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Isolation and Identification of some aerobic bacteria from nose of apparently healthy and clinically sick sheep with rhinitis

A Paper presented to the college of Veterinary Medicine –University of Qadisiyah and is part of veterinary medicine and surgery .

By

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بسم الله الرحمن الرحيم

(فتعلى الله الملك
الحق ولماتعجل
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انيقضي اليك وحيه
وقل رب زدني علما)

صدق الله
العلي العظيم

سورة طه :(الايه١١٤)

Certificate of supervisor

I certify that research entitled (Isolation and Identification of some aerobic bacteria from nose of apparently healthy and clinically sick sheep with rhinitis) was under my supervision at the college of veterinary medicine / university of AL-Qadisiyah.

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Abstract

The study has showed a wide variety of aerobic bacterial species inhibited and colonized the nasal passage of apparently healthy and clinically sick sheep.

A study was carried out from November 2016 to March 2017 in Different regions of AL-Diwaniyah livestock farms to isolate and identify aerobic bacteria from nasal swab specimens taken from the nasal cavity of sheep (females and males).

From a total of 60 swab samples collected from the nasal passage of 11 apparently healthy and 49 clinically sick sheep, all of them contained bacteria.

In general, a total of 97 bacteria was isolated from 60 infected specimens.

The gram stain revealed 39 (65 %) and 21 (35 %) were G +ve and G-ve respectively.

On the other hand, were the least encountered bacterial species among the isolates. The predominant species among the isolates recovered from the nasal cavity of clinically sick sheep were *Staphylococcus aureus* 46.8%.

Gram positive bacteria were dominant over Gram negative in both apparently healthy (65% Vs 35%) and clinically sick sheep (81.6% Vs 18.3%) in this study.

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1-Introduction

Respiratory diseases of various etiologies have been described in different domestic animals. However, the problem is more common in sheep due to the fact that the ratio of the alveolar surface to metabolic weight is very low in sheep compared to other species [1].

Although a single agent may be the primary invader, most respiratory infections are complicated by the presence of secondary or opportunistic invaders.

When the local resistance of respiratory mucus is lowered, bacteria growing in the nose and throat extend down wards, usually producing multiple bacterial infections[9]..

Many specific primary pathogens have been implicated sheep pneumonia. The most common bacterial causes of pneumonia in sheep include *pasteurella spp., Mannheimia haemolytica, Actinomyces pyogenes* and several *mycoplasma species*. *P. multocida* and *m.haemolytica* are important contributory pathogens in enzootic or primary pneumonia in sheep, although their pathogenic effects are enhanced when sheep are infected with viruses [8].

Besides, most of the infectious agents that cause respiratory disease are abundant in nature and are normal inhabitants of the nasopharynx of normal animals. This often creates difficulty with the interpretation of microbiological findings in outbreaks of respiratory diseases. [6].

Respiratory disorders are still serious problem facing sheep raring (Hatem *et al.,* 2003).The importance of respiratory diseases of sheep depends on their prevalence, their effect on productivity, the value of the animal and for some diseases, their international spread [2].

Therefore, the objectives of this study were to determine the type of normal bacterial inhabitants in nasopharyngeal passageways of apparently healthy and clinically sick sheep. [6].

Establish the extent of normal bacterial inhabitants of nasal cavity that serves as a standard in the diagnosis and treatment of respiratory disease out breaks and identify potential bacterial species that may be considered for future research work in etiological identification [5].

2-Literatures Review:

2.1- Anatomy:

Clinically significant upper airway structures in the small ruminant include the frontal and maxillary sinuses, pharynx, larynx, and trachea.

The nasopharynx is the primary path for respiration, but oral respirations are anatomically possible, and “panting” occurs under some fairly normal conditions such as high ambient temperature. [7].

Laryngeal structure is similar to that in other species, with small V-shaped vocal folds just caudal and ventral to the arytenoid cartilages.

The retropharyngeal lymph node is located dorso-caudal to the pharynx and can compress the larynx or trachea when enlarged or abscessed.

The trachea runs down the ventromedial aspect of the neck from the larynx to the bronchial bifurcation in the thorax. It is composed of incomplete tracheal rings connected by a membranous wall. [3].

The tracheal diameter in small ruminants generally is smaller than might be expected and changes at the thoracic inlet: In goats the trachea narrows, whereas in sheep it enlarges. [3].

2.2- The physiology:

The respiratory system permits re-oxygenation of pulmonary venous blood and release of carbon dioxide formed by cellular respiration.

Effective respiration requires both alveolar ventilation and gas diffusion across the respiratory membrane; together, these two processes can be quantified by the ventilation-perfusion ratio, which may be altered during disease.

Alveolar ventilation occurs through movement of gas from the terminal bronchioles and depends on inspiratory tidal volume and expiratory functional reserve in addition to respiratory rate. [12].

Anatomic dead space (e.g., nasal passages, pharynx, trachea, bronchi) does not contribute to alveolar ventilation. Once in the alveolus, respiratory gases must diffuse between the lung and capillaries. Gas movement across membranes is affected by the diffusion coefficient of the gas, the thickness of the septum, and the surface area available for diffusion. [15].

Because carbon dioxide diffuses much more readily than oxygen and is the direct stimulus for respiration, hypoxia may occur without significant increases in respiratory rate.

Alveolar septum thickness can be increased by edema and fibrosis. Surface area can be physically decreased by consolidation and emphysema or physiologically reduced by alteration in the ventilation-perfusion ratio stemming from increased physiologic dead space or shunting of blood away from ventilated alveoli. [14].

The causes of pneumonia in sheep of any age can be broadly described as adverse physical and physiological stresses, combined with a viral, bacterial or parasitic infection. In addition, there are many management factors associated with respiratory diseases in sheep. [2].

2.3- Diagnostic approaches:

2.3.1- Physical Examination.

A thorough and unbiased physical examination is the most important component of the diagnostic evaluation of small ruminants presented for abnormalities of the respiratory tract. [1].

Without a complete physical exam, important primary or secondary physiologic problems may be missed, and the diagnostic plan may be incomplete or result in failure to obtain a definitive diagnosis. [18].

The physical exam should be conducted in a systematic manner and must include all aspects of the respiratory system. Before restraining the

animal, the clinician should spend a few minutes observing its attitude, stance, respiratory rate, and respiratory pattern from a distance, because significant elevations in respiratory rate and pattern can occur after capture and restraint, particularly in animals that are less socialized. [17].

In consequence of the flocking instincts of sheep and goats, animals observed to be standing apart from the rest of the flock or herd are likely to be significantly ill. [4].

Once the animal is caught and restrained, the practitioner should begin by evaluating the respiratory system starting at the head. The nares should be examined for evidence of serous, mucopurulent, or hemorrhagic discharge from one or both nostrils. [13].

Unilateral nasal discharge may provide significant localization of a lesion and should be noted on the examination form. Both nares should be accessed for patency by placing either a small cotton ball or a mirror in front of the nose and observing for movement or fogging, respectively [6].

The remainder of the head should be evaluated for evidence of facial deformity or soft tissue swelling indicative of a localized lesion. The pharyngeal area should be palpated, with particular attention paid to the local lymph nodes. [18].

When possible, the palpation should include an attempt to feel the area lateral and dorsal to the pharynx by placing a hand alongside the trachea and palpating with gentle dorsal pressure. [8].

This area is a common site for retropharyngeal abscesses (often caused by *Corynebacterium pseudotuberculosis*), which may result in considerable respiratory stridor and effort.

The extrathoracic trachea should be palpated from the pharynx down to the mediastinal entrance for any evidence of stricture, dilatation, or external compression. [9].

During this portion of the evaluation, occasional gentle squeezing pressure should be applied to the trachea, to determine how easily coughing can be induced. The mediastinal opening is another area that warrants palpation for evidence of space-occupying lesions or tracheal deviation associated with such findings. [10].

2.3.2- Diagnostic procedure:

Once a complete list of diagnostic possibilities has been generated, the clinician can turn to the development of a useful and cost-effective diagnostic approach specific to the case. In this context, it is important to ascertain the expectations of the client with regard to desired outcome. [13].

Many of the usual procedures for such investigation, as described next, may not be economically feasible or desirable if the producer perceives that the cost does not justify the return on investment. By contrast, if the results can be used to prevent disease in multiple animals, the motivation to pay for the diagnostics may be increased. [5].

The clinical signs in adult sheep include acute onset depression, lethargy and inappetance. Affected sheep typically become separated from the remainder of the group. They show an increased respiratory rate with an abdominal component and a fever (>40.5°C). [11].

2.4- Treatment of respiratory system:

A good treatment response to antibiotic therapy necessitates rapid detection of sick sheep by shepherds. Oxytetracycline is the antibiotic of choice for pasteurellosis as there are few antibiotic resistant strains in sheep.

Lungworm may cause coughing and weight loss in heavy infestations but this is very uncommon. Relative to parasitic gastroenteritis, lungworm
infestation is of no economic significance to sheep farmers. [13].

In some situations, the animals found dead, the identification of predisposing factors and confirmation of the causal pathogens are key steps in designing a control programme for the successful treatment and management of respiratory disease in sheep, particularly when dealing with an outbreak. [9].

2.5- Economic losses:

Respiratory disease can lead to severe financial losses and welfare implications in sheep flocks.

Individual animals may be affected or outbreaks can occur, with losses due to mortality, reduced production – poor or delayed growth in fattening lambs with a greater feed consumption for finishing, and ill economy and poor milk production in adult ewes – and treatment costs. [8].

Because of the clinical economic importance of the disease in sheep, it was a topic of interest of many researchers in the field of small ruminant practice.

But in many instances, most studies were critically focused on the causes of the disease, the diagnostic procedures that can be performed, checklist of potential pathogens to improve diagnosis and assess the potential of therapeutic and preventative strategies, clinical diagnostic methods and treatment options and control measures. [2].

3- Materials and Methods

3.1- Materials:

3.1.1- Instruments and equipment's:

Table (3-1) Instruments and equipment's with their remarks.

	Instrument or equipment	Manufacture
1	Autoclave	Mammert/ Germany
2	Incubator	Mammert/ Germany
3	Hotplate	Heidolph/ Germany
4	Sensitive balance	Sartorius/ Germany
5	Sterilized cotton swabs	Sterile EO. / China
6	Water distillatory	LapTech/ Korea
7	Petri dish	Al-Hani company/ Lebanon
8	Test tubes	Al-Hani company/ Lebanon
9	Light Microscope	Olympus/ Japan
10	Hood	Germany

3.1.2- Culture Media:

The culture media used in this work were listed in Table (3-2). They were prepared according to the manufacturer's instructions on their containers and sterilized according to suitable method.

Table (3-2) Culture media used with their remarks.

	Medium	Manufacturer
1	Nutrient agar	Hi media/ india
2	Nutrient Broth	Hi media/ india
3	Blood base agar	Hi media/ india
4	Chromagar	CHROMagar/ france

3.1.3- Solutions and reagents:

A- Solution: Gram stain

B- Reagent: Catalase

Grams stain procedure :

1. Place slide with heat fixed smear on staining tray.
2. Gently flood smear with crystal violet and let stand for 1 minute.
3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram's iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
6. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
7. Immediately rinse with water.
8. Gently flood with safranin to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.
11. View the smear using a light-microscope under oil-immersion.

Catalase Reagent:

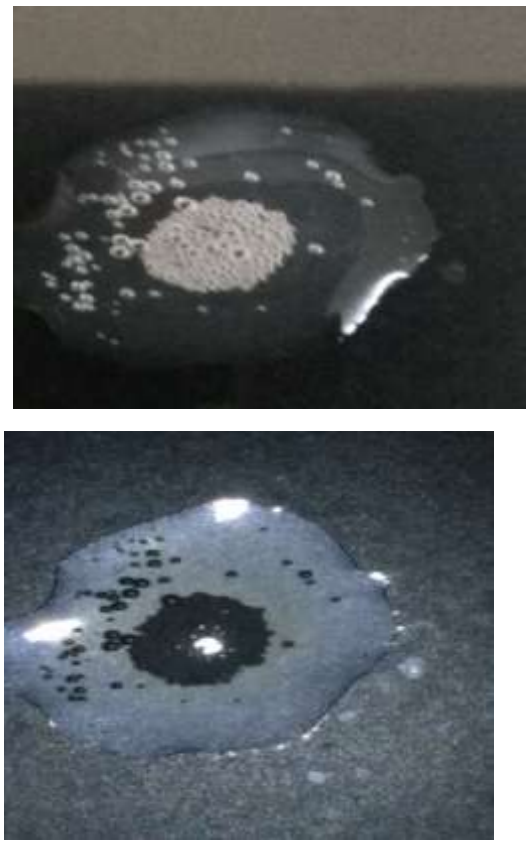


Fig (3-1) The catalase positive

Hydrogen peroxide (3%H2O2) wasused for detection the ability of bacteria to produce catalase enzyme. Fig (3-1) above.

3.2- Methods

3.2.1- Study design:

A study was carried out from November 2016 to March 2017 in Different regions of AL-Diwaniyah livestock farms to isolate and identify aerobic bacteria from nasal swab specimens taken from the nasal cavity of sheep (females and males) each week.

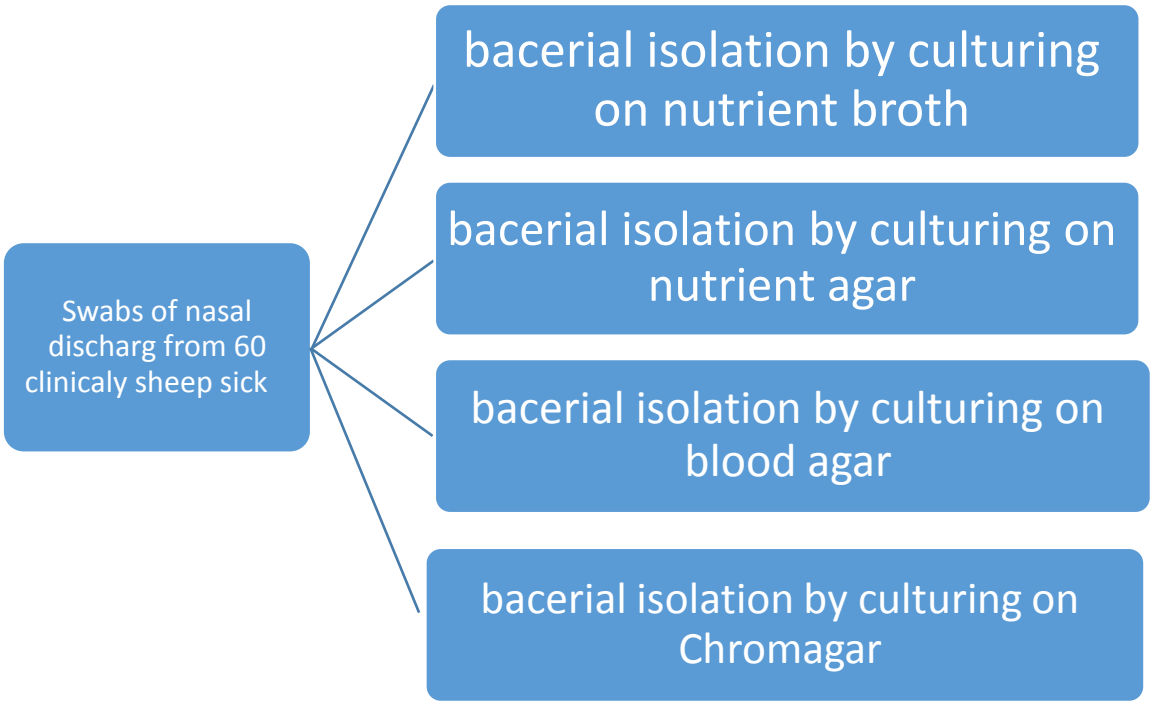


Fig (1) The isolation procedure

3.2.2-Study Animals:

The study animals were 60 local awassi sheep (both males and females), which were examined. Records concerning the exact age and pervious history of management or health status of sheep were not available; however, some of them (11) were found to be clinically normal while others (49) were clinically sick during clinical examination.

3.2.3-Sample Collection:

Nasal swabs were collected in transported swabs sterile tubes after cleaning and disinfecting the external part of the nose using 70% alcohol.

The swabs were replaced back into the tubes to which a transport medium

(3 ml of nutrient broth) was added.

The tubes containing the swab was labeled and kept in an icebox and transported to the University of AL-qadisiyah/ college of Veterinary medicine/ Microbiology Laboratory for further processing.

In the laboratory, samples were immediately incubated aerobically at 37°C for 24 hours [18].

3.2.4-Bacteriological Examination:

The cultured broth samples were thoroughly agitated and mixed. A Loop full of the cultured broth was streaked onto identified blood agar plates supplemented with 7% sheep blood and nutrient agar plates**.**

Pure cultures of a single colony type from blood and nutrient agar were transferred to addition nutrients agar media for purification.

Grams stain were done. [8].

After that, the pure culture transported and cultured on Chromagar media to for isolation and identification of some aerobic bacteria. Fig (3-2).

Fig (3-2) The Chromagar culture media

3.2.5-Data Collection and Analysis:

Colonization rates were recorded for each study animals. Descriptive statistics were used to summarize the data generated from the study.

Finally relative abundance of each bacterial species was expressed as percentage in comparison to the total number of isolates and in relation to the animals' sampled.

4-RESULTS

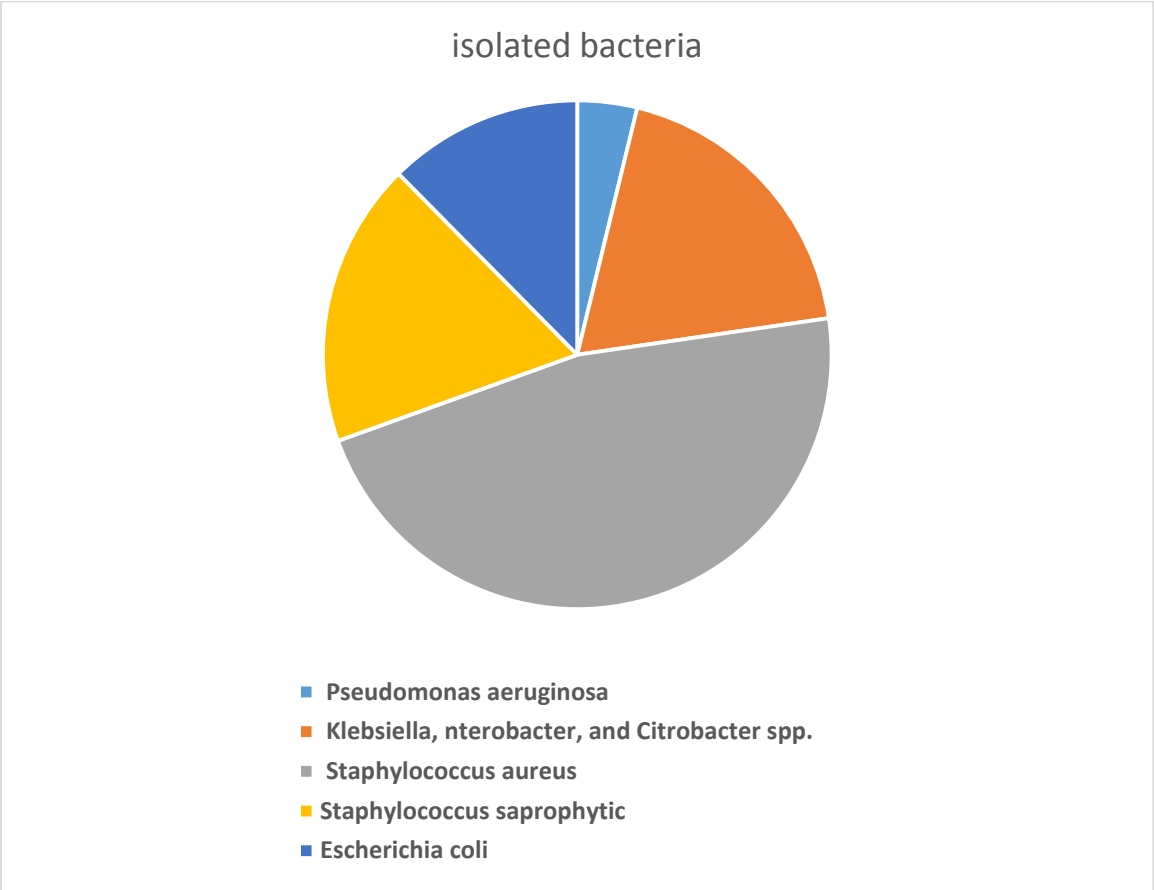
From a total of 60 swab samples collected from the nasal passage of 11 apparently healthy and 49 clinically sick sheep, all of them contained bacteria.

In general, a total of 97 bacteria was isolated from 60 infected specimens, (1.6 bacteria per infected sample).

The gram stain revealed 39 (65 %) and 21 (35 %) were G +ve and G-ve respectively.

The most frequently isolated species from animals were:

1- *Staphylococcus saprophytic* (18.1%)
2- *Escherichia coli* (12.4%)
3- *Pseudomonas aeruginosa* (3.8%)
4- *Klebsiella, Enterobacter, and Citrobacter spp.*18.9%
5- *Staphylococcus aureus* 46.8%



(Fig 1) the percentage of isolated bacteria

On the other hand, were the least encountered bacterial species among the isolates. The predominant species among the isolates recovered from the nasal cavity of clinically sick sheep were *Staphylococcus aureus* 46.8%.

Isolation rate of aerobic bacterial species form nasal cavity of apparently healthy and clinically sick sheep.Healthy (n=11) Sick (n=49)

The majority of isolates colonize the nasal cavity of the examined animals with the exception of *Staphylococcus aureus* 46.8% and *Klebsiella, Enterobacter, and Citrobacter spp.18.9%* .

Gram positive bacteria were dominant over Gram negative in both apparently healthy (65% Vs 35%) and clinically sick sheep (81.6% Vs 18.3%) in this study.

results and discussion. Also the title of the table was concerned in determining the proportion of isolated bacterial species in relation to sampled animals but the calculated figures were in relation with the No. of isolated bacteria (97).

5- DISCUSSION

The study has showed a wide variety of bacterial species inhibited and colonized the nasal passage of apparently healthy and clinically sick sheep.

Several workers isolated similar bacteria from pneumonic caprine lungs [15-13]; apparently healthy respiratory tract and nasal cavity of goats [12,7] and with fewer reports from apparently healthy sheep [18].

The invariable isolation of these organisms from the nasal cavity of apparently healthy and clinically sick sheep in this study reflects their possible role in respiratory syndrome.

The normal bacteria of healthy individual animal can be altered by several factors such as the nutritional and immunological status of the animal or the environment.

The suppression of the normal bacteria frequently allows the development of potential pathogens, leading to the presentation of a variety of pathologies [9].

The pathogenic bacteria isolate, *Staphylococcus aureus, Klebsiella, Enterobacter, and Citrobacter spp* were isolated in higher proportion from the nasal passage of clinically sick than apparently healthy sheep.

Other previous papers [11] reported high incidence rate (55.46%) from pneumonic lungs. *Mannhaemia haemolytica* which is normal flora of upper respiratory tract may play a secondary role after the primary initiating agent suppress the host defense mechanism and favors the multiplication of Pasteurella species leading to bronchopneumonia [16].

In the current study, *Pasteurella multocida* was not recovered from the nasal passage of clinically sick and apparently healthy sheep.

Pasteurella multocida frequently inhabited tonsil and nasal cavity. This is consistent with previous reports from nasal cavity of apparently healthy caprine [17].

The finding that *Staphylococcus saprophytic* was the more predominant species in healthy sheep compared to clinically sick indicating that this organism may be normally inhabits the upper respiratory tract.

This finding conforms well to the previous study similarly isolated relative proportion rate from the nasal cavity of apparently healthy sheep [12].

5- Conclusions and recommendation:

5.1-Conclusions:

1- The normal bacteria of healthy individual animal can be altered by several factors such as the nutritional and immunological status of the animal or the environment.

2- The invariable isolation of these organisms from the nasal cavity of apparently healthy and clinically sick sheep in this study reflects their possible role in respiratory syndrome.

5.2-Recommendation:

1- We are need advance researches to identify and isolate the most dangerous microorganisms from upper respiratory system of sheep and relationship with other infectious diseases in Iraq.
2- Using advanced techniques in microbial isolation and identification of bacteria.

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