Efficacy of Different Dosage Levels and Administration Routes of Tilmicosin in a Natural Outbreak of Infectious Bovine Keratoconjunctivitis (Pinkeye)*

Gustavo C. Zielinski, DVM, PhD\textsuperscript{a}
Hernán G. Piscitelli, DVM\textsuperscript{a}
Hernán Perez-Monti, DVM\textsuperscript{b}
Larry A. Stobbs, DVM\textsuperscript{c}
Alan G. Zimmermann, PhD\textsuperscript{c}

\textsuperscript{a}Estacion Experimental Marcos Juarez, INTA
Córdoba, Argentina
\textsuperscript{b}Elanco Animal Health
Scalabrini Ortiz 3333
Buenos Aires, Argentina
\textsuperscript{c}Elanco Animal Health
P. O. Box 708
Greenfield, IN 46140

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\section*{ABSTRACT}

A total of 120 purebred Hereford cattle were selected from a herd on a ranch in Argentina that had a severe outbreak of infectious bovine keratoconjunctivitis (IBK, pinkeye) caused by \textit{Moraxella bovis}. The animals were separated into six treatment groups: a nonmedicated control group, a group that received oxytetracycline at 300 mg injected intrapalpebrally, and four groups that received tilmicosin (Micotil\textsuperscript{®}, Elanco Animal Health, Indianapolis, IN; one group injected intrapalpebrally at 300 mg and three groups injected subcutaneously at 2.5, 5, and 10 mg/kg body weight, respectively). Animals were individually observed for resolution of lesions associated with IBK (ocular discharge, blepharospasm, and corneal lesions) every 7 days for 3 weeks. Corneal improvement was significantly better ($P \leq .05$) for all doses and for either route of injection for tilmicosin compared with no treatment or treatment with oxytetracycline. Tilmicosin given subcutaneously demonstrated a significant ($P \leq .05$) dose response for overall improvement (one or more score improved, none worsened). Tilmicosin given subcutaneously at 10 mg/kg was significantly more effective than tilmicosin at 2.5 mg/kg, oxytetracycline, and no treatment. Results for tilmicosin at 5 mg/kg were numerically better than no treatment, and tilmicosin at 10 mg/kg was numerically better than the drug given by intrapalpebral injection. Tilmicosin given by subcutaneous injection at 5 or 10 mg/kg was effective against IBK under the conditions of this study.

\section*{INTRODUCTION}

Infectious bovine keratoconjunctivitis (IBK)
is one the most widespread and common infectious diseases of cattle throughout the world. The causative agent is the gram-negative bacteria *Moraxella bovis*, although predisposing agents such as ultraviolet radiation, environmental dust, or infectious bovine rhinotracheitis (IBR) virus infection appear to play a role in the etiopathogenesis of the disease. However, disease replication is possible after inoculating experimental pure cultures of pathogenic strains of *M. bovis*.

Pathologically, the bacterium primarily produces corneal ulceration, with white cell infiltration in the eye’s anterior chamber (hypopyon) and corneal tissue neovascularization. This induces temporary or permanent loss of vision in severely affected eyes. Infrequently, the cornea can be perforated, creating an orifice through which the basal membrane (keratocorne) can protrude. If the process continues, permanent loss of vision in the affected eye can result. Corneal tissue can heal spontaneously or with successful medical treatment, and leukocyte infiltration in the anterior chamber can be eliminated. Sometimes, however, cloudy scars remain in the corneal tissue and corneal recovery is partial.

Economic losses produced by the disease are variable, depending on the severity of the outbreak. In cases with bilateral blindness, the growth rate of steers or calves has been estimated to be reduced by up to 25% in comparison with healthy animals from the same lot and genetic quality. Additionally, administration of medical treatments and animal movement can incur considerable expenses, which can negatively influence production profitability.

The incidence and prevalence of this infectious process among and within herds determine whether preventative and/or therapeutic treatment is necessary. For prevention of IBK in Argentina, bacterins against *M. bovis* are used alone or in combination with inactivated IBR virus. The efficacy of this approach is relatively low due to the complexity of the etiopathogenic and immunologic processes caused by the organism’s virulence factors. Different drugs have been used successfully for treatment via different routes. *Moraxella bovis* is sensitive to a wide variety of antibiotics in vitro, but the organism’s special location limits the efficacy of treatment to only those drugs with good access to the site. One of the antibiotics most frequently used is oxytetracycline, especially the long-acting intramuscular formulation. The main goals of therapy are to reduce healing time of ocular lesions and avoid relapses. Procaine penicillin G is also used, and even though its efficacy is similar to that of long-acting tetracycline, it often has a higher rate of relapses. The chief disadvantage of using the intramuscular route for treatments is the large amount of drug that has to be administered. The subconjunctival route also has been used successfully (most frequently with penicillin), even though it is very difficult and can cause ocular tissue damage. However, subconjunctival treatment requires only a small amount of drug and achieves high antibiotic levels in lacrimal fluid and ocular tissue. Topical treatments, the most popular of which is furazolidone, can be effective in mild cases especially if administration can be repeated several times. The major disadvantage of topical treatment is the short duration of action of the active ingredient in ocular tissues due to the limited lacrimal fluid and low levels of drug achieved in the ocular tissue. Application of a protective patch over the affected eye can also be beneficial in that it protects the eye from irritants, but labor and poor adhesiveness make this procedure less attractive.

The objective of this study was to determine the therapeutic efficacy of different dosage levels and inoculation routes of the macrolide antibiotic tilmicosin (Micotil®, Elanco Animal
Health, Indianapolis, IN) against naturally occurring cases of IBK and to compare this efficacy with a subconjunctival dose of oxytetracycline and a nontreated control group. Before the primary study to investigate the use of intrapalpebrally injected tilmicosin could be performed, it was necessary to conduct a pilot study to determine whether the tissue reaction produced after intrapalpebral injection was strong enough to discourage its use or would be temporary with no secondary and/or permanent consequences.

**MATERIALS AND METHODS**

**Pilot Study**

The pilot study was conducted in 16 young steers that were injected in the eyelid with 150 or 300 mg of tilmicosin (0.5 or 1 ml). Tissue reactions were evaluated at 0, 24, 48, 96, and 120 hours after injection. Cattle treated with 300 mg were injected in both eyes, whereas the group treated with 150 mg was injected in the right eye only. Eyelid skin thickness was measured at all evaluation times for cattle treated with 300 mg of tilmicosin, but this measurement was conducted only at 0, 24, and 96 hours for the group treated with 150 mg.

**Primary Study**

**Animals**

The test animals came from a herd of 1250 purebred Hereford steers approximately 6 months of age on a farm in the province of Santa Fe, Argentina. The animals were recently weaned and were grazing on a new alfalfa pasture with excellent forage availability. When the first cases of IBK were observed, the affected animals were separated from the herd but no therapies were administered. In that way, the 120 animals used in this study were obtained over a 5-day period. Before treatment assignment, the cattle were weighed and identified by a numbered ear tag; individual ocular lesion levels were classified and recorded in a notebook following the classification criteria explained below. An ocular conjunctival swab was obtained from each animal’s eye for isolation of *M. bovis* and determination of the outbreak infection level. The ocular swabs were immediately inoculated on 5% equine blood agar, and the plates were incubated at 37°C for 24 hours under aerobic conditions. The suspect colonies were re-inoculated to permit purification. Once pure cultures were established, different biochemical tests were performed, including Gram’s staining, catalase, oxidase, nitrate reduction, Kligler medium growth, and gelatin liquefaction. The strains were then lyophilized, and the sensitivity to various antibiotics was evaluated.

**Experimental Design**

A randomized block design was used. The 120 affected steers were blocked into 10 groups by severity of ocular lesions. Individual lesion scores for each factor were totaled for each animal. Treatments were allocated approximately evenly across varying levels of disease. Animals within blocks were sequentially allocated to provide approximately 20 animals per treatment. The treatment groups were as follows: negative control (no treatment); positive control (long-acting oxytetracycline at 300 mg by intrapalpebral injection); tilmicosin at 2.5 mg/kg by subcutaneous injection; tilmicosin at 5 mg/kg by subcutaneous injection; tilmicosin at 10 mg/kg by subcutaneous injection; tilmicosin at 300 mg by intrapalpebral injection. For intrapalpebral injections of oxytetracycline or tilmicosin, the animal was immobilized. The eyelid skin was lifted to provide an acute angle with respect to the eye’s surface, and an 18-gauge needle was inserted into the base of the fold created. Subcutaneous injections of tilmicosin were given in the retroscapular region.
Lesion Scoring Criteria

Three types of ocular lesions were considered: ocular discharge, blepharospasm, and corneal opacity. The severity of each type of lesion was given a numeric value using the following scales:

Ocular discharge or weeping: 0 = no discharge or normal weeping; 1 = serous discharge or moderate weeping; 2 = purulent discharge.

Blepharospasm: 0 = normal eyelids; 1 = partially closed eyelids; 2 = swollen and partially closed eyelids; 3 = completely closed and collapsed eyelids.

Corneal opacity: 0 = no opacity; 1 = opacity or moderate ulcers; 2 = opacity or severe ulcers with vision compromise; 3 = protrusion of internal membranes (keratocone).

Clinical observations and lesion scoring were performed 0, 7, 14, and 21 days after treatment. Animals were restrained in a head gate, and each animal was individually observed by a person who was blinded to treatments. The animals were individually weighed before treatment on Days 0 and 21.

The two primary variables considered for determining the efficacy of treatments were corneal improvement and overall improvement. Both were determined for each individual animal, using all affected eyes and ignoring the status of an eye that was not affected at Day 0.

Corneal lesions were considered improved if the lesion score for Day 21 was less than the score on Day 0. For an animal with both eyes affected, one or both had to have a reduced score and neither eye could have an increased score. Corneal lesions were said to have no improvement if the lesion score on Day 21 was greater than the score on Day 0 for one or both eyes or if the score was unchanged from Day 0 to Day 21.

Overall improvement was said to be favorable if one or more scores for ocular discharge, blepharospasm, and corneal lesions on Day 21 were less than on Day 0 and none of the individual scores were greater on Day 21. The outcome was rated unfavorable if one or more scores were greater on Day 21 or all scores were equivalent on Days 0 and 21.

Body Weight

Cattle were individually weighed on Day 0 before treatment and again on Day 21. Average daily gain (ADG) was calculated for each animal.

Statistical Evaluations

Prior to analysis, corneal improvement data were stratified into two groups by initial level of corneal lesions. Animals for which neither eye received a corneal lesion score greater than 1 on Day 0 were considered mildly affected. Cattle with a corneal lesion score of 2 or higher in either eye were considered moderately affected.

Overall improvement data were stratified based on the mean of all three scores for both eyes on Day 0. Eyes with a mean overall initial score of 0.5 or lower were considered mildly affected; those with scores above 0.5 were considered moderately affected. Because some combinations of stratum and improvement result were sparsely represented, treatment comparisons were conducted with exact statistical methods to ensure the validity of $P$ values. The proportions improved for each treatment were compared pairwise by forming $2 \times 2$ tables for each stratum and using a stratified exact analysis of the odds ratio. Homogeneity of odds ratios across the two strata was verified with an exact test.

Average daily gain was compared among treatments using one-way analysis of variance, and pairwise contrasts of treatment means were used to identify differences between individual treatments. Initial severity of the disease was considered as a covariate in the analysis of weight gains; however, this factor was not sig-
The only covariate included in the analysis was initial weight of the animals. Therefore, least squares means were adjusted so that ADG was calculated for all treatments based on the average initial weight for all 120 animals.

■ RESULTS

Pilot study

Results of upper eyelid skin thickness responses to the intrapalpebral tilmicosin injections are summarized in Table 1. There was no appreciable difference clinically between the two dosages in terms of the extent or duration of the swelling of the eyelid; therefore, 300 mg (1 ml) was selected as the intrapalpebral dose of tilmicosin for the primary study.

Primary Study

Bacteriology

Eighteen strains of *M. bovis* were isolated, identified, and lyophilized from cultures from 60 animals. The susceptibility of these isolates

<table>
<thead>
<tr>
<th>Tilmicosin 300 mg</th>
<th>Time 0</th>
<th>24 hr</th>
<th>48 hr</th>
<th>120 hr</th>
</tr>
</thead>
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<tr>
<td>Animal No.</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>11</td>
<td>0.6</td>
<td>0.4</td>
<td>1.9</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>0.8</td>
<td>0.4</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>13</td>
<td>0.7</td>
<td>0.5</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>14</td>
<td>0.8</td>
<td>0.7</td>
<td>2.6</td>
<td>3.6</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>0.4</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td>16</td>
<td>0.6</td>
<td>0.8</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td>17</td>
<td>0.9</td>
<td>0.6</td>
<td>2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>18</td>
<td>0.8</td>
<td>0.7</td>
<td>2.8</td>
<td>2.4</td>
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<tr>
<td>Mean</td>
<td>0.78</td>
<td>0.56</td>
<td>2.53</td>
<td>2.70</td>
</tr>
<tr>
<td>SD</td>
<td>0.13</td>
<td>0.15</td>
<td>0.36</td>
<td>0.44</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Tilmicosin 150 mg</th>
<th>Time 0</th>
<th>24 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal No.</td>
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<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>31</td>
<td>0.6</td>
<td>0.7</td>
<td>2.8</td>
</tr>
<tr>
<td>32</td>
<td>0.6</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td>33</td>
<td>0.9</td>
<td>0.6</td>
<td>2.6</td>
</tr>
<tr>
<td>34</td>
<td>1.0</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>35</td>
<td>0.7</td>
<td>0.4</td>
<td>3.1</td>
</tr>
<tr>
<td>36</td>
<td>0.8</td>
<td>0.7</td>
<td>2.7</td>
</tr>
<tr>
<td>37</td>
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<td>2.2</td>
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<tr>
<td>38</td>
<td>0.7</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mean</td>
<td>0.74</td>
<td>0.59</td>
<td>2.48</td>
</tr>
<tr>
<td>SD</td>
<td>0.15</td>
<td>0.15</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Figure 1. Group mean ocular discharge (A), blepharospasm (B), and corneal lesion (C) scores for cattle (scored 0 to 2) with infectious bovine keratoconjunctivitis before and after treatments. (IP = intrapalpebral injection; SC = subcutaneous injection)
has been previously reported. The minimum inhibitory concentration for 90% of all isolates was below 0.1 µl/ml tilmicosin.

Lesion Scores

Mean ocular lesion scores for affected eyes are presented graphically by individual variable (ocular discharge, blepharospasm, corneal lesion) in Figure 1. Total combined ocular scores are presented in Figure 2. If an animal had two affected eyes, the scores for both were averaged and this was used in the calculation of the group mean.

The numbers of animals with improved corneal lesion scores and the numbers demonstrating overall improvement are shown by severity of the condition within each treatment group in Table 2. The percentages of animals with improved corneal and overall scores are displayed by treatment in Figure 3.

All dosages and forms of tilmicosin provided similar results in terms of the percentage of animals demonstrating improved corneal scores after treatment. All groups treated with tilmicosin had significantly ($P < .05$) better corneal responses than did those treated with oxytetracycline. Corneal lesion scores were numerically more improved for all tilmicosin groups than for the negative control group, but the difference was not significant. Tilmicosin given by subcutaneous injection at 10 mg/kg had significantly ($P < .05$) more positive overall responses than did the control group and the groups treated with either oxytetracycline or tilmicosin at 2.5 mg/kg (Figure 3).

Body Weight

Body weight data are presented by treatment group in Table 3. Examination of ADG during
the 21 days of the trial did not reveal any trends for tilmicosin dosages (i.e., higher dosages of tilmicosin did not produce better weight gain). Cattle treated with tilmicosin at either 2.5 or 5.0 mg/kg had significantly ($P < .05$) better ADG than did cattle treated with long-acting oxytetracycline.

### DISCUSSION

In the pilot study, the irritation process caused by the intrapalpebral tilmicosin injection did not seem to be dose dependent. Eyelid thickness 24 hours after treatment was not clinically different between the two dosage groups. The observed lesions appeared to be only an allergic-type reaction, with some edema in the eyelid's skin. No fluid collection or fistulous tracts were observed. The eyelid lesions decreased in size after 48 hours, and at 96 and 120 hours after treatment the eyelid appeared to be almost normal. Lesions were not observed macroscopically but rather felt like small nodules in the eyelid skin. It could be speculated that lesions would be more evident in smaller animals. However, based on the similarity of lesion severity between the 150- and 300-mg doses, it was hypothesized that lower dose administration would not reduce the tissue reaction at the same rate. It would be necessary to know the tilmicosin concentration in lacrimal fluid after injecting 150 mg and to determine whether this concentration is as active against *M. bovis* as is 300 mg of tilmicosin.

The primary study represents a natural out-
TABLE 3. Body Weight Data for Cattle Treated with Different Medications for Infectious Bovine Keratoconjunctivitis

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Control</th>
<th>Oxytetracycline 300 mg</th>
<th>Tilmicosin 2.5 mg/kg</th>
<th>Tilmicosin 5 mg/kg</th>
<th>Tilmicosin 10 mg/kg</th>
<th>Tilmicosin 300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>156.4</td>
<td>145.5</td>
<td>154.7</td>
<td>156.8</td>
<td>149.9</td>
<td>145.6</td>
</tr>
<tr>
<td>Final</td>
<td>168.8</td>
<td>153.9</td>
<td>170.4</td>
<td>171.6</td>
<td>163.2</td>
<td>159.4</td>
</tr>
<tr>
<td>Average daily gain*</td>
<td>0.59&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.66&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Least squares mean, adjusted for initial body weight for all animals. Average daily gain was calculated for each animal as (final weight – initial weight)/21 days.

<sup>a,b</sup> Values within a row with no common superscript letters are significantly different (P < .05).

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Figure 3. Percentage improvement in corneal lesion and overall scores in cattle treated for keratoconjunctivitis. <sup>a,b</sup> Treatments with different letters are significantly different (P < .05). <sup>c,d</sup> Treatments with different letters are significantly different (P < .05). (IP = intrapalpebral injection; SC = subcutaneous injection)

break of the disease, thus it must be taken into account that lesion severity could be unbalanced among animals at trial initiation. It must also be considered that during the 5 days it took to assemble the 120 animals some spontaneous healing process could have taken place before treatments were given. It is also important to note that the evaluation system used is subjective; however, the statistical analysis performed on corneal and overall improvement
did not utilize a numeric value for the subjective lesion score but rather utilized only the ordering of the lesion score values. In some situations, superposition of an initial lesion with an active infection could exist, in contrast to the evaluation of a healing scar lesion for which medical treatment would be inefficient. However, it has been observed that lesion evaluation is highly reproducible and therefore the potential for error is uniform across all treatments.

Results of this study suggest that the highest dosage of tilmicosin (10 mg/kg) had greater persistence in ocular tissues and this persistence was able to prevent further infections whether due to *M. bovis* or normal flora, which can occasionally complicate the healing process. However, this hypothesis would require confirmation by bacteriologic and pharmacodynamic studies.

Further work is required to determine whether weight gain advantages observed for cattle treated with tilmicosin in this study are attributable to treatment and whether these advantages are sustained through the growing period.

## CONCLUSION

The use of tilmicosin by subcutaneous injection at 5 or 10 mg/kg is therapeutically beneficial for treatment of IBK based on the findings of this study. Clinical improvement achieved by treating cattle with tilmicosin was superior to results obtained with oxytetracycline, one of the most commonly used and researched treatments. Different application strategies should be studied to optimize the cost:benefit relationship, which will differ according to particular farm characteristics, production systems, and aggressiveness of the bacterial strain involved. Administration of tilmicosin by intrapalpebral injection also was effective in this study, but there are insufficient safety data (animal and administrator) to support this route of administration.

## REFERENCES