Republic of Iraq
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& Scientific Research
University of Al-Qadissiya
College of Veterinary Medicine

Cytological analysis of transtracheal washes from healthy stray cats in Al-Qadisiyah province

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ
إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ١١٤

صَدَقَ اللهُ العَظِيمُ

من سورة طه

Certificate of Supervisor

I certify that the project entitled (**Cytological analysis of transtracheal washes from healthy stray cats in Al-Qadisiyah province**) was prepared by **Ali khalaf Jihad** and **Omer Saleh Mahdi** under my supervision at the College of Veterinary Medicine / University of Al-Qadissiya.

Supervisor

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Coll. Of Vet.Med./ Univ. of Al-Qadissiya.

28 / 3 / 2017

Certificate of Department

We certify that **Ali khalaf Jihad and Omer Saleh Mahdi** have finished their Graduation Project entitled (**Cytological analysis of transtracheal washes from healthy stray cats in Al-Qadisiyah province**) and candidate it for debating.

Instructor

Dr. Muthanna H. Hussain

28 / 3 / 2017

Head of Dept of Int. and Prev. Med.

Dr. Muthanna H. Hussain

28 / 3 / 2017

Dedication

To

all people …

who love us…

Summary

This study was conducted to count the cytological parameters; total protein TP, white blood cells count WBCc and differential WBCc; from transtracheal washes TTW (sometimes called transtracheal aspirate) from ten healthy stray cats in Al-Qadisiyah province. Both genders were involved and the cats ranged in 1-3 years long-standing. The total protein measured by the spectrophotometer ranked in (87±0.21) mg/dl, WBCc was (595±12)/µl while the differential WBCc record 98% alveolar macrophages and 2% neutrophils. There was no obvious regard to gender or age on these limitations.

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Chapter One…

Introduction

Cats get Pneumonia just as we humans do. It is always a serious disease for cats and can threaten their lives. Some of the most important things we need to know about cat Pneumonia;

The Disease

Pneumonia is a lung infection. This is typically a bacterial inflammation which begins with one of a number of viral infections such as; feline calicivirus and parainfluenza virus. Some infections arise from introduction of a foreign substance into the lungs, (aspiration) but viruses are a much more common cause.

Symptoms

Feline Pneumonia presents symptoms similar to those seen in humans. Cat will have difficulty breathing and become clearly very sick. We may also see a fever. Cyanosis often appears. (Tongue and gums have a bluish color) The illness is progressive, and as it worsens cat may refuse food and water. This can cause dangerous dehydration.

Diagnosis

If we see these symptoms we should get the cat to the vet quickly because early diagnosis is very important for successful treatment. The vet will take chest x-rays or ultrasounds. If cat has a build-up of fluid in the lungs, the vet may take some of it from the chest for tests using the transtracheal aspirate which will identify bacteria that is causing the infection (1).

Chapter Two…

Review of Literatures

The collection & evaluation of transtracheal aspirate 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 (2).

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 (3).

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 is representative of organisms causing pulmonary disease (4).

Chapter Three…

Materials and methods

Animals: Ten stray cats were hunted and inspected; general examination was done according to (5).

Materials:

Shaving &clipping machines
0.25–0.30 mg/kg xylazine.
sterile 18*11/2" gage needle.
Labeled slides.
99 % methanol
Hemocytometer.
Spectrophotometer.
Cotton & alcohol.
Syringes 10, 20, 50 ml.
Sterile normal saline.
EDTA tubes.
Geimsa stains.
Turck's solution.

Methods:

Cats were sedated with intramuscular injection of xylazine (6). The skin over the selected site (about 10 cm^2) at the ventral aspect of the neck, where the trachea can be grasped & the rings easily palpated, is clipped and surgically prepared. An 18*11/2" gage needle is pushed firmly between tracheal rings with 45 degree angle to the long axis of trachea. A 20 ml syringe used with 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 $4c^o$(2).

Microscope slide method:

Small drop of well-mixed TTW placed on end of the slide, a clean, grease-fresh slide, using an 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 (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 (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 (9). 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 (10).

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 (11).

Chapter Four…

Results & discussion

No previous studies have the same data in which the total protein ranged in (87±0.21) mg/dl, WBCc was (595±12)/µl. The alveolar macrophages were the predominant leukocytes with 98% as shown in figure (1). All the samples don't appear any bacterial growth when incubated 2 days on blood agar & 4 weeks on Lowenstein-Jensen Medium which regarded as aseptic transtracheal washes & it confirm that the inspected cats don't suffering from any respiratory infection.

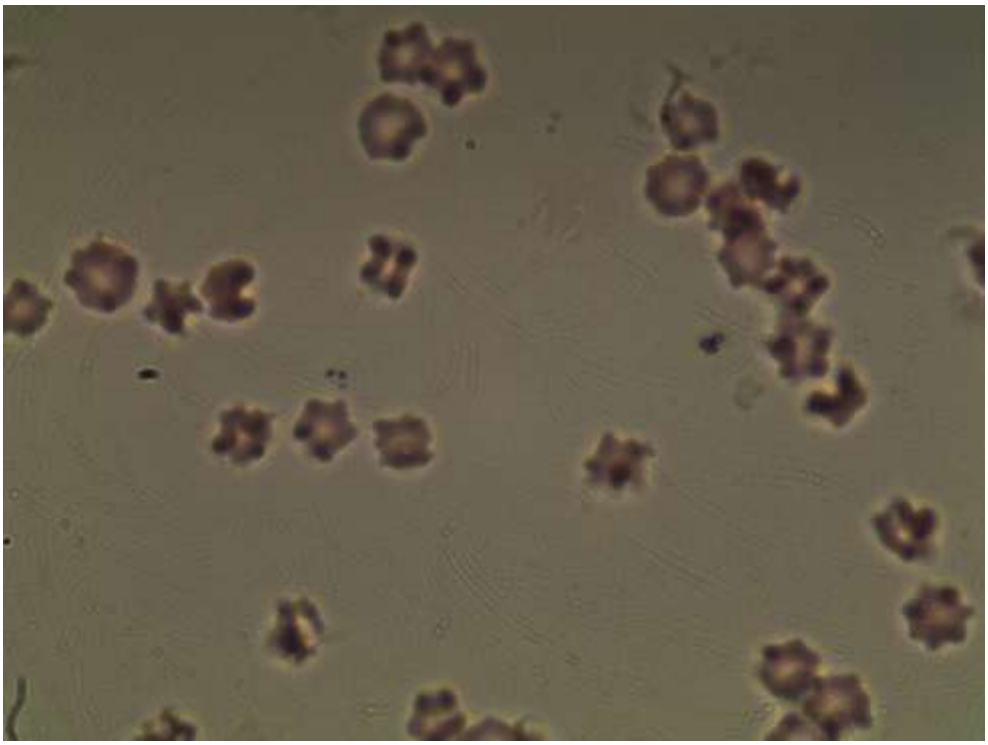

Figure (1); TTW film, Giemsa stain. alveolar macrophages. (3000X)

Conclusions

1- It is the first time to use TTW technique in stray cats in Iraq.
2- TTW could give not only cytological analysis but also a diagnostic and therapeutic guidance.
3- TTW is a precise clinical technique in the diagnosis of lower respiratory tract infections detecting the pathogens without any contaminations.
4- All inspected cases have had no respiratory affection meaning it is so obstinate to infections more than other animals.
5- Cough reflex of cats is so weak even under TTW aspiration.

Recommendations

1- TTW should be the first choice to make a diagnosis of a cats suffering from any pulmonary affection.
2- There is a need to conduct further research on bacterial affections of the lungs and also to distinguish them from those caused by other infectious agents using the TTW technique.
3- Studies of other causes; viral, parasitic, fungal or else of pneumonia should be encouraged toward therapy, prevention and vaccination.
4- Search for more fields of investigations in respiratory system in cats; anatomy, defense mechanisms, pathogenesis, etc.

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