Cytological analysis of transtracheal washes from healthy camels in Al-diوانيya province

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Abstract

This study was conducted to quantify the cytological parameters; total protein TP, white blood cells count WBCc and differential WBCc; from transtracheal washes TTW (sometimes called transtracheal aspirate) from thirty healthy camels in Al-diوانيya province. Both sexes were included and the animals ranged in 5-10 years old. The total protein measured by the spectrophotometer ranked in 6.2-7.9 mg/dl, WBCc was 595-643/ μ l while the differential WBCc record 60% lymphocytes, 28% macrophages, 10% neutrophils, 1% eosinophils and 1% basophils. There was no obvious regard to gender or age on these parameters.

Key words: cytology, trans tracheal, total protein.

التحليل الخلوي لغسول عبر الرغامى من ابل معافاة في محافظة الديوانية

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الخلاصة

استهدفت هذه الدراسة تقييم المعايير الخلوية: البروتين الكلي، عدد خلايا الدم البيض والعد التفريقي لخلايا الدم البيض، من غسول عبر الرغامى من 30 حيوان من الابل المعافاة في الديوانية من كلا الجنسين وبأعمار تراوحت بي 5 الى 10 سنة. تراوحت نتائج تحليل المطياف الضوئي للبروتين الكلي 6.2-7.9 كما اظهر عد خلايا الدم البيض معدلا 595-643 لكل مايكروليتر فيما اظهر العد التفريقي للخلايا البيض نسبة 60% للخلايا اللمفية، 28% للخلايا البلعية، 10% للعدلات، 1% للحمضات و 1% للعدلات. لم يكن هنالك اي تأثير واضح للجنس او العمر على اي من المعايير الانفة الذكر.

الكلمات المفتاحية: التحليل الخلوي ، الغسول عبر الرغامى ، البروتين الكلي.

Introduction

The collection & evaluation of tracheobronchial secretions is useful for assessing lower airway diseases. Although detection of these secretions is a very sensitive indicator of pulmonary disease, cytological & bacteriological analysis is usually required to determine its etiology. Bacteriological evaluation of a transtracheal aspirate may provide useful information on antimicrobial sensitivity & aid in the selection of appropriate drugs (1). Cytology can be a useful diagnostic tool. Inflammation, neoplasia and specific pathogens can be differentiated with cytologic procedures. Ideally, cytology samples should be one cell layer thick to allow for adequate staining and visualization (2). In animals with pneumonia, the nasal flora may not reflect that in the lung and cultures are best taken as transtracheal aspirates of the lower respiratory system. Culture of transtracheal aspirates or (in cattle but not horses) broncho alveolar lavage fluid, is representative of organisms causing pulmonary disease. Transtracheal

aspirates from affected foals reveal neutrophilia Intracellular, Gram -positive pleomorphic rods characteristic of *Rhodococcusequi* may be present in tracheal aspirates but the sensitivity of this observation has not been determined and all tracheal aspirates should be cultured (3). Tracheal aspiration with cytologic studies and quantitative or semi quantitative aerobic and anaerobic culturing of collected samples is principle indicator in the evaluation of patients suspected of having disease of the lungs or pleura particularly if an infectious cause is likely. Tracheal wash samples collected using the percutaneous transtracheal technique are preferred for bacterial culture because these are not contaminated by oropharyngeal organisms (4).

Materials and methods

Animals: Thirty camels were inspected in Al-diوانيya morgue with adequate restraint; general examination was done according to the data sheet below to confirm their healthiness (3).

Food animal			
Physical exam data			
Patient ID: -----	date: -----	age: -----	
Weight: -----	Body condition: thin emaciated normal		
General attitude: depressed somnolent excited convulsing normal			
Lateral body shape: arched gaunt swayback normal			
Posterior body shape: apple pear papple normal			
Gait: normal lame stiff paresis paralysis other			
Hydration: normal mild moderate sever			
Skin: clean & shiny fungi dermatitis parasites			
Ears: warm cold M.M.: pink pale icteric petechiation			
Nose: clean dry moist Discharge:-----			
Mouth & tongue:-----			
L.N.:----- Heart sounds:-----			
J.V.:-----			
Respiration: Rate ----- Rhythm -----			
Quality ----- Nasal discharge -----			
Feces: ----- Urine: -----			
Mammary gland:-----			
Genitalia: -----			
Others:-----			
Clinician: -----		Student: -----	

Data Sheet

Materials:

Shaving and clipping machines, cotton and alcohol, 2% lidocaine, and 0.25–0.30 mg/kg xylazine, syringes 10, 20, 50 ml, 5mm, trocar and sterile plastic catheters, sterile normal saline, labeled slides, EDTA tubes, 99% methanol, Geimsa stains, Hemocytometer, Turck's solution, Spectrophotometer.

Methods:

Adequately restrain animal with intramuscular injection of xylazine was useful as a sedative while the local anesthetic drug was 2% lidocaine (5). The skin over the selected site (about 10 cm²) at the ventral aspect of the neck as shown in fig. (1).

Fig. (1): suitable site to aspirate the TTW in camels.

Where the trachea can be grasped and the rings easily palpated, is clipped and surgically prepared. A trocar and cannula of suitable size (5 mm) is pushed firmly between two tracheal rings perpendicular to the long axis of trachea. The trocar is withdrawn to push the cannula down the tracheal lumen & imbed the catheter distally to the thoracic inlet. A 50 ml syringe filled

with sterile normal saline to be injected and immediately aspirated carrying the respiratory secretions from the lowest point of the trachea to be stored in the EDTA tubes at 4°C (1).

Microscope slide method:

Small drop of well-mixed TTW placed on end of the slide, a clean, grease-free slide, by using of applicator stick or capillary tube. Immediately after placing TTW on the slide, a second slide "spreader" placed in front of the drop of TTW at an angle of approximately 30 degree and it pulled back until it comes to contact with the drop of TTW, and the pause until the TTW spreads along the edge of the spreader. The greater the angle the thicker and shorter TTW smear, and the smaller the angle the thinner and longer smear. Drying the film quickly by waving it in the air. Whenever possible fix and stain TTW films immediately they are prepared, otherwise fix them in absolute methanol for 3-5 minutes and then store in a clean box until they can be stained. Geimsa stain is the choice to be done by sinking the slide at 30-60 minute to be examined under oil immersion objective to see its contents.

White Blood Cells count WBCc:

Hemocytometer is used for enumeration of total leukocytes according to (7). Carefully TTW drawn to the 0.5 mark of the pipette, the diluting fluid (Turck's solution) is then drawn to the mark 11 and well mixed. Discharged onto the hemocytometer counting chamber (neubauer chamber) as

done in erythrocytes count. The total number of WBCs in four squares of larger ruled area in the corner of the counting chamber is determined and multiplied by 50. This value represents the total number of leukocytes per microliter.

Differential WBCc:

Differential leukocytes are counted by TTW film. The TTW film should be made from fresh sample as possible after collection of the TTW; otherwise, best results are obtained if EDTA is used as the anticoagulants.

Spectrophotometer

(CT Chrome Tech) was used to identify the total protein of the samples (8). The unique absorbance property of proteins could be used to estimate the level of proteins. This method is fairly accurate and the assay depends on the presence of amino acids which absorb UV light (9).

Bacteriological evaluation:

Blood agar is the best choice for the cultivation of a variety of microorganisms but mycobacterium is well identified on Lowenstein-Jensen Medium (10).

Results

The result of WBCs examination appeared all type of leukocytes in tracheal wash film in both female and male and the higher percentage was recorded in lymphocyte 60% then macrophages 28% and neutrophil 10%, but the lower level as 1% were in eosinophil and basophil respectively (table 1).

Table (1): Differential leukocytes count

Cell type	percentage	Count /cell
Lymphocytes	60%	372
Macrophages	28%	174
Neutrophils	10%	62
Eosinophil's	01%	6
Basophils	01%	6

In table (2) the total protein ranged in (7 ± 0.02) mg/dl which was (6.9 ± 0.016) mg/dl in female & (7 ± 0.024) in male (without any significant differences), WBCs was $(620 \pm 2.2)/\mu\text{l}$, while in male $(62.89 \text{ cell}/\mu\text{l})$ than in female $(613.27 \text{ cell}/\mu\text{l})$. Columnar cells and mucus were present as well as several portentous materials.

Table (2): TTW & WBCc with no obvious variation in regard to gender.

Case No.	Gender	TP mg/dl	WBCc $/\mu\text{l}$
1	F*	6.2	618
2	M**	6.6	623
3	M	7.1	638
4	M	6.9	628
5	F	7.5	601
6	M	6.9	605
7	F	7.8	609
8	M	7.1	621
9	F	6.4	618
10	M	6.6	627
11	M	6.9	614
12	F	7.9	598
13	M	6.8	619
14	M	7	614
15	M	7	632
16	M	7.9	637
17	F	6.2	595
18	M	7	613
19	F	7	627
20	M	7	632
21	M	6.8	627
22	F	7.1	605
23	M	7.2	614
24	F	6.6	631
25	M	7	643
26	F	7	624
27	M	7	617
28	F	7.1	621
29	M	6.9	622
30	M	6.8	619
Average		7	620

* : female ** : male

Fig. (2); TTW film, Giemsa stain. Macrophage. (3000X)

All the samples didn't show any microbial growth when incubated 3 days on blood agar and 4 weeks on Lowenstein-Jensen Medium which regarded as aseptic transtracheal washes and it confirm that the inspected camels not suffering from any respiratory infection. Different shapes of leukocytes were marked in the TTW film as shown in the figures (2,3 and 4) below. Ciliated columnar cells and mucus were present as well as several portentous materials.

Fig. (3): TTW film. (Right): Ciliated columnar cells. (Left): eosinophil.(3000 X)

Fig. (4): TTW film. (Right): Lymphocyte. (Left down): Basophil.(Left up): Neutrophil. (3000 X)

Discussion

The result was confirm the successful transtracheal wash mechanism that give a complete picture of pathological features of lower respiratory tract which that was agreement with (1,2) and this technique was showed in many small and large animal but no application recorded in camel. Leukocytes in tracheal wash was represent the micro environmental media of bronchial secretion as the important defense line of lower

respiratory mechanism as well as the ciliated epithelial cells which increased in case of pathological changes that accompanied different type respiratory infection (4). Our result in which the total protein ranged in (7 ± 0.02) mg/dl which was (6.9 ± 0.016) mg/dl in female & (7 ± 0.024) in male, WBCs was (620 ± 2.2) / μ l, as shown in table (2) were recorded firstly in Iraq because no previous data in this line of study in camel. The lymphocytes were the predominant leukocytes with 60% as shown in table (1). All the samples microbial growth on blood agar was negatively which that disagreement with (2&3) whom isolate Gram positive and negative bacteria which them are normal inhabitant, while (7&10) revealed the negative and positive result of microbial cultivation of tracheal wash are expectant depending on dose of aspirate wash, pH of saline used, general physical condition of animal and time of technique and others. Also the bacterial culture result was reflect the aseptic transtracheal washes & it confirm that the inspected camels don't suffering from any respiratory infection. Ciliated columnar cells & mucus were present as well as several portentous materials which is closely to (9 & 10) which them describe the bronchial and tracheal secretion have nutrient and immune nature due it contain different metabolites, vitamins, minerals, proteins, carbohydrates and secretory immunoglobulin's.

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