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DIAGNOSTIC VALUE OF RESPIRATORY TRANSTRACHEAL LAVAGE, COMPARATIVE STUDY OF HISTOPATHOLOGICAL FINDINGS AND THE CONSTITUENTS OF LAVAGE FLUIDS IN SOME RESPIRATORY AFFECTIONS IN SHEEP

(With 1 Table and 7 Figures)

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الأهمية التشخيصية لغسيل القصبة الهوائية ، دراسة مقارنة للتغيرات الهستوباثولوجية ومحتويات محلول الغسيل فى بعض الإصابات التنفسية في الخراف

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تمت هذه الدراسة على ٦٩ حالة من الخراف السليمة (١٢) والمصابة بأمراض تنفسية (٥٧) حيث تم تجميع محلول ناتج غسيل القنوات التنفسية عبر القصبة الهوائية وتدويره ثم فحص الراسب مجهريا لتحديد مكوناته وجدولت النتائج إحصائيا. تم أخذ عينات من الأجزاء المريضة للرئة وجهزت للفحص الباثولوجي. هذا وقد تم تقسيم الحيوانات المريضة إلى خمس مجموعات طبقا للتغيرات الهستوباثولوجية بالإضافة إلى المجموعة السليمة. وقد قورنت نتائج فحص محلول الغسيل مع نتائج الفحص الباثولوجي لأنسجة الرئة وذلك لدراسة مدى ملائمة تحليل وفحص محلول ناتج الغسيل لتشخيص أمراض الجهاز التنفسي وقد أوضحت الدراسة أنة بالرغم من أن ناتج الغسيل يعكس التغيرات الباثولوجية في الرئة أحيانا، إلا أنة لايمكن الاعتماد علية كطريقة تشخيصية منفردة لأمراض الجهاز التنفسى.

SUMMARY

Bronchoalveolar lavage fluids (BAL) and lung tissue specimens were obtained from 12 healthy and 57 respiratory affected sheep of both sexes and of various ages (6 months to one year). The respiratory lavage fluids were collected by transtracheal technique and direct wash through the trachea at the Slaughter-House. The lung tissue specimens were obtained after emergency slaughtering of the affected animals for histopathological examination. According to the pathological findings recorded in the lungs, the respiratory affected examined cases were classified into five groups; bronchopneumonia (14), bronchointerstitial pneumonia (11), interstitial pneumonia (16), emphysema and atelectasis (9) and granulomatous pneumonia (7), in addition to the healthy group (12). The total cell count of respiratory lavage fluid was significantly increased by different diffuse respiratory affections. Non-significant changes were recorded regarding the total cell count of granulomatous pulmonary lesions. It is concluded that sometimes, the individual samples of BAL reflect the pulmonary histopathological changes, however it could not be reliable as a diagnostic tool alone for the respiratory affections.

Key words: *Respiratory affections, histopathology, transtracheal lavage, sheep.*

INTRODUCTION

Respiratory diseases constitute one of the most commonly encountered problems in sheep population and lead to great economic losses (Snowder et al., 1990; Bekale et al., 1992 and Malone et al., 1988).

Clinically, the specific diagnosis of such disease in individual cases is often difficult to achieve. On the other hand, histopathological examination is considered to be one of the most definite aids of diagnosis (Trigo et al., 1984).

Moreover, cytopathological and parasitological examination of lavage fluids recovered from the lower respiratory tract has been found to be useful techniques for evaluation of respiratory tract diseases in different animal species. However, little information was reported regarding the examination of respiratory lavage fluids in sheep (Meniai–Belayat et al., 1990 and Berrag et al., 1997).

The present study aimed to evaluate the transtracheal method of washing the lower respiratory tract using cannula and teflon catheter, and to detect whether differential cell counts of the bronchial lavage specimens have predictive diagnostic value in assessing pulmonary pathological conditions or not.

MATERIALS and METHODS

Animals:

Sixty-nine sheep of various ages (6 months to one year) and of both sexes were used during the present study. Ten of them were apparently normally and belonging to the Department of Internal Medicine, Faculty of Veterinary Medicine, Cairo University, 16 were Veterinary Hospital patients that were euthanatized, and the remainder (43 sheep) were sampled by postmortem transbronchial lavage at Cairo Slaughter-House.

Clinical examination:

The living sheep were subjected to full clinical examination using a combination of the clinical history, clinical signs, general clinical examination and physical examination according to Kelly (1984) and Radostitis et al. (1994).

Repiratory transtracheal lavage:

Samples were collected from living sheep by transtracheal aspiration as described by Beach (1981) and Kubesy et al. (1991). Briefly, an area of skin approximately 5 cm in diameter over the lower cervical trachea was clipped. The area was surgically prepared, the subcutis was infiltrated with a local anaesthetic. A 10- gauge needle was inserted through the skin, subcutis and trachea into the tracheal lumen. A teflon catheter was passed down through the needle to the level of carina (tracheobronchial level). Phosphate buffer solution (10 ml) was rapidly injected through the catheter and immediately aspirated. For postmortem lavage, the lungs were removed, taking care to avoid contamination of the trachea with blood or water. Phosphate buffer solution was poured intraluminally while the lungs were held vertically and then recovered by inverting the lungs and collecting the lavage fluid in centrifuge tubes.

Cytological examination:

The in-vivo and in-vitro transtracheal samples were processed in the same manner. The samples were divided into two aliquots. One of which was used for counting the total cells of lavage by using haemocytometer (Mair et al., 1987). The other part of lavage was

centrifuged, and from the sediment blood films were prepared and stained with Field's stain (Bayer, 1968).

pathological examination:

After lavage, the lungs were carefully examined by palpation and serial slicing. Tissue specimens for histological examination were obtained from all detectable pathological lesions and normal adhering tissues. These specimens were processed by the paraffin embedding technique and stained by H & E stain (Bancroft and Cook, 1984).

Clinical management of animals:

Animals subjected to transtracheal lavage were kept under observation for side effect that might occur particularly during the first few days. The observation period was extended to 3 weeks post sampling.

RESULTS and DISCUSSIONS

The transtracheal technique used in this study for collecting the respiratory lavage was well tolerated by sheep. The procedure was simple and easy applicable even in field cases. Manual securing with or without local anesthesia was enough for sampling conscious standing animal. Coughing occasionally occurred during the injection of lavage solution and small jets of fluids were expelled through the nostrils. No complications were resulted from sampling in this study although Beech (1981) reported the development of subcutaneous abscessation and pneumomediastinum as a sequlae of transtracheal sampling in equines. On the other hand, Kubesy (1991) reported no complication of such technique in equine. The mean volume of the recovered respiratory lavage in this study was found to be 1.6 + 0.8 with a percentage of 16 %.

Tracing the available literature, no previous information regarding the percentage of recovered lavage fluids in sheep was observed, however, Mair et al. (1987) reported 37.7 and 22.8 as a percentage of recovered fluids in equines. Blood stained fluids were occasionally recovered during sampling and such samples have to be discarded if accurate cytological finding has to be obtained.

The transtracheal technique provided fewer total cells than lavage, but the proportion of each cell type was similar in both samples. This finding was in accordance with that of Larson and busch (1985). The total and differential cell counts of broncoalveolar lavage fluid (BAL) were recorded in Table (1), based on the histopathological findings of the lungs from which they gained. The histopathological

examination of the pulmonary tissue specimens resulted in six diagnostic classifications. The normal group showed non-significant pathological lesions and the BAL characterized by the presence of normal columnar ciliated epithelial cells with nonactivated macrophages (Fig. 1). The bronchopneumonia group in which the lung tissue exhibited prominent exudative reaction with the presence of fibrinous exudate and suppuration (Fig. 2a). BAL of such cases showed an increased number of neutrophils and colonies of bacteria (Fig. 2b). The broncho-interstitial group represented cases with severe necrotic bronchuolitis and proliferative pneumonia resulted from proliferation of pneumocytes type II, macrophages and fibroblasts (Fig.3a). Neutrophils were also increased in the lavage fluid of these cases with occasionally activated macrophages (Fig. 3b) and syncytial formation (Fig. 4). The proliferative nature of the reaction indicated primarily viral infection complicated with bacteria. Such suggestion was supported by Jubb et al. (1994). The group of interstitial pneumonia comprised cases with marked peribronchial and perivascular lymphoid hyperplasia (Fig. 5a) suggesting viral causes and other without lymphoid hyperplasia suggesting nonspecific causes for pneumonia. This group characterized by an increased number of lymphocytes and macrophages in BAL (Fig. 5b). The emphysema and atelectasis group was mainly associated with parasitic infestation (Fig. 6). Bouljihad et al. (1995) found that parasitic pneumonia of sheep were present in 53% of the lungs examined in Slaughter House and the coexisting lung lesions were emphysema, atelectasis and abscessiation. BAL fluid of these cases demonstrating an increased number of eosinophils and macrophages, although in most cases eosinophils were obscured by neutrophils and macrophages. In some cases parasitic larvae or eggs containing larvae were seen in the lavage fluid but it wasn't a diagnostic finding in all cases. Granulomatous pneumonia group was seen in seven cases. These focal lesions were varied from being pyogranuloma, parasitic granuloma or parasitic cyst. The differential cell counts of this group were highly variable but it simulated the control group to a great extent with an increase in the number of macrophages.

From the present study, we can concluded that sometimes, the individual samples of BAL reflect the pulmonary histopathological changes, however such BAL could not be reliable as a diagnostic tool alone for the respiratory affections.

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LEGENDS OF FIGURES

Fig. 1: Cytological specimen from BAL of normal cases showing sheet of normal bronchial mucosa and nonactivated macrophages. (Field's stain X1000).

Fig. 2a: Lung of sheep showing acute bronchopneumonia, notice the exudative reaction. (H&E, X33).

Fig. 2b: Cytological specimen from BAL of bronchopneumonia group showing highly activated and degenerated neutrophils, mucous and colonies of bacteria. (Field's stain, X 1000).

Fig. 3a: Lung of sheep showing necrotic bronchuolitis with proliferation of pneumocyte type II, macrophages, fibroblasts and lymphocytes. (H&E, X66).

Fig. 3b: Cytological specimen from BAL of bronchointerstitial group showing great number of activated macrophages containing engulphed materials. (Field's stain, X1000).

Fig. 4: Cytological specimen from BAL of brochointerstitial group. Notice the syncytial cell and great number of neutrophils. (Field's stain, X1000).

Fig. 5a: Lung of sheep showing lymphoid interstitial pneumonia. (H&E, X66).

Fig. 5b: Cytological specimen from BAL demonstrating predominent lymphocytes in the lavage. (Field's stain, X1000).

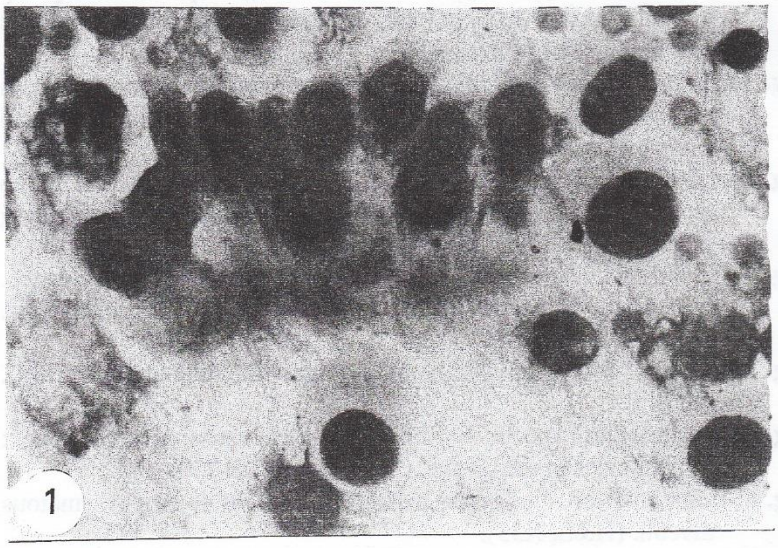
Fig. 6: Lung of sheep. Notice the parasitic larvae inside emphysematous alveoli. (H&E, X33).

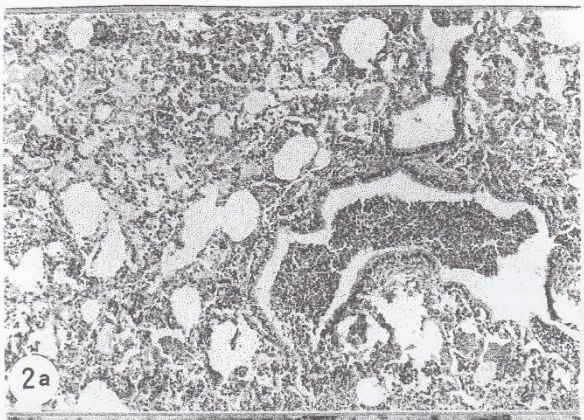
Fig. 7: Lung of sheep showing pyogranuloma. (H&E, X66).

Table 1: Total and differential cell count of transtracheal lavage fluids of normal and respiratory affected sheep

Animal group (No.) / Constituents	Normal (12)	Bronchopneumonia (14)	Bronchointerstitial Pneumonia (11)	interstitial Pneumonia (16)	Emphysema and atlectasis (9)	Granuloatous pneumonia (7)
Total Cell Count (Cells)^3 /ml	6.2±2.8	26.4±5.6*	24.3±3.4*	14.5±2.1*	16.3±1.6*	7.6±2.4*
Epithelial Cells (%)	9.3±1.6	6.4±0.8	5.2±0.7	10.1±0.8	7.2±0.7	7.8±1.4
Macrophages (%)	42.9±2.1	7.8±0.9	11.6±0.7	56.7±1.2*	48.1±0.9	63.3±1.1*
Lymphocytes (%)	8.3±1.1	6.1±1.2	9.6±0.8	12.1±1.3*	8.1±1.2	7.2±0.8
Neutrophils (%)	16.8±0.8	78.2±1.2*	72.1±0.8*	18.6±0.9	17.6±0.6	19.4±0.9
Eosinophils (%)	1.8±0.2	0.8±0.2	0.7±0.3	1.6±0.4	18.2±0.8*	1.8±0.3
Mast cells (%)	0.9±0.1	0.7±0.3	0.8±0.2	0.9±0.2	0.8±0.2	0.8±0.1

* Significant differences at $P \leq 0.05$

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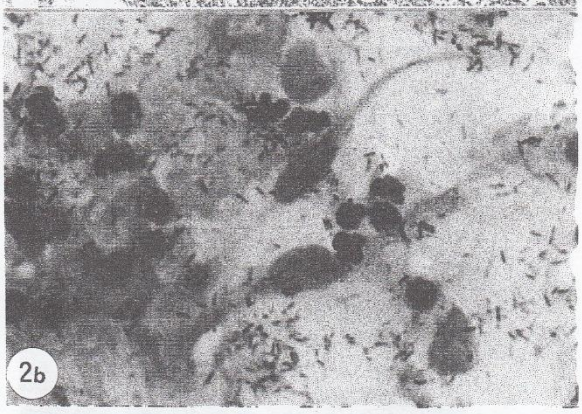
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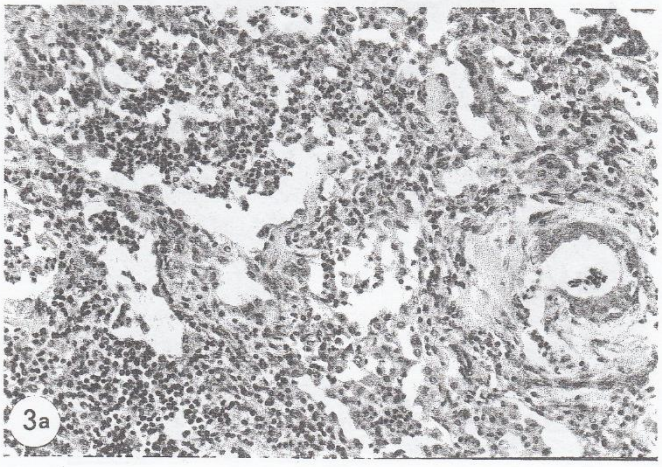
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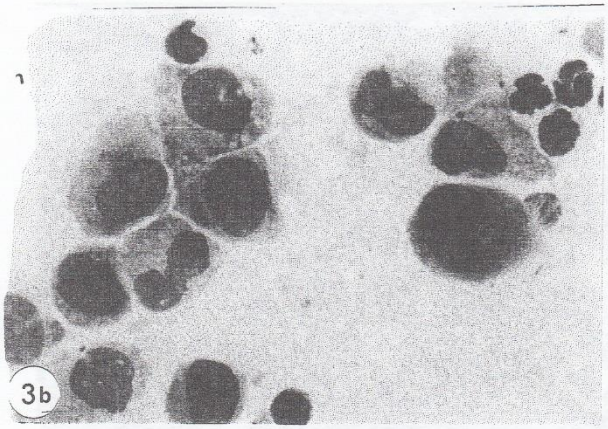

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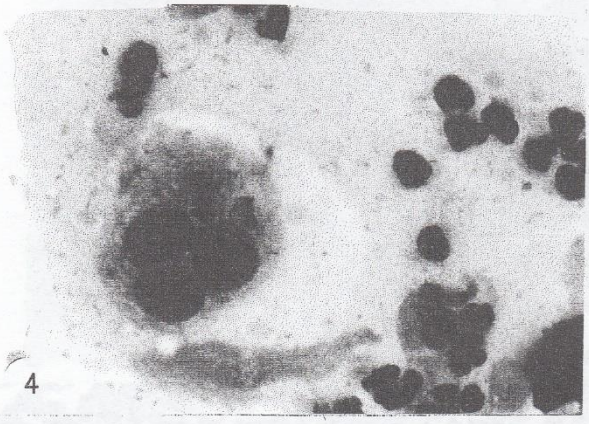

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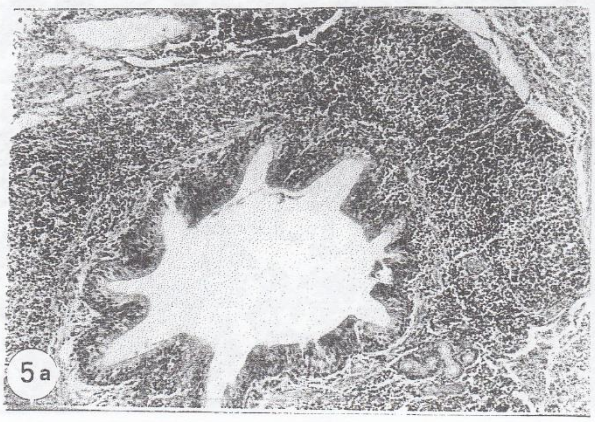

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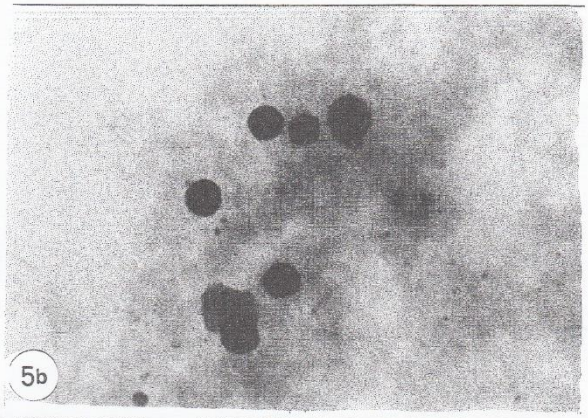

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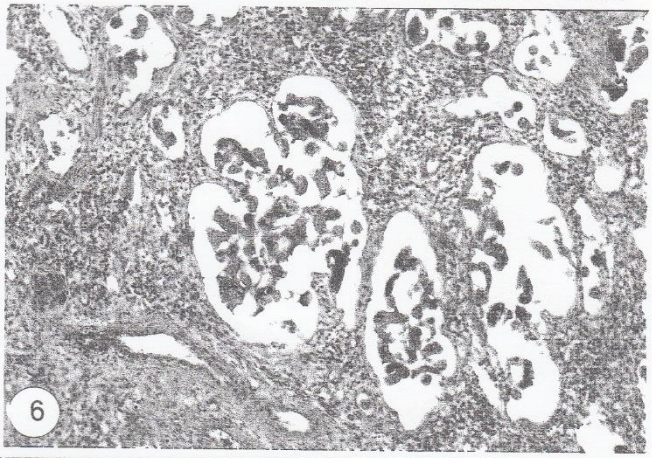

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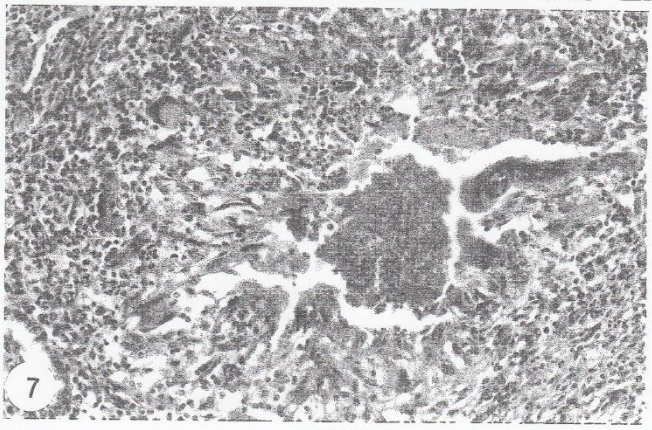

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