Cytological analysis of transtracheal washes from healthy camels in Addiwaniya province

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Abstract

This study was conducted to quantity 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 Addiwaniya 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/µl 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.

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) bronchoalveolar lavage is representative of organisms causing pulmonary disease. Transtracheal aspirates from affected foals reveal neutrophilia Intracellular, Gram -positive pleomorphic rods characteristic of *Rhodococcus equi* 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-Diwaniya 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**: ----------

Materials:

Shaving &clipping machines
Cotton & alcohol.
2% lidocaine & 0.25–0.30 mg/kg xylazine.
Syringes 10, 20, 50 ml.
5mm trocar & 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 cm^2) at the ventral aspect of the neck as shown in figure (1), where the trachea can be grasped & the rings easily palpated, is clipped and surgically prepared. A trocar & 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 & immediately aspirated carrying the respiratory secretions from the lowest point of the trachea to be stored in the EDTA tubes at $4c^o$(1).

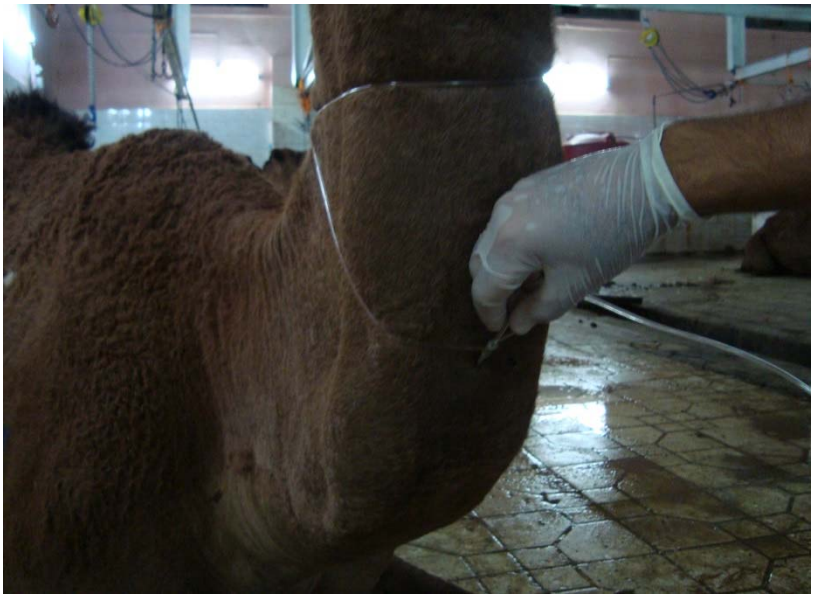

Figure (1): suitable site to aspirate the TTW in camels.

Microscope slide method:

Small drop of well-mixed TTW placed on end of the slide, a clean, grease-fresh 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 the TTW smear, and the smaller the angle the thinner and longer the 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 was used for enumeration of total leukocytes according to Meyer, D. J. & Harvey, J. W. (2004). 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 (neubaure 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 & 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 & discussion

No previous studies have the same data 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, WBCc was (625±2.2) /µl, as shown in table (2). The lymphocytes were the predominant leukocytes with60% as shown in table (1). All the samples don't appear any microbial growth when incubated 3 days on blood agar & 4 weeks on Lowenstein-Jensen Medium which regarded as aseptic transtracheal washes & it confirm that the inspected camels don't suffering from any respiratory infection.

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

Different shapes of leukocytes were marked in the TTW film as shown in the figures (2, 3 & 4) below. Ciliated columnar cells & mucus were present as well as several Proteinic materials.

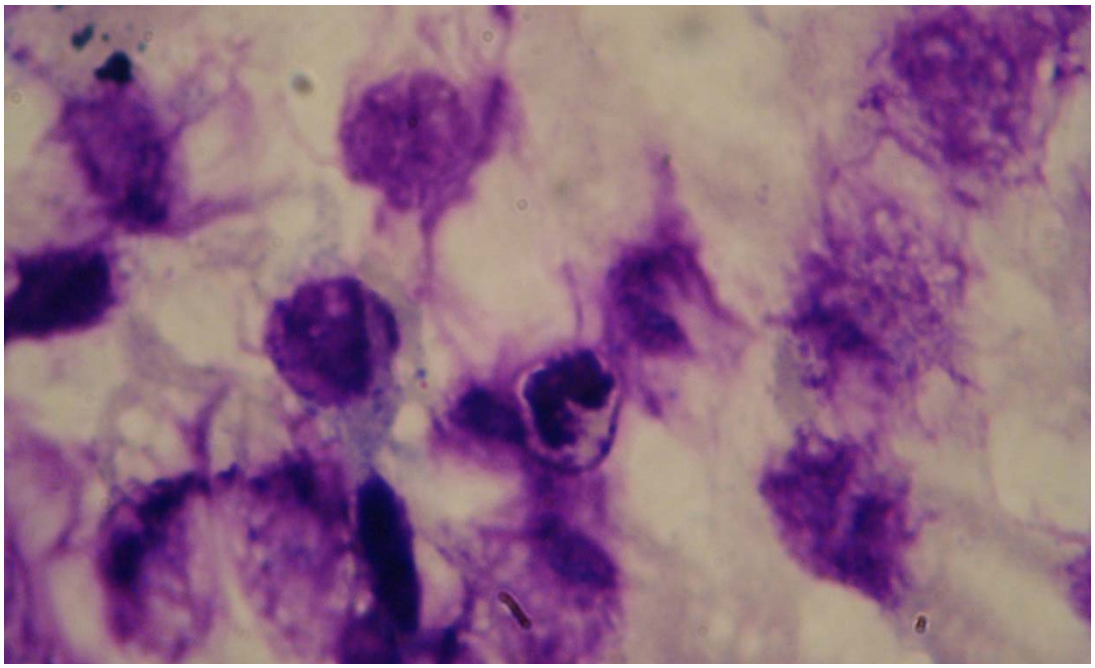

Figure (1); TTW film, Giemsa stain. Macrophage. (3000X)

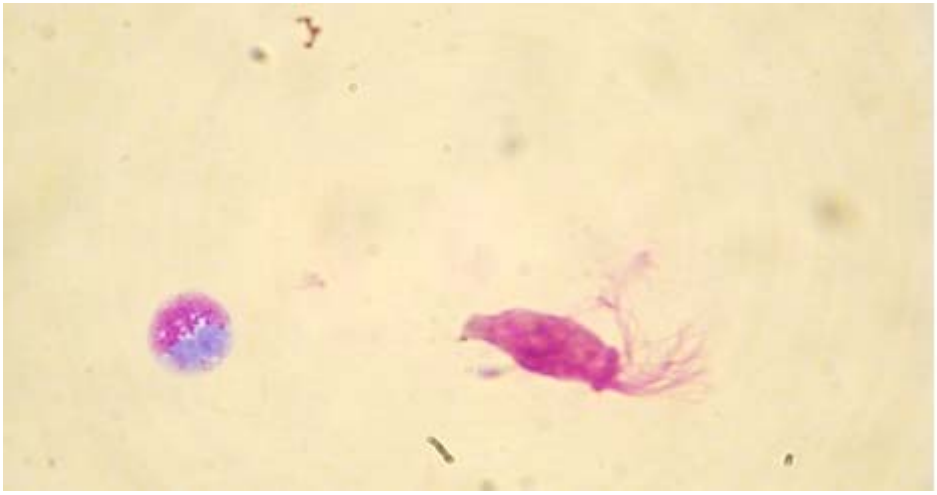

Figure (2): TTW film. (Right): Ciliated columnar cells. (Left): eosinophil. (3000 X)

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

Case No.	Gender	TP mg/dl	WBCc /µl
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±0.02	625±2.2

* : female

** : male

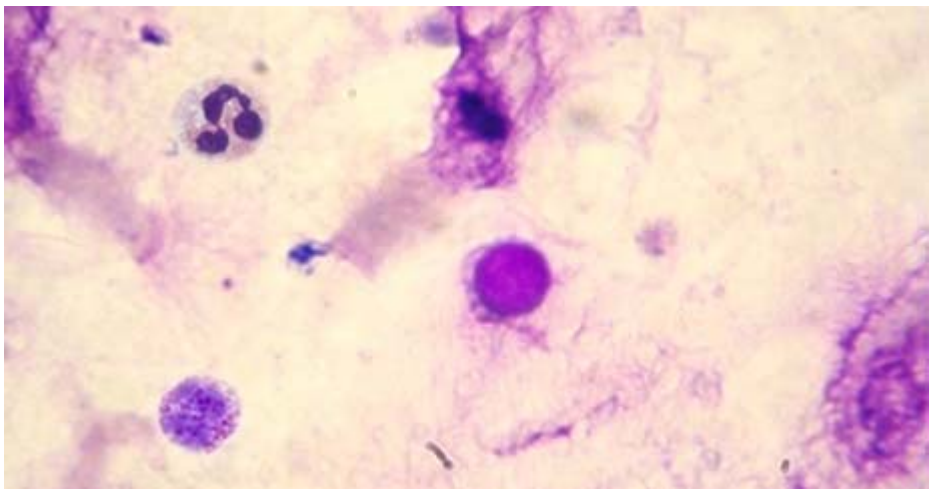

Figure (3): TTW film. (Right): Lymphocyte. (Left down): Basophil. (Left up): Neutrophil. (3000 X)

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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 ٣٠ حيوان من الابل المعافاة في الديوانية من كلا الجنسين وباعمار تراوحت بي ٥ الى ١٠ سنة. تراوحت نتائج تحليل المطياف الضوئي للبروتين الكلي ٦,٢-٧,٩ كما اظهر عد خلايا الدم البيض معدلا ٥٩٥-٦٤٣لكل مايكروليتر فيما اظهر العد التفريقي للخلايا البيض نسبة ٦٠% للخلايا اللمفية، ٢٨% للخلايا البلعمية، ١٠% للعدلات، ١% للحمضات و ١% للقعدات. لم يكن هنالك اي تاثير واضح للجنس او العمر على اي من المعايير الانفة الذكر.