4 ± 0.8	5.2 ± 1.2	29.1 ± 2.7	1.6 ± 1.0	58.3 ± 3.4a
Moderate CPD	(17)	19.3 ± 2.9[b]	0.4 ± 0.3	3.2 ± 0.6	28.6 ± 2.2	2.0 ± 1.5	46.5 ± 3.3b
Severe CPD	(9)	43.2 ± 8.6[c]	1.2 ± 1.1	1.8 ± 0.4	24.4 ± 3.9	0.3 ± 0.2	28.7 ± 4.4c

Within columns means with different superscripts are different ($p < 0.05$).

ciliated columnar epithelial cells and few other cell types. There was a significant overall association between clinical categories and occurrence of different RS cell scores for neutrophils but not for other cell types. In horses with moderate and severe CPD, high neutrophil scores occurred more frequently than in clinically normal horses. When considering

individual horses, only three of 11 horses with mild CPD had more neutrophils in RS samples than healthy horses. However, three other horses with mild CPD had more eosinophils in RS specimens than healthy horses and two other horses in this group had numerous hemosiderocytes. One horse with moderate CPD had few neutrophils but numerous hemosiderocytes. On an individual horse basis, all horses with severe CPD and all but two horses with moderate CPD had more neutrophils in RS than healthy horses.

Based on RS cytology, it was not always possible to assess the severity of the CPD, especially in mildly affected horses.

Bronchoalveolar lavage: Table 4 depicts the differential cell counts for each of the four groups of horses. On a group basis, there was no significant difference in differential cell counts of horses with mild CPD and those of healthy horses. On a group basis, horses with severe CPD had significantly more neutrophils and significantly fewer macrophages in BAL fluid than all other groups.

On an individual horse basis, all healthy horses had BAL differential cell counts which contained predominantly macrophages and few neutrophils. However, overlapping of differential cell counts among the three groups with CPD did occasionally occur so that the severity of the disease based on BAL cytology could not always be determined.

It was evident, after cytological examination of BAL specimens, that a number of horses were incorrectly diagnosed as having CPD. Two horses with mild CPD and one horse with moderate CPD had BAL cytology more consistent with a diagnosis of exercise-induced pulmonary hemorrhage (EIPH). As well, three horses with a clinical diagnosis of mild CPD had elevated eosinophil counts. Pulmonary eosinophilic infiltration may be caused by migrating helminth larvae or represent an immediate-type allergic pulmonary manifestation; however, this syndrome is poorly understood (*Dungworth*, 1985). Thus, eosinophils in BAL fluid may complicate the clinical diagnosis of CPD.

Comparison of RS and BAL cytology in individual horses revealed that there were sometimes discrepancies between the results of these two methods or between cytological and clinical respiratory examination results. Three horses with a clinical diagnosis of mild CPD had normal RS and BAL cytology. One horse with mild CPD had normal RS cytology but an increase in eosinophils in BAL cytological specimens. Another horse with mild CPD had more eosinophils than normal in RS but the BAL cytological results were normal. Three horses with moderate CPD had RS cytology compatible with their diagnoses; however, their BAL cytological results were similar to those of horses with mild CPD. One horse with moderate CPD had RS cytology similar to that of a healthy horse and BAL cytology compatible with its clinical diagnosis. One horse with severe CPD had RS cytology which agreed with its clinical diagnosis; however, the BAL cytology was similar to that of a horse with mild CPD.

Hemosiderocytes were observed in BAL fluid from one horse with mild CPD and from one horse with severe CPD but not in RS from these two horses. Hemosiderocytes were seen in RS from six horses with moderate CPD; however, only two of these horses had hemosiderocytes in BAL fluid.

DISCUSSION

Similar to other reports (*Viel*, 1983; *Dyer* et al., 1983; *Zink* and *Johnson*, 1984; *Mair* et al., 1987) no adverse effects were noted in any of the horses used in this study after BAL. Although not significantly different, the percentage of lavage fluid recovered decreased with an increase in the severity of CPD. *Viel* (1983) found a significantly lower recovery rate of BAL fluid in horses with severe chronic small airway disease. The mean total cell count and mean percent viability of BAL cells for healthy horses fell within previously reported ranges (*Viel*, 1983; *Derksen* et al., 1985, 1989; *Mair* et al., 1987). However, as *Viel* (1983) pointed out earlier, total cell counts should be interpreted cautiously since they depend on the amount of lavage fluid recovered.

The results of this study indicated that cytological examination of BAL fluid offers no advantage over that of RS samples as an aid in the clinical diagnosis of equine CPD except when RS are not present in the trachea for sampling. In addition, the clinical severity of the CPD did not always correlate with either RS or BAL cytology.

Other researchers have reported that due to the large variability in tracheal cell populations of transtracheal aspirates from normal horses, this technique has limited clinical use when evaluating horses with CPD (*Larson* and *Busch*, 1985; *Derksen* et al., 1989). Contrarily, in this study all healthy horses had RS cytology typical of normal horses (*Beech*, 1975; *Whitwell* and *Greet*, 1984; *Winder* et al., 1989). As well, it has been reported that transtracheal aspirates are not as reliable as BAL in diagnosing pulmonary hemorrhage (*Derksen* et al., 1989). In this study, cytological examination of RS more often identified horses with pulmonary hemorrhage than BAL specimens. The difference in sampling techniques may explain the discrepancies in these results since RS refers to an undiluted sample of tracheal mucus whereas transtracheal aspirates involve lavage of the caudal portion of the trachea. With regard to pulmonary hemorrhage, this usually occurs in the caudodorsal areas of lungs (*O'Callaghan* et al., 1987). It is

conceivable that in horses with evidence of pulmonary hemorrhage in RS specimens but not in BAL samples that the BAL fluid did not reach the caudodorsal lung regions. This aspect represents perhaps the greatest disadvantage of BAL since focal lung lesions may be missed (*Sweeney* et al., 1988). The fact that BAL only samples part of the lung may also explain why the clinical diagnosis obtained from cytological examination of BAL specimens sometimes disagreed with that of RS samples.

It has been observed that in horses with mild chronic small airway disease, histological lesions are invariably more severe in the caudal lung regions than in the anteroventral areas (*Winder* and *von Fellenberg*, 1987). Thus, if only the anteroventral part of the lung is lavaged, a correct clinical diagnosis may be missed.

It was evident in this study that cytological examination of either RS or BAL specimens sometimes aided in a more precise diagnosis. In several horses, results of routine pulmonary examinations were compatible with a diagnosis of CPD; however, cytological results of either RS or BAL samples revealed an increase in eosinophils in some horses and evidence of EIPH in others. Increased numbers of eosinophils may indicate parasitic infection or an immediate-type allergic pulmonary manifestation.

In this study it was noted that three horses with a clinical diagnosis of mild CPD had RS and BAL cytology similar to that of healthy horses. In cases such as these, results from the clinical respiratory examination and cytological evaluation should probably be reconsidered. A secondary clinical respiratory examination, including either BAL or RS sampling, at a later date might also be of help in determining whether or not respiratory disease is present.

The recognition of horses with mild or subclinical chronic pulmonary disease remains a challenge for the clinician. This study indicated that cytological examination of RS and BAL fluid did not always agree with the clinical diagnosis. Percutaneous lung biopsy would perhaps be a valuable tool for diagnosing difficult cases. This procedure has been shown to provide reliable pulmonary tissue which accurately reflects the absence or presence of lung pathology (*Viel*, 1983).

In human medicine, BAL is a very useful technique in the diagnosis of pulmonary disease. However, the clinician knows where the lavage fluid is going. If the same were true in equine medicine, BAL could prove to be a much more valuable tool than it is at present.

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Comparaison des résultats cytologiques de liquides de lavages broncho-alvéolaires et de sécrétions bronchiques chez les chevaux souffrants de maladies pulmonaires chroniques

39 chevaux et 3 poneys ont été répartis en 4 groupes selon le degré de leur maladie pulmonaire: sain (4 chevaux, 1 poney); légèrement atteint (11 chevaux); moyennement atteint (16 chevaux, 1 poney); gravement malade (8 chevaux, 1 poney). La cytologie des liquides de lavage broncho-alvéolaires et des sécrétions bronchiques de 39 chevaux a été étudiée et comparée. Les résultats montrent que la cytologie des deux liquides peut être utilisée pour le diagnostic des maladies pulmonaires chroniques. Le prélèvement de liquide de lavage broncho-alvéolaire n'est indiqué qu'au cas où il n'est pas possible de prélever des sécrétions bronchiques. Les résultats cytologiques ne sont pas toujours en corrélation avec le degré de gravité de la maladie.

Paragone fra i risultati citologici di lavaggi bronco-alveolari e di secreti bronchiali in cavalli con malattie polmonari croniche

39 cavalli e 3 pony furono suddivisi, sulla base dei risultati clinici dei polmoni, nei seguenti gruppi: sani (4 cavalli, 1 pony); malattia polmonare leggera cronica (11 cavalli); malattia polmonare media (16 cavalli, 1 pony); malattia polmonare forte (8 cavalli, 1 pony). Dei 39 animali furono fatti esami citologici dei lavaggi bronco-alveolari e del secreto bronchiale ed in seguito paragonati fra di loro.
I risultati mostrano che la citologia, sia dei lavaggi bronco-alveolari che dei secreti bronchiali sono adatti per la diagnosi di malattie polmonari croniche. Il lavaggio bronco-alveolare è solo indicato nel caso in cui non è possibile ottenere del secreto bronchiale. I risultati citologici non corrispondevano in tutti i casi con la gravità della malattia.

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