Bacteriological study of infectious Keratoconjunctivitis in Iraqi Sheep

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Abstract

The study was conducted to investigate the bacterial causes of ovine Infectious keratoconjunctivitis, Since the knowledge of its etiology is limited, In a field present study was performed to determine the microorganisms involved. Conjunctiva swabs were collected from Apparently normal eyes (n=160) and infected (n= 40). All samples were cultivated for bacteria, Antimicrobial susceptibility of bacterial isolates were also done. Totals of (9) and (8) genera of bacteria were recovered from non-infected and infected eyes, respectively. *Staphylococcus aureus* was the most predominant bacteria as it was isolated from 30 (30.6%), 12 (24%) from apparent normal eyes and infected eyes respectively. While *Staphylococcus coagulase negative* (CNS) was isolated from 10 (10.2%), 9 (18%). *Streptococcus SPP* were 8 (8.16%), 4 (8%). *Corynebacterium SPP*. 6 (6.1%), 7 (14%). *Bacillus SPP*. 6 (6.1%%), 3 (6%). *Escherichia coli* 15(15.3%), 4(8%). *Klebsiella SPP*. 11 (11.2%), 6 (12%) from apparent normal eye and infected eye respectively. While *Proteus SPP*. 9 (9.18%). *Pasteurella SPP*. 3 (3%) were isolated from apparent normal eye only but *Moraxella (Branhamella) ovis*. 5(10%) were isolated from infected eye only. High percentages of bacteria were susceptible to Chloramphenicol and ciprofloxacin antibacterial drugs and show resistance to other antibacterial. The *Staphylococcus coagulase negative* (CNS) appear sensitive to Erythromycin 14(73.6%). *Moraxella SPP*. and *Pasteurella SPP*. bacteria were sensitive to Gentamycin at 4(80%) and 3(100%) respectively. The *Staphylococcus aureus* and *Staphylococcus coagulase negative* were the most predominant bacteria. Long-term monitoring of sheep populations provides a basis for understanding the impacts of disease outbreaks and improves management decisions.

Key words: Infectious keratoconjunctivitis, Bacteriology, Iraqi ewes.

دراسة بكتيرية لمرض التهاب القرنية والملتحمة المعدي في الأغنام العراقية

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الخلاصة

أجريت الدراسة للتحري عن المسببات البكتيرية لمرض التهاب القرنية والملتحمة المعدي في الأغنام، والذي هو من الأمراض الواسعة الانتشار في هذه الحيوانات، حيث ان المعلومات المتوفرة عن مسببات هذا المرض لازالت محدودة، أنجزت الدراسة الحقلية الحالية لمعرفة المسببات الجرثومية لهذا المرض، لذلك تم جمع 160 مسحة من عيون الحيوانات السليمة ظاهريا وكذلك 40 مسحة من عيون الحيوانات المصابة، جميع العينات تم زرعها للتحري عن البكتريا، كما اجري فحص حساسية الجراثيم للمضادات البكتيرية. عزلت 9 أجناس بكتيرية من عيون الحيوانات السليمة ظاهريا وكذلك 8 أجناس من عيون الحيوانات المصابة. كانت بكتريا المكورات العنقودية الذهبية الأكثر شيوعا حيث تم عزل 130 عزلة وبنسبة 30.6% وكذلك تم عزل 12 عزلة وبنسبة 24% من العينات السليمة ظاهريا والعينات المصابة بينما بكتريا المكورات العنقودية السالبة لخميرة التجلط كان عددها 10 وبنسبة (10.2%) وكذلك 9 عزلات وبنسبة 18%، بكتريا المكورات السبحية 8 (8.16%) 4 (8%)، بكتريا الوتديات 6 (6.1%) 7 (14%)، بكتريا العصيات 6(6.1%) و3 (6%) بكتريا الايشريكيا القولونية 15(15.3%) و4(8%) بكتريا الكلبسيلا 11(11.2%) و6 (12%) من العينات السليمة ظاهريا والعينات المصابة على التوالي، أما بكتريا

الزوائف الزنجارية 9 (9.18) بكتريا الباستوريلا 3 (3%) فقد عزلت من العينات السليمة ظاهريا فقط أما بكتريا المورکسلا 5 (10%) من العينات المصابة فقط. كذلك أظهرت الدراسة ان نسبة عالية من العزلات البكتيرية كانت حساسة للمضادات البكتيرية مثل الكلورومفينيكول والسايبروفلوكساسين، كما أظهرت مقاومة لباقي المضادات البكتيرية، بكتريا المكورات العنقودية السالبة لخميرة التجلط كانت حساسة للاريثرومايسين 14(73.6%)، أما بكتريا المورکسلا والباستوريلا كانتا حساستان للجنتامايسين 4(80%) و3(100%) على التوالي. المكورات العنقودية الذهبية والمكورات العنقودية السالبة لخميرة التجلط كانت البكتريا الأكثر شيوعا، المراقبة الطويلة الأمد لقطعان الأغنام تزودنا بقاعدة معلومات لفهم تأثير انتشار الأمراض وتحسين قرارات الإدارة.

الكلمات المفتاحية: التهاب القرنية والملتحمة المعدي، دراسة بكتيرية، الأغنام العراقية.

Introduction

Keratoconjunctivitis is an infection which affects the eyes and membranes of many animals, including cattle, sheep, goats, dogs and cats. It is a highly contagious disease that is usually acute and tends to spread rapidly affecting one or both eyes. When the conjunctiva is inflamed it is called conjunctivitis, however when both the cornea and conjunctiva are inflamed it is called keratoconjunctivitis (1).

Keratoconjunctivitis in sheep is a harmful disease. It may cause temporary or permanent blindness in severe cases. The first signs are hyperaemia, serous lachrymation, increased blinking and blepharospasm with purulent discharge followed by keratitis and corneal opacity. The conjunctival blood vessels become dilatated and migrate across the cornea. The cornea may become blackened or greyish, especially in the periphery. After 2 to 5 days the exudate becomes purulent. Occasionally a corneal ulcer develops, which may occur in severe cases. Nasal discharge, photophobia and pannus are seen (2). Both eyes usually become affected, although the clinical signs may start in one eye only. Later, the conjunctival blood vessels become dilated and migrate across the cornea. Usually animals recovers after a week, but some lambs remain ill for few weeks with weakness and fever and they are anorectic resulting in weight loss and reduced slaughter weights (3). Many conditions and factors can cause Keratoconjunctivitis which fall into two main categories; non-infectious causes and infectious causes. Non-infectious causes include injury or trauma to the eye that may occur due to dust, allergies or foreign material etc. Other irritants such as chemicals can also be non-infectious triggers and in some cases congenital defects (4). Keratoconjunctivitis is caused by either viral or bacterial infections. The bacterial infection are the most common form of this infection and is typically spread by contact. (5) isolated very few species of bacteria from the conjunctival sac of sheep but (6) found a diverse flora after sampling sheep once. (3) described *Neisseria ovis*, today termed *Moraxella (Branhamella) ovis*, while (7) demonstrated *Rickettsia (Colesiota)* conjunctivae from conjunctival smears (8) isolated *Listeria monocytogenes* while (9) were isolated *S. aureus*, *Corynebacterium* spp. and *E. coli*. The objective of this study was isolation and identification of different bacterial causes of Keratoconjunctivitis in sheep and study the sensitivity test of these bacteria to different antibacterial drugs.

Materials and Methods

The study conducted in the period from October 2014 to May 2015. from different sheep farms in Baghdad and Diyala province participated in the study. The eye swabs were taken from sheep with clinically apparent unaffected and from animals with outbreaks of keratoconjunctivitis. Both affected and unaffected animals were sampled. The apparently normal conjunctival samples showed no signs of keratoconjunctivitis and had not been treated with antibiotics systemically or locally during the 3 weeks prior to sampling. Two hundred samples were collected from 100 ewes, 60 sample from

30 ewes affected with clinical cases and 140 apparently normal samples from 70 ewes. According to (10) Sampling from the conjunctiva was carried out on the lower part of eyelid of two eye with 2 sterile cotton swabs under septic precaution and transporting immediately to the laboratory of College of Veterinary Medicine/ Department of internal and preventive medicine in University of Diyala by a cooling box. Isolation and identification of bacteria from samples were performed according to (11). The sample swab was cultural on nutrient broth for 24 hrs then all samples from clinical keratoconjunctivitis and apparent normal samples were cultured on blood agar, mac Conkey agar and nutrient agar, incubated at 37 C° for 24 hrs, Diagnosis depends on morphological character (shape, color and size of colony). The growth of the plates was examined visually. If no growth was observed, the plates were incubated for a further 24 h. The numbers of colonies for each type of bacteria present was recorded as rich, moderate or poor. Representative colonies of bacteria were sub cultivated onto blood agar plates and identified through biochemical tests. A single colony from nutrient agar was spreader on a clean slide and fixed with heat and staining with gram stain according to (12) and then the bacterial cell was examined under oil immersion. The bacterial isolates were identified by catalase, oxidase, Gelatin liquefaction, urease, O/F test, tube coagulase test and indol test. The isolated bacteria were tested for their susceptibility in vitro to many antibacterial drug like Erythromycin 15 µg, Amikacin 30 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, Kanamycin 30 µg, tobramycin 10 µg and Ampicillin 10 µg, by the standard agar disc-diffusion method (Kirby-Bauer) using the discs obtained from Hi-Media, and Mueller Hinton agar (13). All isolates after definitive isolation were cultured on sterile brain heart infusion broth and glycerol 20% and incubated at 37 C° for 24 hours, then after turbidity occurred, stored in a freezing. The data collected were subjected to statistical analysis using ANOVA were done using the program Sigma Sat for Windows.

Results and Discussion

Our results show that the main clinical signs which recorded in the case of keratoconjunctivitis in sheep includes lacrimation, mucopurulent occular discharge, conjunctivitis, congestion of corneal blood vessels, corneal opacity and blindness. The results were agreement with that of (7, 14).

- **Bacterial isolation according to gram stain:** The results showed a significant variance at ($P \leq 0.05$) between G^+ bacteria 60 isolates and G^- bacteria 38 isolates which isolated from normal apparent eye at percentage (61.2, 38.7%) respectively, also between infected eye (35), (15) isolates at percentage (70%), (30%) from G^+ and G^- bacteria respectively. The G^+ bacteria isolates showed high ratio comparing with G^- bacteria. (Table 1) this result was in agreement with (15) and (14), Also our result disagreed with. (16) which recorded that G^+ and G^- bacteria from infected eye was 55%, 40% respectively.

Table (1) Number and percentage of bacterial isolate according to gram stain

Type of bacteria	isolates from normal eye	%	isolates from infected eye	%	Total	%
G^+ bacteria	60	61.3	35	70	95	64.18
G^- bacteria	38	38.7	15	30	53	35.81
Total	98	100	50	100	148	100

- **Bacterial isolation:** Results of culturing and isolation of the causative agents revealed that the isolated bacteria were 148 isolates (74%) from specimens and No bacteria were cultivated from 26 sheep (5 sick animals and 21 apparent normal animals) that *S.aureus* were the most predominant bacteria as they were isolated from 30 (30.6%), 12 (24.5%) from apparent normal eye and infected eye respectively, and there was significant difference at ($P \leq 0.05$) between apparent

normal eye and infected eye. while, *Staphylococcus coagulase negative* (CNS) was isolated from 10 (10.2%), 9 (18%). *Streptococcus SPP* were 8 (8.16%), 4 (8%). *Corynebacterium SPP*.6 (6.1%), 7 (14%). *Bacillus SPP*. 6 (6.1%), 3 (6%), No significant difference at (P≤0.05) was found between apparent normal eye and infected eye. E. coli 15(15.3%), 4(8%). *Klebsiella SPP*. 11 (11.2%), 6 (12%). from apparent normal eye and infected eye respectively. There was significant difference at (P≤0.05) between apparent normal eye and infected eye. *Proteus SPP*. 9 (9.18%) and *Pasteurella SPP*. 3 (3%) were isolated from apparent normal eye only but *Moraxella (Branhamella) ovis*. 5 (10%) were isolated from infected eye only (Table 2). The present study conduct to detect the role of various microorganisms in keratoconjunctivitis of sheep. Ovine keratoconjunctivitis has a worldwide distribution and Many factors like Flies and sun light have been considered as predisposing factors and a variety of microorganisms have been listed as causative agent in sheep (9). These results were agreed with the most studies on eye infection where many investigators demonstrated that *S.aureus* were the most common pathogens isolated from eye and the common bacteria isolated from the eye conjunctiva (1, 2, 4). The predominance of *Staphylococcus* spp. was also agreed with results of (9) who found that (5%) of animals with keratoconjunctivitis infected with *S.aureus*, while 4% were infected with *CNS*. The presence of *S.aureus* on the eye conjunctiva and their ability to resist the penicillins, and other antibiotics, as well as the arbitrary use of the antibiotics in treatment of keratoconjunctivitis may explain the high rate of *S.aureus* incidence in this study. Our results isolated other gram positive bacteria like. *Streptococcus SPP* were 8 (8.16%), 4 (8%). *Corynebacterium SPP*.6 (6.1%), 7 (14%). *Bacillus SPP*. 6 (6.1%), 3 (6%). This result were agreed with that of (13) which found that *Bacillus* species (19%). *Pasteurella* species (6%), *Corynebacterium* species (6%), *Streptococcus* species (6%) and *Moraxella* species (4%). (15) demonstrated *M. (B.) ovis* in ovine infectious keratoconjunctivitis, but was uncertain as to its etiological role. In some reports *Moraxella (Branhamella) ovis.* was isolated more often from eyes with keratoconjunctivitis than from healthy eyes. (14) isolated *M. (B.) ovis* equally often from animals with signs of keratoconjunctivitis as from animals in the same farms without signs of the disease. Enterobacteriaceae were also isolated from several cases of apparent normal eye and infected eye, this results agreed with these obtained from other studies (9, 17). The prevalence of cases caused by these bacteria may be ascribed to their abundant presence in the environment of the animals as a cause of keratoconjunctivitis, it is worthy to mention that the frequency of bacterial species isolated from apparent normal eye and infected eye was ascribed to many factors such as breed differences, different hygiene and management practices followed in each farm (10).

Table (2) Number and percentage of bacterial isolate from infected and normal apparent eye

Bacterial isolates	Bacterial isolates from apparent normal eye		Bacterial isolates From infected eye		Total	%
	Number	%	Number	%		
S.aureus	30	30.6 %	12	24%	42	28.3%
Staphylococcus coagulase negative	10	10.2%	9	18%	19	12.8%
Streptococcus SPP.	8	8.16%	4	8%	12	8.1%
Corynebacterium SPP	6	6.1%	7	14%	13	8.7%
Bacillus SPP.	6	6.1%	3	6%	9	6%
E. coli	15	15.3%	4	8%	19	12.8%
Klebsiella SPP.	11	11.2%	6	12%	17	11.4%
Proteus *SPP.*	9	9.18%	-	-	9	6%
Moraxella SPP.	-	-	5	10%	5	3.3%
Pasteurella SPP.	3	3 %	-	-	3	2%
Total	98	66.2%	50	33.7%	148	100%

- **Sensitivity test:** As shown in tables (3) the susceptibility test results for amikacin, chloramphenicol, ciprofloxacin, gentamicin, Erythromycin, Kanamycin, Tobramycin and Ampicillin were considered for analysis as they are routinely used for ocular infections in the region, *S.aureus* isolates were highly sensitive to ciprofloxacin (83.3%), chloramphenicol (71.4%) and resistant to the remainder antibacterial, these results agreed with some results of (18) who found that *S.aureus* moderate to very high resistance to ampicillin (53.4%), penicillin (45.3%) and polymixin B (97.7%); also agrees with (19) who showed the highest frequency of resistance to penicillin. (20) demonstrated that 61.4% and 59.5% of *S. aureus* isolates were resistant to penicillin and ampicillin, respectively. The higher resistance of *S.aureus* isolate to most antibacterial may be attributed to continuous using of these antibiotics in systemic and local treatment, this may lead to development of resistance. On the other hand, the little use of the other antibacterial like Ciprofloxacin, chloramphenicol, in systemic and local treatment may lead to decrease the resistance of the *S.aureus* isolates to these drugs. In the other hand *Staphylococcus coagulase negative* (C-NS) were sensitive to, Chloramphenicol (78.9%) and Erythromycin (73.6%) while, resistant to the remainder, this result agrees with (20) who reported that C-NS showed resistance to ampicillin, erythromycin and lincomycin (34.4, 14.9 and 17.6)% respectively. Also agree with (22) & disagree with (21). (20) reported that (34%) of the CoNS isolates cultured from 18 eyes were resistant to 5 of the 14 antibiotics tested. *Streptococcus spp*. were highly sensitive to Chloramphenicol (91.6) and Ciprofloxacin (83.3) and resistant to the other antibacerials, this result disagrees with (22) & (21) in Iraq. Also (20) found that all streptococci isolate were susceptible to penicillin, ampicillin and Cephalothin, except for one isolate of *S.uberis*, while 19.8% of *S. dysgalactiae* isolates, were classified with intermediate susceptibility and 32.2% with resistance to tetracycline. Also our result agree with (19) who found that *Streptococcus spp* resistant to tetracycline, gentamicin and erythromycin. The present study reported that *Corynebacterium SPP* and *Bacillus spp* bacteria were resistant to all type of antibacterial this result was agree with study of other researchers like (23), (21). *E.coli*, *Klebsiella* and *proteus* isolates in our study were highly susceptible to Chloramphenicol and Ciprofloxacin (100, 100, 88.2, 76.4, 88.8 and 77.7)% respectively. while, in *E. coli* they were moderately susceptible to Gentamicin (68.4%) and resistant to the remainder. This result was disagreed with (20) who found that *E. coli* represents, the highest percentage of isolates resisted to ampicillin, neomycin, streptomycin and tetracycline. *Moraxella SPP* isolates in present study showed highly sensitive to Chloramphenicol (100%), Ciprofloxacin (80%) and Gentamycin (80%) but resist to other antibacterial. This results were agreement with study of (9). Isolates of *Pasteurella SPP* were highly sensitive to Gentamycin (100%) and moderately sensitive to the Chloramphenicol (66.6%) ampicillin (66.6%), This result was disagreed with study of (19) where all isolates of *Pasteurella multocida* in Finland and most of those isolated in Denmark, England (and Wales), Italy and Sweden were susceptible to the majority of the antimicrobials. It is recommended that ciprofloxacin, Chloromphenicol and gentamicin should be used in treatment of Keratoconjunctivitis in sheep in Iraq. Microbial resistance to antibiotic agents are becoming more common in ocular infections (24). The past 2 decades have witnessed changes in antibiotic susceptibility patterns on a worldwide basis. Guidelines that have been developed to help slow the escalation of systemic antibiotic resistance and encourage prudent use of antibiotic agents also apply to the management of ocular infections. Clinicians

should prescribe antibiotic agents only when clearly indicated and should order susceptibility testing whenever possible to prescribe the most appropriate agent. The excessive use of antibiotic agents is a primary cause of resistance. In addition, physicians should select agents that have rapid bactericidal activity, high attainable concentrations at the site of infection compared with the organism MIC, a relatively low incidence of antibacterial resistance, and a broad spectrum of activity.

Table (3) Antibacterial susceptibility test of isolated bacteria

Antibiotic	Antibacterial	NO. of isolates	Erythromycin (15 µg)	Amikacin (30 µg)	Chloramphenicol (30 µg)	Ciprofloxacin (5 µg)	Gentamicin (10 µg)	Kanamycin (30 µg)	Tobramycin (10 µg)	Ampicillin (10 µg)
	S.aureus	42	20(30.9)	0	30(71.4)	35(83.3)	6 (14.2)	0	0	10(23.8)
	C-NS	19	14(73.6)	5 (26.3)	15(78.9)	8 (42)	0	4 (21)	0	6 (31.5)
	Streptococcus spp	12	4 (33.3)	3 (25)	11(91.6)	10(83.3)	0	2 (16.6)	3 (25)	6
	***Corynebacterium SPP* (%)**	13	4 (30.7)	0	5 (38.4)	6 (46)	2 (15.3)	2 (15.3)	0	5 (38.4)
	Bacillus SPP.	9	3 (33.3)	0	4 (44.4)	6 (66.6)	0	3 (33.3)	2 (22)	4 (44.4)
	E. coli	19	10(52.6)	5 (26.3)	19(100)	19(100)	13(68.4)	0	0	8 (42)
	Klebsiella SPP.	17	5 (29.4)	3 (17.6)	15(88.2)	13(76.4)	7 (41)	0	0	9 (52.9)
	Proteus *SPP.*	9	3 (33.3)	0	8 (88.8)	7 (77.7)	4 (44.4)	2 (22.2)	0	3 (33.3)
	Moraxiella SPP	5	2 (40)	0	5 (100)	4 (80)	4 (80)	0	2 (40)	0
	Pasteurella SPP.	3	0	0	2 (66.6)	1 (33.3)	3 (100)	0	0	2 (66.6)

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