Introduction

Infectious bovine keratoconjunctivitis (IBK) is caused by a Gram-negative bacterium, *Moraxella bovis*. It is characterised by acute contagious ophthalmia and it spreads rapidly among cattle in a herd. *M. bovis* is generally present on the mucous membranes of healthy animals and is considered to be an opportunistic pathogen (Quinn et al., 1994; Gyles and Thoen, 1993). The bacterium is transferred to susceptible animals by direct contact with contaminated material, by insects and by particles in the air (Brown et al., 1998). Unfortunately, other than fly control, little can be done to halt the spread of the disease, and currently available vaccines have limited efficacy (Smith et al., 1990). Thus, treatment of individual animals is often necessary to reduce economical losses caused by IBK. Several studies on IBK have shown that florfenicol, a derivative of chloramphenicol, can be used successfully in the treatment of cattle (Cosgrove et al., 1998; Varma et al., 1998; Angelos et al., 2000). When the drug was injected intramuscularly (im) and subcutaneously (sc), its effectiveness was 98% and 93%, respectively (Cosgrove et al., 1998a; Angelos et al., 2000). Oxytetracycline preparations have been widely used in animals for the treatment of IBK (George et al., 1988; Eastman et al., 1998) and other bacterial infections (Johnson et al., 1998; Ayling et al., 2000). However, studies have indicated that several bacterial species may have developed resistance against oxytetracycline (Erdeger and Aydin, 1991; Eastman et al., 1998).

A comparison of the efficacy of florfenicol and oxytetracycline in the treatment of naturally occurring infectious bovine keratoconjunctivitis

H. Ibrahim Gokce¹, Mehmet Citil¹, Oktay Genc², H. Metin Erdogan¹, Vehbi Gunes¹ and Orhan Kankavi³

¹ Department of Internal Medicine, Faculty of Veterinary Medicine, The University of Kafkas, KARS-36040, Turkey.
² Department of Microbiology, Faculty of Veterinary Medicine, The University of Kafkas, KARS-36040, Turkey.
³ Department of Surgery, Faculty of Veterinary Medicine, The University of Kafkas, KARS-36040, Turkey.

Thirty calves naturally infected with *Moraxella bovis* were divided into two equal groups. One group was injected with florfenicol, the other group with long-acting oxytetracycline. The animals in each group were examined over 10 weeks to compare differences in the progress of eye lesions and in their respective healing periods. Animals treated with florfenicol recovered more rapidly than the animals treated with oxytetracycline. In contrast to the animals in the florfenicol group, eye lesions reoccurred in some animals six weeks after the injections of oxytetracycline. Antibiotic sensitivity of *M. bovis* isolated from the conjunctival sacs of infected animals was determined and florfenicol was found to be the most effective antibiotic tested. Thus, florfenicol was found to be a more effective antibiotic against *M. bovis* in vivo and in vitro than long-acting oxytetracycline.

Keywords
Thus, new therapeutic agents are required in veterinary practice to avoid microbial resistance. Florfenicol is one of the new drugs marketed for veterinary use in Turkey in recent years. However, prior to this study it was used only in the treatment of respiratory disease caused by bacteria (Aslan et al., 2000) and it has not yet been used in the treatment of IBK which frequently occurs in the Kars region of Turkey. Therefore, the purpose of the present study was to compare the efficacy of florfenicol and long-acting oxytetracycline for the treatment of calves with naturally occurring IBK.

Materials and methods
Calves
A naturally occurring outbreak of IBK affected 30 Swiss Brown calves, six to 12 months old, in a herd in Kars region of Turkey.

Experimental procedure
Calves were randomly divided into two equal groups (Group A and Group B).

The animals in Group A received injections of florfenicol (20 mg/kg), im, on hours 0 and 48. Calves in Group B received injections of long-acting oxytetracycline (20mg/kg), im, on hours 0 and 48. Calves in each group were examined daily for the first week and then every three days for a further three weeks. The animals were examined for lacrimation, photophobia, blepharospasm, conjunctivitis, ulcers, keratitis and opacity. Clinical recovery was assessed and the remaining eye lesions were recorded at weekly intervals for four weeks. Treatment of the calves was considered to be successful when the ulcers completely healed within four weeks. All the animals were kept indoors for four weeks to avoid the adverse effects of ultraviolet light and they were then moved to paddocks for six weeks of further observations.

Bacteriologic studies
On day 0, prior to the injection of florfenicol or oxytetracycline, ocular secretions were collected by sterile swabs from the conjunctival sac of each of the 30 calves with suspected corneal ulcers. Swabs samples were sent to the microbiology laboratory, Faculty of Veterinary Medicine, in thioglycolate broth. In addition, 45 flies from the animals' eyes and from the barn were also collected and sent to the laboratory.

Eye swabs and smashed flies were streaked on PPLO agar to isolate *Mycoplasma* spp and *Ureaplasma* spp. Swabs were also streaked on blood agar containing 7% defibrinated oxine blood and on MacConkey agar to isolate *Moraxella* spp and *Branhamella* spp. Blood agar and MacConkey agar were incubated for 48 hours at 37°C, while PPLO agar was incubated microaerobically (containing 10% CO2) for five days at 37°C. Catalase, oxidase, gelatinase, glucose, nitrate reduction, limbus milk, phenylalanine deaminase and motility tests were also performed for the identification of isolates (Bilgehan, 1995; Arda, 2000).

Antibiogram tests
Isolates identified as *M. bovis* were suspended in skim milk-glycerol medium and stored frozen at –80°C. Frozen *M. bovis* isolates were recultured onto blood agar, then transferred to nutrient agar and used to determine their sensitivity to various antibiotics. For this purpose, the Kirby-Bauer disc diffusion method (Bauer et al., 1966) was used. Commercially available florfenicol (30mg), oxytetracycline (30 mg), penicillin (6 IU), kanamycin (30mg), streptomycin (10mg), neomycin (30mg), enrofloxacin (5mg), lincomycin (2mg) and erythromycin (15mg) discs were used in the test.

Results
Clinical findings
Increased lacrimation, photophobia, blepharospasm, conjunctivitis, keratitis and corneal opacity were observed in all of the calves prior to treatment. In each calf corneal opacity was observed in only one eye. Calves without opacity were not used in the study.

In Group A, photophobia and lacrimation disappeared 24 hours after the injections, whereas in Group B photophobia and lacrimation decreased slightly at 48 hours after the injections as compared with the pre-injection period.

Mean (±SEM) time of disappearance of corneal opacity was 13.14 ± 3.39 days in calves that received florfenicol and 18.56 ± 6.18 days in nine of 15 calves that received oxytetracycline. Six calves treated with oxytetracycline still had spot-like corneal opacity four weeks after treatment.

All the animals were moved to paddocks in the fifth week of the study. New infection occurred in the previously unaffected eye in three of the 15 calves that received oxytetracycline: one during the sixth week and two during the seventh week after treatment. There was no reinfection amongst the calves treated with florfenicol.

Isolation and identification of *Moraxella bovis*
*Moraxella bovis* was isolated from ocular secretions of all the calves and from the 24 of 45 flies collected from animals' eyes and the barn. There was no growth in PPLO agar and MacConkey agar. However, characteristic haemolytic pure colonies, 1 to 3mm diameter in size, were grown on blood agar. Gram-stained smears from colonies showed Gram-negative rods in pairs. Colonial growth was autoagglutinated when suspended in saline. All isolates were non-motile and biochemically positive for catalase, oxidase, gelatinase and litmus milk. They were negative for glucose, nitrate reduction and phenylalanine deaminase. The bacteria were also able to be differentiated from *Branhamella ovis* because they tested positive for oxidase, gelatinase and litmus milk. Based on these findings, the bacteria
In this study, there was no fly control, and calves were treated for a maximum of 48 hours. It is possible that reinfection might be due to depletion of tissue antibiotic concentrations. Another possibility is the presence of bacterial resistance to oxytetracycline. Foot-and-mouth disease and respiratory disease are common problems in cattle in the Kars region of Turkey. Oxytetracyclines are haphazardly used for the treatment of these diseases. A study indicated that 41.5% of the isolates of *M. bovis* were resistant to tetracycline, suggesting the presence of bacterial resistance to tetracyclines in Turkey (Erdeger and Aydin, 1991). In this study, antibiogram tests indicated that 16.67% of the isolates of *M. bovis* were resistant to oxytetracycline, while all of the isolates were sensitive to florfenicol, suggesting the presence of bacterial resistance to oxytetracyclines in the region. Florfenicol is a new therapeutic agent for Turkey and resistance has not developed (Aslan et al., 2000).

In conclusion, florfenicol was a more effective antibiotic than long-acting oxytetracycline for the treatment of naturally occurring IBK in calves.

### References


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**TABLE 1: The antibiogram test results of *M. bovis* strains (n=30) isolated from calves**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive (n)</th>
<th>Relatively sensitive (n)</th>
<th>Resistant (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin</td>
<td>27 (90.00%)</td>
<td>3 (10.00%)</td>
<td>0</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>28 (93.33%)</td>
<td>2 (6.67%)</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin</td>
<td>26 (86.67%)</td>
<td>4 (13.33%)</td>
<td>0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>25 (83.33%)</td>
<td>5 (16.67%)</td>
<td>0</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>25 (83.33%)</td>
<td>5 (16.67%)</td>
<td>0</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>19 (63.33%)</td>
<td>6 (20.00%)</td>
<td>5 (16.67%)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>13 (43.33%)</td>
<td>11 (36.67%)</td>
<td>6 (20.00%)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>7 (23.33%)</td>
<td>11 (36.67%)</td>
<td>12 (40.00%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6 (20.00%)</td>
<td>11 (36.67%)</td>
<td>13 (43.33%)</td>
</tr>
</tbody>
</table>

were identified as *M. bovis*.

### Antibiotic test

The sensitivities of the isolates are shown in Table 1. Twenty-eight of the 30 isolates (93.33%) were sensitive to florfenicol, while 19 isolates (63.33%) were sensitive to oxytetracycline and five isolates (16.67%) were resistant to that antibiotic.

### Discussion

The clinical signs (i.e., lacrimation, blepharospasm, conjunctivitis, keratitis and opacity) observed in the study are the common clinical signs of IBK in calves. The clinical diagnosis was confirmed by isolation of *M. bovis* from the conjunctival swabs. The bacteria were isolated also from flies collected from the animals’ eyes and the barn. It is well-known that flies are the mechanical vectors and have a role in the transmission of *M. bovis* from animal to animal (Brown et al., 1998).

Healing times of eye lesions were shorter in calves treated with florfenicol than in calves treated with oxytetracycline. However, studies have indicated that *M. bovis* was isolated from cattle treated with florfenicol (Cosgrove et al., 1988; Eastman et al., 1998). These studies indicated that oxytetracycline was unable to completely eliminate *M. bovis* from animals, which may effect healing time (Edmondson et al., 1989). A previous study of calves with IBK that were treated with oxytetracycline suggested that the corneal epithelium may regenerate at a constant rate following clearance of *M. bovis* infection which is considered important for achieving resolution of IBK (Edmondson et al., 1989; George et al., 1988).


After the initial appearance of porcine reproductive and respiratory syndrome (PRRS) in the USA in 1987, the causative virus has spread rapidly across much of the globe. A commercial antibody ELISA (IDEXX HerdChek, IDEXX Laboratories, Maine, USA) is extensively used for surveillance, diagnosis and certification. The manufacturers reported a sensitivity of 100% and a specificity of 99.5% on testing 450 sera, though lower specificities have been reported when testing field sera (Nodelijk et al., 1996). This communication reports the prevalence and magnitude of non-specific reactions encountered when testing 38,152 porcine sera received in this laboratory since early 1997.

Thirty thousand surveillance sera, collected mainly at slaughter and 8,152 sera collected on-farm were examined for antibodies to PRRS virus using the IDEXX HerdChek ELISA. As recommended by the manufacturer, sera with a sample to positive (S/P) ratio of \( \geq 0.4 \) were deemed to be positive. The immunoperoxidase monolayer assay (IPMA; Wensvoort et al., 1991) is considered to be the gold standard for PRRS serology (Drew, 1995; Nodelijk et al., 1996) and was used to confirm the specificity of the ELISA reactions (O’Connor et al., 2002). A total of 1,691 sera were positive in the ELISA. One hundred and eighty one of these ELISA-positive sera were not confirmed by the IPMA, yielding an overall specificity of 99.5% (36,461/36,642). The on-farm specificity of 99.7% was slightly better than the specificity of 99.4% recorded for the surveillance sera. The lower specificity in sera obtained at slaughter may be due to the difficulties involved in collecting the samples and to delays sometimes experienced in transporting them to the laboratory. In addition, many of the samples collected at slaughter were from breeding animals, where up to 2.2% false positive reactions have been reported (Dec et al., 2001).

The S/P ratio range of the 181 ELISA-positive, IPMA-negative sera is shown in Table 1. The mean S/P ratio was 0.62. One hundred and nineteen (66%) sera had S/P ratios between 0.4 and 0.99 and 48 (27%) sera had S/P ratios between 1.00 and 1.99. Only 2 (1%) sera had S/P ratios \( \geq 2.0 \).

<table>
<thead>
<tr>
<th>Range of S/P ratios</th>
<th>Number of sera positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 - 0.59</td>
<td>119 (66)</td>
</tr>
<tr>
<td>0.6 - 0.99</td>
<td>48 (27)</td>
</tr>
<tr>
<td>1.00 - 1.99</td>
<td>12 (7)</td>
</tr>
<tr>
<td>( \geq 2.0 )</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>
On a routine basis, S/P ratios of <0.6 are reported to be considered doubtful or weakly positive by the manufacturer (Dea et al., 2000). The false-positive results occurred as singleton reactors in the majority of cases. Two false-positive samples per serum batch (10 to 25 sera) were detected on 10 occasions, while three false positives per batch were detected twice.

In contrast, examination of sera in this laboratory from infected herds has detected a high prevalence of antibody (up to 100%) and high S/P ratios (many sample >1.0) in fatteners at slaughter. Antibody levels to PRRS virus decline relatively rapidly (Yoon et al., 1995), yet many culled breeding animals from infected herds still had detectable antibody, though at lower S/P ratios (many samples <1.0) than were found in fatteners.

The occurrence of five false positives per 1000 negative sera tested could lead to pig herds being wrongly classified as PRRS virus-positive, especially as some of these sera had high S/P ratios. However, the low prevalence of false positive reactions compared to the high prevalence of antibody in infected herds is a useful guideline. Resampling can eliminate many false positives (Dec et al., 2001), but where interpretation continues to be difficult, such as when examining sera from weaners (Nodelijk et al., 1996) or breeding animals (Bierk et al., 2001) or when infection is fading-out in a herd (Nodelijk et al., 2000), the use of the IPMA will help to determine the specificity and significance (Yoon et al., 1995; O’Connor et al., 2002) of ELISA results.

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References


