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Isolation and identification of some genera and species of bacteria from infected eye in cattle

A Research

Submitted to the Council of the College of the College of Veterinary Medicine/ University of AL-Qadissiya in Partial Fulfillment of the Requirements For The Degree of Bachelors of Science in Veterinary Medicine .

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بسم الله الرحمن الرحيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا)

صدق الله العلي العظيم

سورة طه/ الآية (١١٤)

Certificate of Supervisor

I certify that the research entitled **(**Isolation and identification of some genera and species of bacteria from infected eyes in cattle **)** was prepared under my Supervision at the college of Veterinary Medicine/ University of AL-Qadissiya**.**

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27 / 3 / 2017

Certificate of Department

We head of Dept. of Int. & prev. Med. Certify that (**Ali Ahmad Abd Amer and Hussein Hamed Obaid**) is adequate for the debate of Graduation Project of Bachelor Degree in Science in Veterinary Medicine

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Dedication …

To the function of patience and optimism and hope

To each of the following in the presence of God and his Messenger , my mother dear

To the big heart my dear father

To those who have demonstrated to me what is the most beautiful of my brother's life

To the people who paved our way of science and knowledge

All our teachers Distinguished

To the taste of the most beautiful moments with my friends

I guide this research

Ali & Hussein

Abstract

This study was designed to identify **some** species of bacteria that infect eyes in cattle , as the studies on this subject are few somewhat . This study included examination of (30) eye swabs , from cows from different ages and regions in diwaniyah city. This study extended from 1/11/2016 – 1/3/ 2017.

The samples were collected from infected eyes of animals, (5) bacterial species were isolated they included , *E. coli* 19(63.3%) , , *Proteus Spp* 11(36.6%) {*P. mirabilis* 4 (13.3%) & 7(23.3%) *p.Vulgaris* } *Klebsiella pneumonia* 10 (33.3%) , *Staphylococcus aureus* 8(26.6%) and *Enterobacter spp* 6 (20%) .

This means the highest isolation rate was *E. coli* , lowest percentage was *Enterobacter spp*

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Introduction

, especially ruminants The eye diseases in large farm animals including cattle, buffalo, sheep, goats, recorded a high rate in recent years as a result of lack of attention to these conditions and for the role played by the factories and its waste as a source of exciting and scarification of the eyes (1)

Bacterial eye infection is a common problem which has been reported . The most obvious clinical features of bacterial eye infections world wide , keratoconjunctivitis, canaliculitis, cellulitis include conjunctivitis , keratitis and endophthalmitis (2)

The most common bacterial agents in ocular infections include ***Staphylococcus spp***, ***Streptococcus spp***, ***Corynebacterium spp*** , ***Moraxella spp, Pseudomonas spp, Haemophilus spp, Neisseria spp*** and enteric bacteria (3)

Inflammation of the eye Cause excitement and temporary blindness and permanent blindness and then effect on the length of grazing, which in turn effects on the growth and weight gain in developing animals and weight loss in adult animals and the occurrence of the loss after showing weakness and wasting (4)

Aim of these study :

- Isolate and diagnose some species of bacteria from infected eyes of

cows

٢ .1 The eye:

٢ .1.1Anatomy of the eye:

The eye lies in the front half of the orbit surrounded by fat and connective tissue and it is supported by a fascial hammock (5.)

The eyes are set indeep cavities known as the bony orbits , which edges are prominent and form protection to the eye ball. In the dogs and cats the edges of bonyorbits are not complete posterialy, but in the other domestic animals it forms a complete circle lying inside the orbit (6).

The eyeball comprises three layers. From the outside to inside, these tissues are the sclera, choroid and retina. The anterior portion of the sclera is the cornea, which is transparent and has no blood vessels. A mucous membrane called conjunctiva lines each eyelid and extends onto the surface of the eye itself.

The large interior space of the eyeball is divided into two sections the anterior and posterior cavities ,The anterior cavity is filled with watery substance called aqueous humor; the posterior cavity is filled with a soft gelatin – like substance called vitreous humor.

The major components of eye lacrimal apparatus include the lacrimal gland, lacrimal canaliculi and lacrimal sac. (3)The cornea which is largely responsible for the refraction of light entering the eye is in contact posteriorly with aqueous humor. The retina is the light sensitive membrane at the back of the eye. The outer surface of the retina is in contact with the choroids and its inner surface is in contact with the vitreous body. (7)

2.1.2 **Defense mechanisms of the eye**:

The eye has a number of defense mechanisms. The eye lids are composed of an outer layer of skin , a middle layer of muscles and inner layer of moist conjunctiva tissues so they protect the eye from the environment, injury and light (8.).

The eyelids protect the eye and keep the cornea healthy and moist. Each time a person blinks, the tear is spread across the surface of the cornea. (9)

The nictitating membrane or third eye lid, found in the medial can thus of domestic animals aids in protection of cornea, and its gland produces

tears. It is larger and more mobile in domestic birds than in domestic mammals. The frequency of blinking varies among domestic animals. Cats tend to blink the least. In domestic mammals the upper eyelid is most movable; in domestic birds the lower lid is more movable.

Normal blinking maintains the tear film over the corneal surface, help toremove debris, and assists in drainage of tears into the lacrimal apparatus(10), the eye lashes prevent entry of foreign materials in to the eye. (3)

2.1.3 The tears:

It is the important defense mechanism of the eye , the constant secretion of the lacrimal fluid flowing from the upper eyelid to the inner canthus and then to the nasal cavity. Lacrimal secretions also contain lysozyme which is capable to lyse certain bacterial species (11)

The tear film has antibacterial properties function through the action of lysozyme, lactoferrin and the immunoglobulins, mainly secretary lgA . Tears film provides oxygen interiorly to the avascular cornea , as well as removes

debris and foreign particles from the ocularsurface through the flow of tears(12)

2.1.4 **Eye Infections**:

Because of their location, externalocular structures such as conjunctiva and cornea are frequently challenged by a variety of microorganisms (12)

Pathogenic microorganisms of numerous types can invade the eye from the external surface, the adjacent orbital tissue or via blood stream. Primary infection by blood-borne bacteria is important, but more so are those resulting from secondary complications of accidental or surgical trauma. The eye is particularly vulnerable because the lens and vitreous are avascular and protein-rich structures; thus ideal media for the proliferation of many pathogenic bacteria (13)

2.2 Some microorganisms Associated with eye infections:

Staphylococcus aureus is known as anaerobic , Gram-positive cocci and commonly causes Staph infections , Staphylococci often represent part of normal bacterial flora of the skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tracts of mammals and birds. Staphylococcus aureus is one of the most prevalent and clinically significant pathogens worldwide. It causes a variety of illnesses ranging from superficial skin eruptions to life-threatening infections with bacteremia, endocarditis , pneumonia and toxic shock syndrome (14). Since, methicillin-resistant Staphylococcus aureus (MRSA)was first identified in 1961, it has become

the most common cause of nosocomial and community infections worldwide (15)

Generally, the coagulase-positive Staphylococci are known as *Staphylococcus aureus* and have been regarded as opportunistic pathogens. Furthermore, some coagulase positive and negative Staphylococci strains are erroneously identified as Micrococci and are generally regarded as non-pathogens (16)

Staphylococci are easily spread between animals and under certain conditions to humans through contact with excretions such as saliva or aerosols released during sneezing and coughing. Moreover, Staphylococci spread by animal products such as non-pasteurized milk (17)

The characteristics used to identify Staphylococcus aureus include Gram stain morphology, cell morphology, production of catalase, coagulase production, pigment production, susceptibility to lysostaphin and lysozyme, and production of acid from glucose (17)

Staphylococci produce heat-resistant toxins that act at the digestive level. There are seven kinds of enterotoxins , A, B, C1, C2, C3, D, and E. Toxins A and D are more frequently present in food intoxication. ***Staphylococcus aureus*** may cause skin infection (acne). Staphylococci could be present in, e.g., dairy, meat, sausages, fish, and eggs (18)

K. pneumoniae is a gram negative bacterium. It is facultative anaerobic. It is rod-shaped and measures 2 μm by 0.5 μm.) (19)

Klebsiella spp. grow readily on ordinary media commonly used to isolate Enterobacteriaceae ,e.g., Nutrient agar, Tryptic casein soy agar, bromocresol purple lactose agar, blood agar, as well as more differential plating media for Enterobacteriaceae, such as Drigalski agar, MacConkey agar, eosin-methylene blue agar (EMB), and bromo-thymol blue agar (BTB). *Klebsiella pneumoniae* and *K. oxytoca* colonies are lactose positive, more or less dome-shaped, 3–4 mm in diameter after overnight incubation at 30°C or 37°C, with a mucoid aspect and sometimes stickiness, depending on the strain and the composition of the medium.

K. pneumoniae grow more slowly on the same media, yielding voluminous, rounded, very mucoid , translucent and confluent colonies in 48 h at 30°C or 37°C (20).

The reason for its pathogenicity is the thick capsule layer surrounding the bacterium. It is 160 nm thick of fine fibers that protrudes out from the outer membrane at right angles (21).

Proteus *spp* is a genus of Gram-negative Proteobacteria. *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections. (22)

P. mirabilis causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins. *P. vulgaris* is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immunosuppressed individuals. *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of

animals, and in manure, soil, and polluted waters. *P. mirabilis*, once attached to the urinary tract, infects the kidney more commonly than *E. coli*. *P. mirabilis* is often found as a free-living organism in soil and water. (23)

Proteus species do not usually ferment lactose, but have shown to be capable lactose fermenters depending on the species in a triple sugar iron (TSI) test. Since it belongs to the family Enterobacteriaceae, general characters are applied on this genus. It is oxidase-negative but catalase- and nitrate-positive. Specific tests include positive urease (which is the fundamental test differentiate *Proteus* from Salmonella)and phenylalanine deaminase tests. (*23)*

Enterobacter **spp**. are in the family Enterobacteriaceae (24) *Enterobacter* spp. are facultatively anaerobic Gram-negative bacilli, 0.6-1 µm in diameter and 1.2-3 µm long, motile by means of peritrichous flagella and have class 1 fimbriae and grow rapidly on the usual enteric media , In general, the strains from environmental sources grow better at 20-30 degrees C, whereas strains from clinical sources grow better at 37 degrees C. Colonial morphology differs greatly among *Enterobacter* spp. ranging from smooth, irregularly round to rough "cauliflower" type colonies. Anaerogenic strains often exhibit yellow pigmented colonies. (25)

, are methyl red negative, They produce acid upon glucose fermentation and Voges-Proskauer positive, with an optimal growth temperature of 30 °C), 80 % are encapsulated (24

The *Escherichia coli* genus belongs to the enterobacteracea family and it is usually a sign of fecal contamination. *E. coli* is a bacillus with the following

characteristics : Gram negative, mobile or nonmobile , oxidase negative , catalase positive, and glucose and lactose fermentation positive. E. coli is found in the human and animals intestine , some strains are enterohemorrhagic pathogens. (26)

Chapter three……..…….…… Materials and Methods

3. Materials and Methods

3. 1. Materials

3.1.1. Equipments and Instruments

Table (3-1): The equipment and instruments used in this study with their companies and countries of origin:

No.	Equipment & instrument	Company
1	Autoclave	Mammert/Germany
2	High Speed Cold centrifuge	Eppendorf /Germany
3	Incubator	Mammert/Germany
4	Oven	Mammert/Germany
5	Light Microscope	Olympus/Japan
6	Sensitive Balance	Sartorius/Germany
7	Refrigerator	Concord /Lebanon
8	Disposable Petri dishes	Al-Hani company / Lebenon
9	Sterilized cotton swabs	Sterile EO. / China

10	Disposable syringe 10 ml, 5ml and 3ml	Sterile EO. / China

3.1.2. Cultural Media

Table (3-2): Represented cultural media that used in this study with their companies and countries of origin:

No.	Cultural media	Company / origin
1	Nutrient broth	Hi media / India
2	MacConk"y agar	Hi media / India
3	Eosin Methylene Blue Agar	Hi media / India
4	Orientation CHROMagar	Hi media / India
5	Nutrient agar	Hi media / India
6	Salmonella shigella agar	Hi media / India
7	Blood base agar	Hi media / India
8	Urea base agar	Hi media / India

9	Manitol salt agar	Hi media / India

All these cultural media were prepared as described by the provided company and sterilized by autoclaving at 121c for 15 minutes and 15 pound cm3 pressures .except OrintationCHROM agar & S.S agar prepared as described by the provided company and sterilized by heating on heater in (100 C°)

3-1-3 Solutions , Reagents and stain :

A-Urea solution

B- Reagents

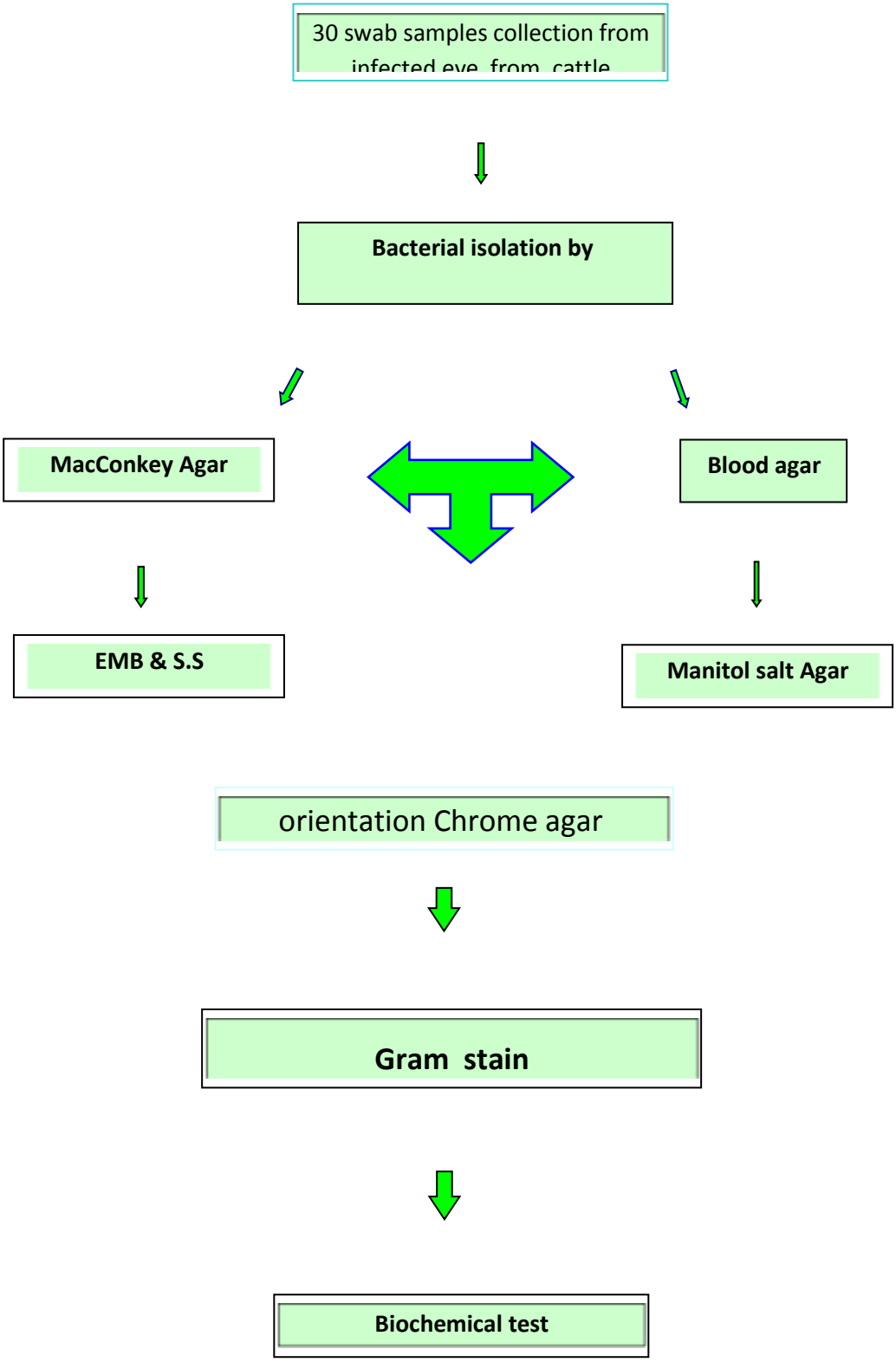
1- Catalase Reagent: Hydrogen peroxide (3% H2O2) was used for detection the ability of bacteria to produce catalase enzyme (Cloeckaert, A. and Chaslus -Dancla ,E. (2001). Mechanisms of quinolone resistance in Salmonella .Vet. Res., 32:291300.).

2- Kava's Reagent

C-stain : Gram Stain (27) **.**

3.2. Methods

3.2.1. Study design



3.2.2. Sampling and isolation of bacteria

1-Animal and Sample Collection:

Thirty swab Were collected from the eyes of infected cattle and of all ages for a period of (1/11/2016- 1/3/2017)

samples were taken by entering a sterile cotton swab in to the conjunctival sac and then transferred to a sterile test tubes contain sterile nutrient broth volume of 5 ml , and then the samples transferred to bacteriological Laboratory of the Veterinary Medicine collage of Qadissiya University.

2- Isolation of bacteria :

The tubes were incubated aerobically in the incubator at a temperature 37 ° C for 24 hours , then was culturing these samples on each of the blood agar containing 5% blood of sheep and MacConkey agar and incubated aerobically at a temperature 37 ° C for 24 hours, after that it was studying the form of developing colonies then culture on selective media (EMB,S.S agar, OrintationCHROM agar & Manitol salt agar) and incubated aerobically at a temperature 37 ° C for 24 hours, after that it was studying the form of developing colonies , and germs interaction that taken from it to Gram stain ,

Pure colonies saved on nutrient agar for the purpose of conducting biochemical tests and know the different types of it. (28)

3.2.3. Biochemical Tests:

1- Indole Test:

Peptone water medium was inoculated with new culture colonies and at incubated at 37C˚. for 24 hrs. , then few drops from Kovacs reagent were added A red color in the alcohol layer indicated a positive reaction (29).

2- Simmons Citrate Utilization Test:

Medium was inoculated by streaking from saline suspension of the organism to be tested and Incubate for 24 - 48 hrs. at 37C Positive result was indicated by blue color and streak of growth while negative result was indicated by its green color with no growth (29).

3- Urease Test:

The test was done by inoculation of urea agar medium with new culture colonies and incubated aerobically at 37C˚ for 24 hrs. A positive result was recorded by changing the color of the media from yellow to pink due to the ability of an organism to split urea , forming two molecules of ammonia by the action of the urease enzyme (30).

4- Catalase Test:

A small amount of the bacterial growth was obtained and suspended in a drop of hydrogen peroxide 3% on a glass slide, and observed for evolution of bubbles as a positive result (30)

4. Results and Discussion

4.1. Agricultural traits

All the *E. coli* isolates were able to produce bright pink colonies on MacConkey agar fig4-1. these result agree with (30), characteristic metallic sheen colonies on the EMB agar fig 4-2. these result agree with all (31)

While the colony of Enterobacter rounded and lactose fermentation While on orientation CHROMagar the colonies characteristic in rounded & blue fig 4-3. these result agree with (32) Showed the result of culture *Proteus spp* colony appear on MacConkey agar rounded & pale , while in the blood agar & nutrient agar swarming Phenomenon appeared& produce h2s on S.S agar . fig 4-4 , fig 4-5 (33) *Klebsiella pneumonia* colonies appear as pink , rounded , mucus textures on macConkey agar (fig 4-5) while appear Blue , mucous , rounded colonies on orientation CHROMagar agree with(32)

Staphylococcis aureus, B hemolysis colonies show on blood agar & rounded Colden colonies appear on manitol salt agar(34)

Figure (4-1): Characterization of E. coli colonies on MacConkey agar (bright pink colonies)

Figure (4-2): Characterization of E. coli colonies on EMB agar(metallic sheen)

Figure (4-3): Characterization of *Enterobacter spp* colonies on Orientation CHROMagar (rounded & blue)

Figure (4-4) : swarming Phenomenon for *proteus spp* on blood agar

Figure (4-5) : produce H2S form *proteus spp* on salmonell.shigella agar

4.2 Results of Biochemical Tests :

The identification of bacterial spp. Was confirmed based on their biochemical reaction that are Shawn in tables (4-1) . the result of biochemical test agree with(35)

Table 4-1 Differentiating characters of bacteria by biochemical test

+ = positive reaction - = negative reaction v= variable reactions

Chapter four …….. Results &Discussion

4.3. Bacterial isolation ratios : Out of 30 samples 19 were found positive for *E coli* isolates the prevalence of *E coli* from eye samples was (63.3%) while (36) recorded only (24.8%) (37) recorded only (8.57%) for this reason I don't agree with him.

Staphylococcus aureus 8(26.6%) The percentage of isolation for approach to (37,38,) 23% & 17.94% respectively and

Klebsiella pneumonia and *Enterobacter* The percentage of isolation for *spp* (33.3% - 20%) respectively While studying of (38) about the isolation of *Klebsiella pneumonia* and *Enterobacter* from *Conjunctivitis* was (2.2% - 1.3%)respectively, Regarding *Proteus Spp in the current study the percentage was* 11(36.6%) { while (36) recorded 3.3 % and (37) recoded 7.69% 0nly.

5.1 CONCLUSION:-

- ✓ From the present study we can conclude that Gram-Negative bacteria were the most common causative agents of eye infections .
- ✓ Staphylococcus was found to be the important bacteria in eye infections.

5.2 RECOMMENDATIONS:

- ✓ Further studies must be done on Chlamedial , Mycoplasmal , viral and fungal eye infections.

- ✓ In animals further studies should be done on normal flora of the eye and the area of the skin around the eye

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خلاصة

صممت هذه الدراسة لتحديد بعض أنواع البكتيريا التي تصيب عيون الماشية ، والدراسات حول هذا الموضوع هي شيء قليل وشملت هذه الدراسة فحص (٣٠) مسحة عينيه من الأبقار بمختلف الاعمار والمناطق في الديوانية ،امتدت الدراسة للفترة من ٢٠١٦/١١/١ -٣/١/ ٢٠١٧ جمعت العينات من عيون الحيوانات المصابة ، تم عزل (٥) اجناس من البكتريا وتضمنت الأشريكية القولونية١٩(٦٣.٣%) ، المتقلبات ١١(٣٦.٣%) (متقلبات ميرابيليس ٤ (١٣.٣٪) ومتقلبات فولكارس ٧(٢٣.٣%) ، الكليبسيلا الرئويه ١٠(٣٣.٣%) ، المكورات العنقودية ٨ (٢٦.٦%) والبكتيرية المعوية ٦(٢٠ %) وهذا يعني ان اعلى نسبة للعزل الأشريكية القولنية واقل نسبة البكتيرية المعوية

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة القادسية

كلية الطب البيطري

عزل وتشخيص بعض الاجناس والانواع البكتيرية من العيون المصابة في الابقار

رسالة مقدمة الى مجلس كلية الطب البيطري في جامعة القادسية وهي جزء من متطلبات نيل درجة البكالوريوس في الطب والجراحة البيطرية

من قبل

علي احمد عبد عامر

حسين حميد عبيد

اشراف

م. جنان ناظم صادق

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