

# Isolation and identification of some genera and species of bacteria and fungi from conjunctiva in cattle in Al-Diwaniya city

H. H. H. Handool

Coll. of Vet. Med./Univ. of Al- Qadisiya

e-mail : hamshussainhashim@Gmail.com

## Abstract

This study was designed to identify some genera and species of bacteria and fungi that infect conjunctiva in cattle, as the studies on this subject are few somewhat, especially those that include isolating fungi that infect the conjunctiva as rare cases and that may be caused by low body immunity after topical corticosteroid and antibacterial therapy to an inflamed eye, This study included examination of (100) eye swabs, from cows from different ages and regions in diwaniya city. This study extended from 1/5/2012 – 27/11/ 2012. The samples were collected from clinically healthy and infected eyes of animals. (7) bacterial species were isolated they included *Staphylococcus spp.* 26.4% , *E. coli* 24.8%, *Staphylococcus aureus* 19% , *Moraxella bovis* 9.9% , *Streptococcus spp.* 8.3%, , *Pseudomonas spp.* 8.3%, *Proteus spp.* 3.3%. The isolated bacteria were 121, Gram positive bacteria were 63 (52.1%) while Gram negative bacteria 58 (47.9%). Many types of bacteria isolated from healthy swabs 75 (62%) and 46 (38.1%) from infected swabs. The study also included isolation and identification of some species of fungi associated with the mycotic conjunctivitis in cattle, the observed study *Aspergillus niger* was the most frequent species isolated and was accounted to 37.5% of all isolates, while other fungi included *Aspergillus fumigates* 25% , *Alternaria spp.* 25% and *Penicillium spp.* 12.5%.

## Introduction

The eye diseases in large farm animals, especially ruminants 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 (1.2). The inflammatory eye contagious disease is one of the common cases occurrence in field animals and happen randomly, and the highest incidence rate is in small animals, as well as the possibility of the occurrence of disease and severely in adult animals, especially in the event of a disease for the first time in the herd, it is clear that this disease has immunity linked with age as a result of previous exposure to infection, and a lot of animals carrying the disease that bacteria are found in the nasal lacrimal duct and in

the pharynx (3). Eye diseases include conjunctivitis, keratitis and congestion cornea and abscesses under the conjunctiva (4). inflammation of the eye Cause excitement and temporary blindness and permanent blindness and then affect on the length of grazing, which in turn affects 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 (5.6). The main clinical signs of inflammation of the eye in the calves and cows are profuse Altadma and watery and serous and purulent thick secretions of the eye, swelling eyelids, adhesion eyelashes, keratitis with Keratohelcosis (7), there are many factors that lead to a proliferation of bacteria in the conjunctival sac Either be pathological specific conditions such as dropsy cornea

and uveitis or may be of natural flora of the eyes or eyelids or skin and reach eye (8). numerous studies Recorded present of microscopic organisms linked to cases of keratitis and conjunctivitis including *Mycoplasmas*, *Listeria*, *Moraxella*, *Streptococcus*, *Staphylococcus*, *E.coli.*, *Micrococcus*, *Corynebacterium*, and germs negative bacteria (9, 10), and there are an unspecified number of studies of the natural flora for the eyes and have been isolated bacteria, including *Staphylococcus*, *Bacillus*, *Moraxella*, *Pseudomonas*, *Proteus*, *Corynebacterium*. the infection by opportunistic fungi has increased significantly, especially in recent times, and the filamentous fungi which from the important fungi in cause recurrent infections, as a fungus *Aspergillus fumigates* of the most important of these fungi ( 11), note that many of these germs

### Materials and methods

#### Collection of samples:

One hundred swab Were collected from the eyes of healthy and infected cows and of all ages for a period of (1/5/2012- 27/11/2012) from multiple areas of Al- Diwaniya city Included (Al-furat Dist. , Al-hakeem/3 Dist. , Al-chalabia Dist. , Rifaat Dist. , A;- Askarii Dist. ), and cases brought to the Veterinary Teaching Hospital in Qadisiya Province , samples were taken by entering a sterile cotton swab into 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 Qadisiya University.

#### 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 7% blood of sheep and MacConkey agar and

have been isolated from cases of satisfactory eye infection of cows and the bacteria of natural flora often be present in the conjunctiva or on the skin and nostrils and up to the eye and the infection occur because many of these germs is opportunistic (13.12 ). A lot of predisposing factors to an inflammation of the eye, including direct contact with lacrimal and nasal secretions contaminated with germs that have a significant role to spread infections that cause inflammation of the cornea and conjunctiva and have domestic flies, insects and stable flies has mechanical role in transfer of pathogens, as well as sunlight, dust and often eye infections high During the summer months (9). The purpose of this study was to isolate and diagnose some species of bacteria and fungi from the healthy and infected eyes of cows and ratios presence.

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 , also was culturing of questionable colonies on different selective media , including Manitol salt agar, Edward agar, MacConkey agar , Salmonella shigella agar , Eosin methylene blue agar and blood agar , pure colonies saved on brain heart infusion agar for the purpose of conducting biochemical tests and know the different types of it.(18).

#### Isolation of fungi :

swabs were culturing on the Sabouraud dextrose agar equipped by MEDIA company in a sterile Petri dishes and at the rate of 3 dishes per sample , dishes incubated at a temperature of 28 C for two weeks , and then examined developing colonies according to (15,16,17).

## Results

One hundred twenty one isolates for different bacteria have been diagnosed from swabs taken from the eyes of cows from (100) swab of clinically healthy animals and other with infection eye, where the pathological clinical signs is Altadma profuse and irritation and thick secretions, and was 47 (47%) are swabs of cases an infection eye and 53 (53%) of the cases healthy, from the Total examined

swabs (100) swab. The bacterial isolates were classified to 7 bacterial genera which is *Staphylococcus spp.* (26,4), *E.coli* (24,8%) is the highest percentage, and *Staphylococcus aureus* bacteria (19%), which were positive for coagulase test, while the *Proteus spp.* (3.3%) at least, and the bacterial genera differ in the isolation rates, as shown in Table (1).

Table (1): Genera and numbers of bacteria isolated from eye swabs in cattle

Genera of isolated bacteria	Number	Percentage
<i>Staphylococcus spp.</i>	32	26.4
<i>Escherichia coli</i>	30	24.8
<i>Staphylococcus aureus</i>	23	19
<i>Moraxella bovis</i>	12	9.9
<i>Streptococcus spp.</i>	10	8.3
<i>Pseudomonas spp.</i>	10	8.3
<i>Proteus spp.</i>	4	3.3
Total	121	100

*Aspergillus fumigatus* (25%), *Alternaria spp.* (25%), *Penicillium spp.* (12.5%) are the lowest ratio, as shown in Table (2).

The fungal isolates were classified into 4 genera where were *Aspergillus niger* (37.5%) are top ratio, and the fungus

Table (2): Genera and numbers of fungi isolated from swabs eye in cattle

Genera of isolated fungi	Number	Percentage
<i>Aspergillus niger</i>	12	37.5
<i>Aspergillus fumigatus</i>	8	25
<i>Alternaria spp.</i>	8	25
<i>Penicillium spp.</i>	4	12.5
Total	32	100

recorded the proportion of Gram positive isolated bacteria 63 (52.1%) which is higher than the proportion of Gram negative bacteria 58 (47.9%).

The bacteria isolated from healthy swabs Formed (75) 61.9% while the bacteria were isolated from inflammatory swabs (46) 38.1%, as shown in Table (3). The study

Table (3): the numbers and percentage of bacteria isolated from healthy and inflammatory smears

Genera of isolated bacteria	Healthy smears		Inflammatory smears	
	Number	Percentage	Number	Percentage
<i>Staphylococcus spp.</i>	32	26.4	0	0
<i>Escherichia coli</i>	24	19.8	6	5
<i>Staphylococcus aureus</i>	9	7.4	14	11.6
<i>Moraxella bovis</i>	0	0	12	9.9
<i>Streptococcus spp.</i>	4	3.3	6	5
<i>Pseudomonas spp.</i>	3	2.5	7	5.8
<i>Proteus spp.</i>	3	2.5	1	0.8
Total	75	61.9	46	38.1

from inflammatory smears (23) 71.9%, as shown in Table (4).

And isolated fungi from healthy swabs formed (9) 28.1% while the fungi isolated

Table (4): the number and percentage of fungi isolated from healthy and inflammatory smears

Genera of isolated fungi	Healthy smears		Inflammatory smears	
	number	Percentage	Number	Percentage
<i>Aspergillus niger</i>	4	12.5	8	25
<i>Aspergillus fumigatus</i>	3	9.3	5	15.6
<i>Alternaria spp.</i>	2	6.3	6	18.8
<i>Penicillium spp.</i>	0	0	4	12.5
Total	9	28.1	23	71.9

The bacterial species involved for more than one type per swab has up to four genera per swab in some swabs.

### Discussion

Showed The results of study for the swabs taken from the eyes of cows that bacteria isolation ratio was 100%, where bacteria isolated from all swabs examined (100) swab. The total bacterial isolates (121) isolation for 7 different genera of bacteria, as shown in Table (1)., The results of our study agreed with each of (13,18) Where the bacterial species was *Staphylococcus aureus*, *Moraxella bovis*,

*Streptococcus spp.* , *Pseudomonas spp.* , *Proteus spp.* , Similar to what has been isolated in this study. The proportion of 52.1% of germs positive bacteria are the highest and the proportion of bacteria *Staphylococcus spp.* Are the most percentage of 26.4%, followed by bacteria *Escherichia coli* (24,8%) which was agreed with (13,19). Either from healthy animals have been isolated bacteria *Streptococcus*

*spp.* and it's One of the bacteria that are isolated from healthy cases (20)., Pointed out (21) to different types of bacteria isolated from the eyes of clinically healthy cows and these bacteria *Staphylococcus aureus* , *Moraxella bovis* , *Proteus spp.* *Pseudomonas spp.* , *Pasturella*, while mentioned each of the (7-12) to this bacterial species isolated from infected animals with contagious keratitis and conjunctivitis, this agreed with our study in most of these bacteria isolated from healthy and inflammatory cases of the eyes. The isolate of bacteria *Staphylococcus spp.* (26.4%) and *E.coli*. By (24.8%), its high proportion as these bacteria are part of the Bacterial environment for humans and animals worldwide its located on the skin and in the introduction nostrils , in the gut of humans and animals, as they are found in polluted air, water and soil as well as The fact that many of them severely pathogens particularly pathogenic bacteria ( *Staph. aureus* ) which producing coagulase enzyme, which accounted for 19% in this study. The bacteria *E.coli*, *Proteus* are germs belonging to the family Enterobacteriaceae and endemic mostly gastrointestinal tract of humans and animals, and some types exist on other parts of the body and on plants, in soil and live in saprophytes or commensals or pathogenic to humans and animals (22). Formed bacteria *Moraxella bovis* (9,9%) which commensals germs located on the mucous membranes of humans and mammals and considers conjunctiva, nose and pharynx the storage places for these germs, especially in cattle older than 2 years of age , which bacteria do not live long outside the body of the host and directly transmitted by flies and insects ,

These germs cause pink eye disease (Infectious bovine keratoconjunctivitis) and virulence strains of these bacteria secrete hemolysin and contain cilia and secrete Cytotoxin this toxin break lipopolysaccharide of neutrophils (23). The results of the study for the swabs taken from the eyes of cows also showed that fungal isolation ratio don't was 26%, with no isolated fungi from all examined swabs (100) swab. All the fungal isolates (32) isolates for 4 different genera of fungi as shown in Table (2). The results of our study agreed with each of the (24), where the fungal species *Aspergillus spp.* And *Penicillium spp.* Similar to what has been isolated in this study. Either from healthy animals have been isolated fungi *Aspergillus niger* and *Aspergillus fumigatus* , *Alternaria spp.* is a fungus that is isolated from healthy cases , Fungi found in the conjunctival sac of cows might represent transient seeding from the environment, as suspected in other species (25) .. Also the results of our study showed that Although various fungal agents can be recovered from the conjunctiva, fungal conjunctivitis is rarely observed clinically. In comparison to fungal keratitis, relatively few organisms have been implicated in fungal conjunctivitis. *Candida spp.* can cause conjunctivitis after topical corticosteroid and antibacterial therapy to an inflamed eye(26). noted a lot of studies to resistance of bacterial isolated from the healthy and infected eyes to many antibiotics known, hence these bacterial resistance to antibiotics has lead to a lot of therapeutic problems and thus may conditions take time without response to treatment (13,18, 27, 28).

## Reference

- 1-Punch, P.I.; Slatter, D.M.(1984). A review of infectious bovine keratoconjunctivitis. *Vet Bull.* 1984;54:193-207.
2. Aly ,M.S.; Mohammed,M.H.(1995). Bacteriological studies of infection keratoconjunctivitis In dairy calves. *Assuit Vet Med J.* 1995;32:88-95.
3. Scott, P. (1990).Infectious bovine keratoconjunctivitis *Vet Practice.* 1977;9:301- 303. ed. Philadelphia W.B. Saunders Company;1990. pp:43- 52.
4. Slatter, D. *Fundamentals of veterinary ophthalmology.* 2
5. Ruehl ,W.W.; Mars ,C.F.; George, L.; Banks, S.J.M.; Schoolnik, G.K. (1993). Infection rate, disease frequency, pilin gene rearrangement, and pilin expression in calves in inoculated with *Moraxella bovis* pilin-specific isogenic variants. *Amer Vet Res.* 1993;54:248-253.
6. Willoughby, R.A. (1977).The prevalence of bovine keratoconjunctivitis in beef cattle herd in north eastern Queensland. *Aust Vet J.* 1977;53:128-131.
7. Knot ,J.B.; Ajinkya ,S.M. (1980).A study on infection bovine keratoconjunctivitis in the young calves on the organized farms in and around Bombay. *India Vet J.* 1980;57:87-88.
8. Slatter, D.H; Edwards ,M.E.; Wilcox, G.E.; Ezekie, D.(2008). Ocular inserts for application of drugs to bovine eye-effects of hydrophilic contact lenses. *Aust Vet J.* 2008;52:1-3.
9. Barber, D.M.; Jones, G.E.(1988). An attempt to induce clinical infections keratoconjunctivitis in calves with naturally occurring *Moraxella bovis* infection. *Vet Rec.* 1988;122:210.
10. Evans, K.; Smith, M.; McDonough, P.; Wiedmann ,M. (2004).Eye infections due to *Listeria monocytogenes* in three cows and one horse. *J Vet Digen Invest.* 2004;16:464-469. nd
11. Scott ,P. (1977).Infectious bovine kerato-conjunctivitis. *Vet Practice* 1977; 9: 301303.
12. Gouws, J.J.;Coetzer, A.W.; Howell ,P.G.(1995). A comparative microbiological study of clinically healthy eyes and those affected by ophthalmia in cattle and the association of noctuid eye frequently moths. *South Africa Vet J.* 1995; 66:160-169.
13. Zafer, O.; Ekrem, K.; Fikrullah ,K.; Mahir ,K.(2005). Screening conjunctival bacterial flora and antibiogram teat in cattle. *J Animal Vet Advance.* 2005;4:845-847.
14. Alfred ,E.; Barown,(2005). *Benson's microbiological applications.* McGraw Hill Companies;2005.
15. Raber ,K.B. and Fennel ,D.I.(1977). *The genus Aspergillus.* New York: Robert E Krieger Pub Co Huntington 1977: 238, 293.
16. Koneman, E.W.; Robert, G.A. and Wright, S.F.(1979). *Practical laboratory mycology.* USA: Williams and Wilkins Co 1979: 33-64.
17. Pitt, I.I. and Holking ,A.D. *Fungi and food spoilage.* 2 ed. New York: Blakia Academic and Professional 1997: 26-53. nd.
18. Al- Rashidy , S.D.H. (1998). Common bacterial eye infections in cattle and sheep in the Mosul area

- (Master). University of Mosul, Mosul 1998.
19. Dietz, O.J; Wehr ,R.; Thelmann, A. ;Popp Buschmann, W. New investigation on aetiology and treatment of bovine keratoconjunctivitis. Vet Rec. 1983;38:843-847.
  20. Ali, H.H. ; Al-Abidy,H.F. ; Mechael,N.S.(2011). Isolation and Identification of conjunctival bacteria in cattle in Mosul. Iraqi Jornal of Vet. Science. Vol.25,Numb.1 , 2011 (11 – 14 ).
  - 21 .Haddad, Gaspe Jassim.(1991). Veterinary Microbiology, Mosul, Iraq: Wisdom House for Printing and Publishing, 1991. 171 p.
  22. Quinn, P.J.; Markey, B.K.; Carter ,M.; Donnelly, W.J; Leonard, F.C. (2002).Veterinary microbiology and microbial disease. Blackwell Publishing Company;2002.pp:106-123.
  23. Quinn, P.J.; Carter, M.E.; Markey, B.; Carter, G.R. (2004).Clinical veterinary microbiology. An imprint of Elsevier limited;2004. 284-286p.
  24. Samuelson ,D.A.; Andresen, T.L.; Gwin, R.M. (2010). Conjunctival fungal flora in horses, cattle, dogs, and cats. (2010).Department of Veterinary Clinic, Veterinary Ophthalmology56010 San Piero a Grado, Pisa, Italy. 13(4):227-34.
  - 25.Sgorbini,M.; Barsotti, G.; Nardoni,S.; Brombin, M.; Sbrana,A.; Mancianti, F.; Corazza,M. (2010). Seasonal prevalence of fungi in the conjunctival fornix of healthy cows during a 2-year study.(2010).Department of Veterinary Clinic, Veterinary Ophthalmology56010 San Piero a Grado, Pisa, Italy. 13(4):227-34.
  26. Mandell, G. L. , Bennett, J. E. , Dolin, R. (2010). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases.2010.7<sup>th</sup> ed.
  27. Turnes ,C.; Albuquerque, I.B. Serotypes and antibiotic sensitivity of *Moraxella bovis* isolated from outbreak of infectious bovine keratoconjunctivitis. Can J. Comp Med. 1984;48:428-430.
  28. Sayed ,A.M.; Adel Fatah, A.M.; Manao, A.M. Epidemiological view of infectious bovine keratoconjunctivitis in calves, Clinical symptoms, microbiological examinations and treatment. Assuit Vet Med J.1995;100-105.

## عزل وتشخيص بعض الأجناس و الأنواع البكتيرية والفطرية من ملتحة العين لدى الأبقار في مدينة الديوانية

همس حسين هاشم هندول

كلية الطب البيطري – جامعة القادسية

e-mail : hamshussainhashim@Gmail.com

### الخلاصة

صممت هذه الدراسة لتحديد بعض الأجناس والأنواع البكتيرية والفطرية التي تصيب ملتحة العين لدى الأبقار , حيث إن الدراسات التي تناولت هذا الموضوع تعد قليلة نوعا ما وخاصة تلك التي تتضمن عزل الفطريات التي تصيب الملتحة باعتبارها من الحالات النادرة الحدوث والتي ربما يكون سببها انخفاض مناعة الجسم بعد العلاج بالمضادات البكتيرية والعلاج بالكورتيكوستيرويد للعين الملتهبة , وتم في هذه الدراسة فحص (100) مسحة من عيون الأبقار بمختلف الأعمار من مناطق عدة من مدينة الديوانية للفترة من 1/5/2012 - 27/11/2012 من حيوانات سليمة ظاهريا وأخرى مصابة بالتهاب العين . تم عزل 7 أجناس من البكتريا وهي *Escherichia* , ( $26,4\%$ ) *Staphylococcus spp.* , ( $24,8\%$ ) *coli* , ( $19\%$ ) *Staphylococcus aureus* , ( $9,9\%$ ) *Moraxella bovis* , ( $8,3\%$ ) *spp.* , ( $8,3\%$ ) *Pseudomonas spp.* , ( $3,3\%$ ) *Proteus spp.* , وكانت مجموع العزلات البكتيرية المعزولة 121 عزلة , حيث سجلت الجراثيم الموجبة لصبغة كرام 63 ( $52,1\%$ ) بينما كانت الجراثيم السالبة لصبغة كرام 58 ( $47,9\%$ ) , عدة جراثيم تم عزلها من المسحات السليمة 75 ( $62\%$ ) , أما الجراثيم المعزولة من المسحات الالتهابية فهي 46 ( $38,1\%$ ) . كما وشملت الدراسة أيضا عزل وتشخيص بعض الأنواع الفطرية المترافقة مع التهاب ملتحة العين للأبقار حيث أظهرت نتائج العزل إن فطر *Aspergillus niger* كان الأعلى نسبة من بين الفطريات المعزولة بنسبة عزل  $37,5\%$  أما الأنواع الفطرية الأخرى فقد كانت *Aspergillus fumigates*  $25\%$  , *Alternaria spp.*  $25\%$  و *Penicillium spp.*  $12,5\%$  .