

Cytological and Bacterial Analysis of Transtracheal Aspirate from Stray Cats in Al-Qadisiyah Province/ Iraq

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Abstract

This study was conducted to count the cytological parameters; total protein (TP), white blood cells count WBCs and differential (WBCs); from transtracheal aspirate (TTA) and microbial detection from thirty cats in Al-Qadisiyah province. Both genders were involved and the cats ranged in 1-3 years old. The mean total protein measured by the spectrophotometer was (89 ± 0.04) mg/dl, mean WBCs was (925 ± 0.7) cell/ μ l and the mean differential WBCs record 61% alveolar macrophages, 31% eosinophils and 8% neutrophils. Microbial investigation has revealed that respiratory tract of cats have no flora and we couldn't observe any respiratory infection in the captured cats; it may be related to the physiological and immunological adaptation of the respiratory system through the presence of the alveolar macrophage with a high percentage in regard to other WBCs. There was no obvious regard to gender or age on these limitations.

Introduction

In animals with pneumonia, the nasal flora may not reflect that in the lung and cultures are best taken as the transtracheal aspirates or the tracheobronchial secretions of the lower respiratory system. Culture of transtracheal aspirates is representative of organisms causing pulmonary disease (1).

Cats have right and left lungs, just like humans. Both sides of the lungs are further divided into sections, or lobes. Inside the lungs, the bronchi divide into smaller and smaller tubes, called 'bronchioles,' much like branches of a tree divide into smaller and smaller branches. At a microscopic level, the bronchioles end in small structures called 'alveoli'. It is here that the blood makes contact with the

individual cells in the lungs and oxygen is exchanged for carbon dioxide. Alveoli are supplied by a vast network of microscopic blood vessels known as capillaries (2).

The collection & evaluation of tracheobronchial secretions is useful for assessing lower airway diseases of the respiratory system. Although assessment of these secretions is a very sensitive indicator of pulmonary disease, cytological & microbial analysis is usually required to determine its etiology. Microbial evaluation of a transtracheal aspirate may provide useful information on antimicrobial sensitivity & aid in the selection of appropriate drugs (3).

Cytology can be a useful diagnostic tool. Inflammation, neoplasia and specific pathogens can be differentiated with cytologic procedures. Ideally, cytological samples should be one cell layer thick to allow for adequate staining and visualization.

The WBC and differential counts in cats are more variable. This may be caused in part by a higher percentage of leukocytes in the marginated pool, estimated to be 70%, than in other domestic species. Increased blood flow caused by anxiety shifts leukocytes from the marginated pool to the circulating pool, resulting in higher and more variable WBC and differential counts (physiologic leukocytosis) (4).

This study has conducted to perform the clinical technique of sampling the TTA for the First time in Iraq from stray cats captured randomly to evaluate the consistency of the respiratory secretions and to find out the cases of pneumonia; if present; with its possible causative agents.

Materials and methods

Animals

Thirty stray cats were captured with a local made hunter and inspected with a general examination according to (5) from November 2016 to March 2017.

Methods:

Cats were captured and sedated with intramuscular injection of 0.5-1.0 mg/kg xylazine. Age estimate was made according to the teeth formula and a

general examination was done according to (5) to find out any respiratory affection. The skin over the selected site (about 10 cm²) at the ventral aspect of the neck, where the trachea can be grasped & the rings easily palpated, was clipped and surgically prepared. A needle of 18* gage was inserted firmly between tracheal rings with 45 degree angle to the long axis of trachea. A 50 ml syringe filled with 20-30 ml of sterile warm 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° (6).

Microscope slide method:

Small drop of well-mixed TTA placed on the slide, a clean, grease-free slide, using of applicator stick or capillary tube. Immediately after placing TTA on the slide, a second slide "spreader" placed in front of the drop of TTA at an angle of approximately 30 degree and it pulled back until it comes to contact with the drop of TTA, and the pause until the TTA spreads along the edge of the spreader. The greater the angle the thicker and shorter the TTA smear, and the smaller the angle the thinner and longer the smear(8).

Drying the film quickly by waving it in the air. Whenever possible films immediately fixed and stained as soon as they are prepared, otherwise fixed in absolute methanol for 3-5 minutes and then stored in a clean box until they can be stained. Geimsa staining was done by sinking the slide at 30-60 minute to be examined under oil immersion objective to see its contents (8).

White Blood Cells count WBCs: Hemocytometer was used for enumeration of total leukocytes according to (9).

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

Measurement of Total Protein: Spectrophotometer (CT Chrome Tech) was used to estimate the total protein of the samples (10). The unique absorbance property of proteins could be used to count the level of proteins. This method is

fairly accurate & the assay depends on the presence of amino acids which absorb UV light (11).

Microbial evaluation: Blood agar was used for the cultivation of a variety of aerobic bacteria, but mycobacterium should be identified on Lowenstein-Jensen Medium (12).

Results

The general examination has revealed that all captured cats have no respiratory or other defect except being emaciated due to the bad nutrient circumstances. Despite the cold weather during the period of the study (Nov. 2016/ Mar.2017), we couldn't observe any respiratory infection in the captured cats; it may be related to the physiological and immunological adaptation of the respiratory system through the presence of the alveolar macrophage with a high percentage in regard to other WBCs.

The cytology of the TTA has shown that the total protein ranged in (89 ± 0.04) mg/dl, WBCs were (925 ± 0.7) cells/ μ l. The alveolar macrophage was the predominant leukocytes with 61% as shown in table (1). All the samples don't appear any bacterial growth when incubated at $37^{\circ}\text{C}/48\text{hr}$ on blood agar & $37^{\circ}\text{C}/4$ weeks on Lowenstein-Jensen medium. In the same time; the smear showed highly spread macrophages as shown in figure (1) below.

Table (1): Mean Differential leukocytes count

Cell type	percentage
Macrophages	61
Eosinophils	31
Neutrophils	8
Lymphocytes	0
Basophils	0

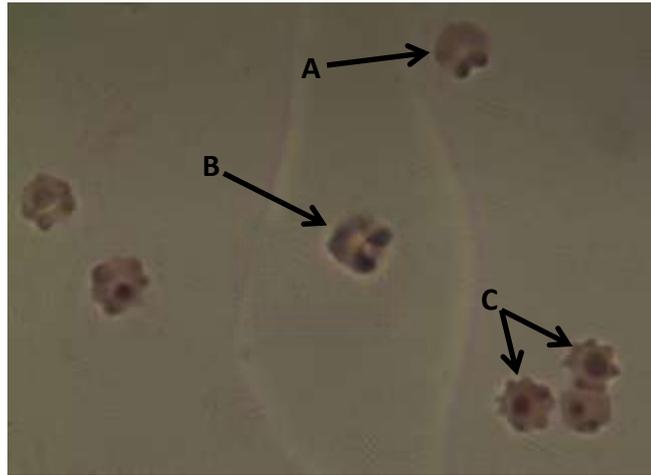


Figure (1):- Film of TTA of cats, A- Eosinophils, B- Neutrophils, C-Macrophage.
Giemsa stain. (100X)

Discussion

The study was the first in Iraq and other close countries and the results had regarded the transtracheal aspirate as aseptic as the first choice of samples in researches related to the infections of respiratory system in cats & it confirms that the inspected cats didn't suffer from any respiratory infections. Being agreed with (6); the alveolar macrophage was highly presented which may be the reason of the advanced immunity of the respiratory system, consequently; it was impossible to find a cat with pneumonia despite the way of living in stray.

Eosinophils were highly presented in the TTA, it may be related to the high presence of eosinophils in feline blood as compared with other domestic animals (13). Ciliated columnar cells & mucus were never seen as well as proteinic materials which was different from TTA from other species like camels (14).

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