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# Evaluation of the clinical efficacy of subconjunctival injection of clindamycin in the treatment of naturally occurring infectious bovine keratoconjunctivitis

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**Abstract**

*Objective* To determine the clinical efficacy of subconjunctival injection of clindamycin in the treatment of naturally occurring infectious bovine keratoconjunctivitis (IBK).

*Animals studied* Clinically, out of 81 animals examined, 46 were found to be suffering from IBK of variable severity. The ocular secretions were collected and cultured for *Moraxella bovis*. The study included 36 Holstein cattle from which *M. Bovis* was isolated. These animals ranged between 4 and 28 months of age.

*Procedures* The severity of the clinical findings were scored as normal, mild, moderate, and severe. Clindamycin was injected subconjunctivally at a total dose of 150 mg (1 mL), once daily for 3 days to the test group ($n$ = 18); isotonic saline solution (1 mL) was administered to the control group. After treatment, all cattle were re-examined and clinical response was evaluated on days 3, 7 and 15 post-treatment.

*Results* Compared with the control group and prior to treatment, all active lesions such as blepharospasm, epiphora, photophobia, chemosis, corneal edema, and corneal ulceration were generally resolved by day 15 after subconjunctival injection of clindamycin. Severity of IBK lesions increased on days 3 and 7, compared to baseline in the control group administered isotonic saline solution.

*Conclusions* The results of this study suggest that subconjunctival injection of clindamycin is effective in the treatment of naturally occurring infectious bovine keratoconjunctivitis.

**Key Words:** cattle, clindamycin, IBK, keratoconjunctivitis, *Moraxella bovis*, subconjunctival injection

## INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is an infectious and contagious ocular disease of cattle characterized by conjunctivitis and ulcerative keratitis. Outbreaks of epidemic proportions can affect grazing herds during the summer and autumn months.[1,2] *Moraxella bovis* is identified as the main causative agent of IBK; however, other factors, including ultraviolet light, concurrent disease status, and other ocular bacterial organisms influence the disease. The disease is transmitted by direct contact, inanimate fomites and face flies (*Musca autumnalis*).[1–3]

Medical treatment alternatives include topical, subconjunctival, and parenteral administration of antibiotic drugs.[1] Numerous antibiotic drugs including gentamicin, penicillin, neomycin – bacitracin – polymyxin B, sulfonamides, tetracyclines, furazolidone, chloramphenicol, florfenicol and ceftiofur have been used in the treatment of IBK.[1,3–5] Antibiotic resistance may develop in *M. bovis* species because of frequent usage of antimicrobials.[6] Thus, new antimicrobials are required for the treatment of IBK to avoid microbial resistance. Clindamycin is the 7(S)-chloro-7-deoxyderivative of lincomycin. It binds to the 50-s ribosomal subunits of bacteria and inhibits protein synthesis.[7,8] Clindamycin accumulates in leukocytes, which allows it to act on intracellular bacteria and be transported to and liberated at the sites of infection.[7–9] Clindamycin is a bacteriostatic antibiotic. However, it has bactericidal action against some bacteria at achievable tissue levels. Clindamycin is widely distributed into most tissues, including respiratory tissue, soft tissue, bones, and joints.[7,8] This agent is used commonly for the treatment of most aerobic Gram-positive cocci, including staphylococci, and many anaerobic and microaerophilic Gram-negative and Gram-positive anaerobic infections.[10–12]

The aim of this study was to assess the efficacy of subconjunctival injection of clindamycin in the treatment of naturally occurring infectious bovine keratoconjunctivitis.

## MATERIALS AND METHODS

The study was conducted in Holstein Friesian beef breeding farms around the town of Yenisehir in Bursa, Turkey. Clinically, out of the 81 animals examined, 46 cattle aged 4–28 months were found to be suffering from different degrees of IBK. The study livestock included 36 Holstein cattle, from which *M. bovis* was isolated. These animals were randomly assigned to test ($n$ = 18) and control ($n$ = 18) groups after routine clinical examination. Body temperature, pulse and respiration rates and ruminal motility were determined by clinical examination. Ophthalmic examinations (cornea and conjunctiva) were performed using a penlight in all beef cattle prior to treatment, and findings were noted. Animals with any degree of ocular disease were identified from their tag number. These animals underwent further close examination and their lesions were categorized as described previously.[13–15] Clinical findings were categorized as mild (lacrimation, congestion of bulbar and palpebral conjunctiva), moderate (chemosis, photophobia, small, pale white to yellow opacity on the center of the cornea but without vision impairment), and severe [chemosis, corneal opacity, corneal ulceration (intact or perforated), purulent ocular discharge, pain, photophobia and blepharospasm]. In addition, degrees of clinical findings were scored as follows: clinically normal, 0; mild signs, 1; moderate signs, 2; severe signs, 3.

### *Sample collection and bacteriologic laboratory examination*

*Samples* For isolation purposes, 46 samples were obtained from the conjunctival sac of eyes of cattle with symptoms compatible with IBK using sterile cotton-tipped swabs. These samples were placed in screw-capped tubes containing sterile Stuart transport medium (Oxoid) and transported to the laboratory.

*Isolation* Swabs were streaked on 10% sheep blood agar plates[16] and tween 80 agar plates,[17] which were then incubated at 37 °C for 48 to72 h under aerobic conditions.

*Identification* After incubation, isolated strains were identified on the basis of the following criteria: colony characteristics; Gram-staining reaction and morphology; cellular elongation in the presence of penicillin, growth characteristics in tryptic case soy broth (TSB); motility; oxidase; catalase; growth on MacConkey agar; growth at 20 °C; tolerance to 5% NaCl; oxidation-fermentation (O-F); carbohydrate (glucose, lactose, arabinose, xylose, mannose) fermentation; indole and hydrogen sulfide production; nitrate reduction; urease; tween 80 and casein hydrolysis; coagulated serum; and gelatine liquefaction.[15–18]

All tests were performed as described by Wilcox[16] except for cellular elongation, tween 80 hydrolysis and O-F carbohydrate fermentation tests, which were carried out as defined by Catlin,[19] Boure,[20] Mattinson and Cox[17] and Cowan and Steel,[21] respectively.

Antibiotic sensitivity testing was performed using the disk diffusion method[19] with the following antibiotics: penicillin G (1 unit), oxytetracycline (30 μg), neomycin (30 μg), erythromycin (30 μg), clindamycin (2 μg), sulfamethozale/trimethoprim (25 μg), gentamicin (30 μg), cephazolin (30 μg), ampicillin (30 μg), and enrofloxacin (25 μg). Diagnosis of IBK was based on history, clinic examination and bacteriologic identification.

### *Treatment and evaluation of clinical efficacy*

Clindamycin (Klindan 300®, Bilim, Istanbul, Turkey), 150 mg (1 mL), was administered subconjunctivally to the treatment group at the dorsolateral limbus once daily for 3 days. Similarly, isotonic saline solution (1 mL) was administered to the control group. Clinical response (lacrimation, blepharospasm, photophobia, corneal opacification, conjunctival hyperemia, keratitis) was evaluated on days 3, 7 and 15. All the affected animals were kept indoors for 15 days to avoid the adverse effects of solar ultraviolet radiation. Bacteriologic cultures were performed on day 3 following treatment.

Assessment of efficacy was based on signs of clinical resolution in affected beef cattle. For the detection of significant differences between clinical resolution at different time points the Friedman nonparametric repeated measures test and the Mann–Whitney test were used for the differences between the groups at a given time. Instat statistical program version 2.02 (Graph-Pad Software Inc., San Diego, CA, USA) was used for the statistical analyzes.

## RESULTS

Prior to treatment, lesions of IBK generally included blepharospasm, epiphora, photophobia, chemosis, corneal edema, and corneal ulceration. Twenty-four animals had unilateral lesions while 22 were bilateral cases. Prevalence of IBK associated with age, and the severity of IBK in *Moraxella bovis*-positive cattle are shown in Tables 1 and 2, respectively. It was observed that prevalence of IBK and the degree of severity of the disease was significantly associated with age; younger animals were more severely affected than older animals.

In comparison with the control group, all active lesions such as blepharospasm, epiphora, photophobia, chemosis, corneal edema, and corneal ulceration were generally resolved at the end of 15 days after subconjunctival injection of clindamycin. However, it was observed that keratitis and corneal opacity continued to some degree in two of the cattle with IBK. Severity of active lesions was increased on days 3 and 7, compared to baseline in the control group administered isotonic saline solution. Adverse reactions were not observed in any cattle, whether given clindamycin or isotonic saline solution. Assessment of clinical severity of *M. bovis*-positive beef cattle suffering from IBK before and after treatment is presented in Table 3.

**Table 1.** Age distribution in Holstein Friesian beef cattle with IBK

| Age (years) | Affected animals | No. of all animals | Percentage of affected animals in same age groups | Percentage of affected animals in various age groups |
|---|---|---|---|---|
| < 1 | 31 | 37 | 83.7% | 67.3% |
| 1–2 | 9 | 21 | 42.8% | 19.5% |
| 2 < | 6 | 23 | 26% | 13.2% |
| Total | 46 | 81 | 56.7% | 100% |

**Table 2.** The severity of IBK in various age groups of *Moraxella bovis*-positive cattle

| Disease severity (score) | Affected animals | | < 1 year old | | 1–2 years old | | 2–3 years old | |
|---|---|---|---|---|---|---|---|---|
| | Test group | Controls | Test group | Controls | Test group | Controls | Test group | Controls |
| Mild (1) | 4 | 5 | 1 | 2 | 1 | 1 | 2 | 1 |
| Moderate (2) | 11 | 10 | 10 | 8 | 1 | 1 | – | 1 |
| Severe (3) | 3 | 3 | 3 | 2 | – | 1 | – | – |
| Total | 18 | 18 | 14 | 12 | 2 | 3 | 2 | 2 |

**Table 3.** Mean (± SE) assessment of clinical severity in *Moraxella bovis*-positive beef cattle before and after treatment

| | Group | 0 days (mean + SE) | Three days (mean + SE) | Seven days (mean + SE) | Fifteen days (mean + SE) |
|---|---|---|---|---|---|
| Scores of clinical findings | Test | 1.96 ± 0.1† | 1.00 ± 0.1‡,** | 0.56 ± 0.1§,** | 0.23 ± 0.1*,¶,** |
| | Control | 1.94 ± 0.1† | 2.27 ± 0.1‡,†† | 2.19 ± 0.1‡,†† | 1.77 ± 0.1*,§,†† |

*$P < 0.05$. †, ‡, §, ¶: Differences between the values involving different letters on the same row are found to be statistically significant.
** and †† indicate a significant difference between test and control group measurements at the same time point.

**Table 4.** *M. bovis* strains isolated from cattle with symptoms of infectious keratoconjunctivitis and their antibiotic sensitivities

| No. of isolates sensitive to antibiotics (no. of *M. Bovis* strains = 36) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CFP | ENR | AMP | CN | SXT | CL | OT | N | E | P |
| 36 (100) | 36 (100) | 34 (94.44) | 34 (94.44) | 34 (94.44) | 34 (94.44) | 34 (94.44) | 32 (88.88) | 30 (83.33) | 22 (61.11) |

CFP, cephazolin; ENR, enrofloxacin; AMP, ampicillin; CN, gentamicin; SXT, sulfamethoxazole/trimethoprim; CL, clindamycin; OT, oxytetracycline; N, neomycin; E, erythromycin; P, penicillin G.

*Moraxella bovis* was detected in 36 (60.7%) out of 46 samples taken from cattle with symptoms of IBK. Of the 36 strains examined, 36 (100%) were sensitive to cephazolin and enrofloxacin; 34 (94.44%) to clindamycin, ampicillin, gentamicin, sulfamethozale/trimethoprim, oxytetracycline; 32 (88.88%) to neomycin; 30 (83.33%) to erythromycin; and 22 (61.11%) to penicillin G. The antibiotic susceptibility of the bacteria isolated from cattle with symptoms of IBK is presented in Table 4. In these animals bacteriologic cultures were negative at the end of treatment.

## DISCUSSION

In this study, 36 *Moraxella bovis* strains were isolated from 46 samples obtained from affected eyes of cattle with symptoms of IBK. Varied susceptibility between various breeds of cattle and ages of animals has been reported.[22,23] In the present study, the findings indicated that younger animals were more susceptible to the disease than adults. This is probably because young animals lack immunity, owing to the absence of previous exposure. Enhancement of IBK by solar ultraviolet irradiation could be responsible for the high incidence of the disease in summer months.

Treatment of IBK is dictated by economic considerations, intended animal use, and feasibility of administration. Antibacterial therapy is aimed at achieving drug concentrations in tears to meet or exceed the minimum inhibitory concentration (MIC) for prolonged periods.[22] *Moraxella bovis* is susceptible to most antibacterial agents, such as oxytetracycline, florfenicol, penicillin, gentamicin, cloxacillin and ceftiofur.[1,2,4,5] Antibacterial agents may be used topically, by subconjuntival injection, and parenterally. Topically applied antimicrobials may not be very effective owing to the frequent administration required to maintain therapeutically effective levels in the precorneal tear film. In addition, ocular discharge and blepharospasm render their application difficult.[3,24] Parenterally used antimicrobials may result in greater drug residues in milk or tissues than subconjunctival administration. Some

parenteral antibacterial injections may not reach MIC values in the lacrimal gland or tears.[24,25] Punch and others[26] reported that lipophilic drugs such as chloramphenicol, erythromycin and oxytetracycline diffused into the tears in higher concentrations than drugs that were not lipophilic such as gentamicin and procaine benzyl penicillin. However, erythromycin, chloramphenicol and oxytetracycline may result in edema at the injection site, a marked pain response, and tissue damage in the carcasses of cattle after intramuscular injection.[27]

Subconjunctivally administered medications usually maintain higher corneal drug concentrations for longer periods of time than topical or parenteral applications.[24] Based on this characteristic, in the present study clindamycin was administered as a subconjunctival injection. Clindamycin penetrates well into abscesses and white blood cells. It has a spectrum of activity that includes *Staphylococcus* species, *Streptococcus* species, and *Mycoplasma* species, as well as anerobic organisms, such as *Fusobacterium* species, *Bacteroides* species, *Clostridium perfringens*, *Actinomyces* species, and *Pronobacterium* species.[7–9] In our study, antibiogram tests indicated that 94.44% of *M. bovis* strains isolated from beef cattle with IBK were sensitive to clindamycin. In comparison with the control group, treatment with clindamycin of the 16 *M. bovis*-positive cattle was classified as successful at the end of 15 days. Clindamycin administered subconjunctivally showed an excellent response in these cattle. In addition, no recurrence was observed in animals during the 15 days' monitoring period following clindamycin therapy. However, treatment was considered as a failure in two animals. Lack of clinical response to treatment may be associated with resistance to clindamycin in these cattle, which had severe ulcerative keratitis.

In conclusion, the results of this study suggest that subconjunctival injection of clindamycin is effective in the treatment of naturally occurring IBK. In addition, it is safe for cattle with IBK at the dosage used in the study.

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