Efficacy of difloxacin in calves experimentally infected with *Mannheimia haemolytica*

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Objective—To determine the efficacy of difloxacin, a novel fluoroquinolone antibiotic, in calves experimentally infected with *Mannheimia haemolytica* (formerly *Pasteurella haemolytica)*.

Animals—Seventy-two 3-month-old Holstein calves.

Procedures—Calves were inoculated with *M haemolytica* intratracheally; after they developed clinical signs of pneumonic pasteurellosis, they were randomly assigned to 1 of 6 groups (n = 12/group). Calves in each group were treated with 10% difloxacin (2.5 or 5 mg/kg of body weight), 5% difloxacin (2.5 or 5 mg/kg), enrofloxacin (5 mg/kg), or saline (0.9% NaCl) solution (control group), once daily for 5 days, and clinical signs were scored daily. On day 15, calves were euthanized, and the percentage of diseased lung tissue was calculated. Swab specimens of the lungs were submitted for bacterial culture.

Results—Mortality rate and percentage of diseased lung tissue were significantly higher and cure rate and average daily gain were significantly lower for control calves, compared with calves in the treatment groups; however, no significant differences were found among treatment groups. *Mannheimia haemolytica* was isolated from the lungs of 10 control calves and from at least 2 calves in each of the treatment groups.

Conclusions and Clinical Relevance—Results suggest that difloxacin and enrofloxacin were equally effective for treatment of calves with experimentally induced pneumonic pasteurellosis. However, treatment of infected calves with difloxacin or enrofloxacin may not eliminate the organism. (*Am J Vet Res* 2000;61:710–713)

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A variety of bacterins and vaccines have been developed to protect calves from infection with *Mannheimia haemolytica* (formerly *Pasteurella haemolytica)*. Unfortunately, experimental and field studies of the efficacy of these products have yielded inconsistent results,[1,2] and protection from disease can be inconsistent,[3-5] particularly because of stress associated with common cattle management practices such as weaning, dehorning, and transportation.[6] Therefore, antibiotic treatment will remain an important part in the management of calves with pneumonic pasteurellosis. The purpose of the study reported here was to determine the efficacy of difloxacin, a novel fluoroquinolone antibiotic, in calves experimentally infected with *M haemolytica*.

Received Jan 29, 1999.
Accepted May 26, 1999.
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Funds provided by Fort Dodge Animal Health.
The authors thank Agam Sinha for assistance with statistical analyses.

Materials and Methods

Animals—Weaned male Holstein calves (mean weight, 76.4 kg) free of clinical signs of disease that were approximately 3 months old and had been purchased from local dairies were used. Calves were acclimatized to individual slatted-floor hutches and provided water and a pelleted complete calf-grower ration[a] ad libitum. The feed did not contain probiotic, growth-promoting, or antibiotic additives. Throughout the study, calves were examined daily by a single individual (TNT) who was not aware of group assignments of the calves.

Bacterial challenge exposure procedure—*Mannheimia haemolytica* A1 (isolate D92-06252)[b] grown in brain-heart infusion broth at 37 C was used for inoculation of calves. Calves were administered 2 doses of bacteria in the log phase of growth by the intratracheal route. The first inoculum (10 ml with 6.4×10^8 **colony-forming units [CFU]**/ml) was acidified with acetic acid to a pH of 4.5 immediately before administration. The second inoculum (15 ml with 8.7×10^{10} CFU/ml) was administered approximately 4 hours later. Calves were examined 24 and 48 hours after inoculation with *M haemolytica*, and calves with rectal temperature > 40 C, respiratory score > 1, and appetite or attitude score > 2 **(Appendix)** were included in the study. Approximately 72% of the calves inoculated with *M haemolytica* were included in the study.

Experimental procedure—Calves included in the study were randomly assigned to 1 of 6 groups (n = 12/group) by use of random numbers generated with a computer software program.[c] Forty-two calves (7/group) were included in the study 24 hours after bacterial inoculation; 30 (5/group) were included 48 hours after bacterial inoculation. For all calves, experimental treatments were begun the day the calf was included in the study; the first day of treatment was considered day 1. Treatments were administered by an individual (RLH) who was not involved with clinical assessments of the calves. Treatment dosages were based on body weight the day prior to bacterial inoculation.

Calves in each of the groups were treated with difloxacin (10%)[d] at a dosage of 2.5 mg/kg of body weight, SC, every 24 hours for 5 days; difloxacin (10%) at a dosage of 5 mg/kg, SC, every 24 hours for 5 days; difloxacin (5%)[e] at a dosage of 2.5 mg/kg, SC, every 24 hours for 5 days; difloxacin (5%) at a dosage of 5 mg/kg, SC, every 24 hours for 5 days; enrofloxacin (5%)[f] at a dosage of 5 mg/kg, SC, every 24 hours for 5 days; or saline (0.9% NaCl) solution at a dosage of 0.1 ml/kg, SC, every 24 hours for 5 days (control group).

Rectal temperatures were measured, and respiratory, appetite, and attitude scores were assigned daily for 15 days (Appendix). Calves were considered to be cured if all scores were 1, and rectal temperature was < 40 C by day 6. Calves were considered to have had a relapse if at any time after they were considered cured they had 2 consecutive days when rectal temperature was ≥ 40 C, respiratory score was > 1, attitude score was > 2, or appetite score was > 2.

On day 15, calves were euthanatized and necropsied. Lungs were removed for gross examination, and the amount of diseased tissue in each lung lobe was estimated to the nearest 10% (or to the nearest 5% if < 10% of the lobe was diseased) by visual observation and palpation. Total percentage of diseased lung tissue was calculated by use of the following equation[7]:

Total diseased lung = (A X 0.05) + (B X 0.06) + (C X 0.32) + (D X 0.04) + (E X 0.35) + (F X 0.07) + (G X 0.05) + (H X 0.06)

where A is the diseased percentage of the cranial part of the left cranial lung lobe, B is the diseased percentage of the caudal part of the left cranial lung lobe, C is the diseased percentage of the left caudal lung lobe, D is the diseased percentage of the accessory lung lobe, E is the diseased percentage of the right caudal lung lobe, F is the diseased percentage of the right middle lung lobe, G is the diseased percentage of the caudal part of the right cranial lung lobe, and H is the diseased percentage of the cranial part of the right cranial lung lobe.

Swab specimens were aseptically collected from a diseased portion of each calf's lungs and submitted for bacterial culture. In calves without visible evidence of pulmonary disease, a swab specimen was collected from the right middle lung lobe. Specimens were plated on blood, chocolate, and MacConkey agar. Colonies that grew were identified on the basis of hemolytic character, gram-staining reaction, oxidase and catalase production, indole and urea use, and growth characteristics on triple sugar iron media slants.

Statistical analyses—Rectal temperature, respiratory score, appetite score, attitude score, average daily gain, and percentage of diseased lung were compared among groups by use of ANOVA, followed by the least-significant difference test. Respiratory, appetite, and attitude scores were tested for normal distribution by use of the Shapiro-Wilk test[8]; all scores underwent rank-based normal score transformation prior to statistical analyses. For each experimental group, mortality, cure, and relapse rates were compared with values for the control (saline solution) group by use of 1-sided Fisher exact tests. For all analyses, a value of $P < 0.05$ was considered significant.

Results

Clinical assessment—For all calves, clinical abnormalities were limited to signs of respiratory tract disease. Five control calves died of acute fibrinous pneumonia during the study, whereas all 60 calves treated with difloxacin or enrofloxacin survived (**Table 1**). Mortality rate for the control group was significant-

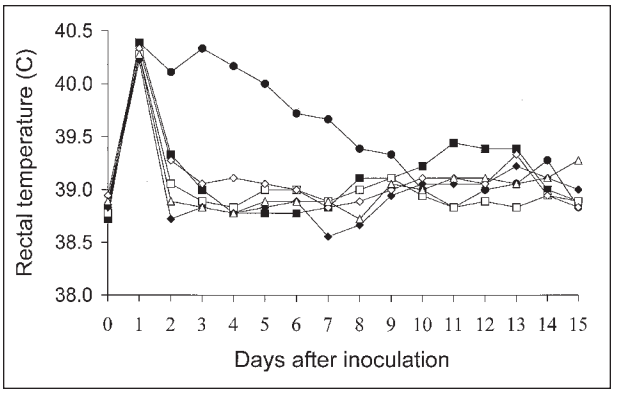

Figure 1—Mean rectal temperature of calves (n = 12/group) with experimentally induced pneumonic pasteurellosis treated with 10% difloxacin (2.5 mg/kg of body weight, ■; 5 mg/kg, □), 5% difloxacin (2.5 mg/kg, ◆; 5 mg/kg, ◇), 5% enrofloxacin (5 mg/kg, Δ), or saline (0.9% NaCl) solution (●), SC, every 24 hours for 5 days.

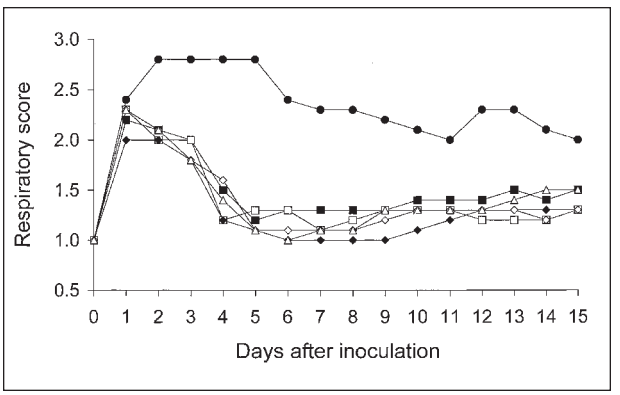

Figure 2—Mean respiratory scores of calves with experimentally induced pneumonic pasteurellosis treated with difloxacin or enrofloxacin. *See* Figure 1 for key.

Table 1—Efficacy of difloxacin and enrofloxacin in treatment of calves (n = 12/group) with experimentally induced pneumonic pasteurellosis

Treatment*	Mortality rate†	Cure rate‡	Relapse rate§	Average daily gain (kg/calf/d)	Percentage of diseased lung	Culture-positive rate‖
Difloxacin 10%						
2.5 mg/kg	0/12	8/12	4/8	0.67	15	3/12
5 mg/kg	0/12	11/12	2/11	0.79	18	5/12
Difloxacin 5%						
2.5 mg/kg	0/12	12/12	3/12	0.90	14	5/12
5 mg/kg	0/12	10/12	3/10	0.82	14	2/12
Enrofloxacin 5%						
5 mg/kg	0/12	10/12	5/10	0.77	16	4/12
Saline (0.9% NaCl) solution						
0.1 ml/kg	5/12¶	3/12¶	1/3	−0.34¶	36¶	10/12

*All drugs were given SC every 24 hours for 5 days. †No. of calves that died/No. of calves in group. ‡No. of calves cured/No. of calves in group; calves were considered to be cured if respiratory, attitude, and appetite scores were 1, and rectal temperature was < 40 C by day 6. §No. of calves that relapsed/No. of calves cured; calves were considered to have had a relapse if at any time after they were considered cured they had 2 consecutive days when rectal temperature was ≥ 40 C, respiratory score was > 1, attitude score was > 2, or appetite score was > 2. ‖No. of calves from which *Mannheimia haemolytica* was isolated at necropsy/No. of calves in group. ¶Significantly ($P < 0.05$) different from values for other groups.

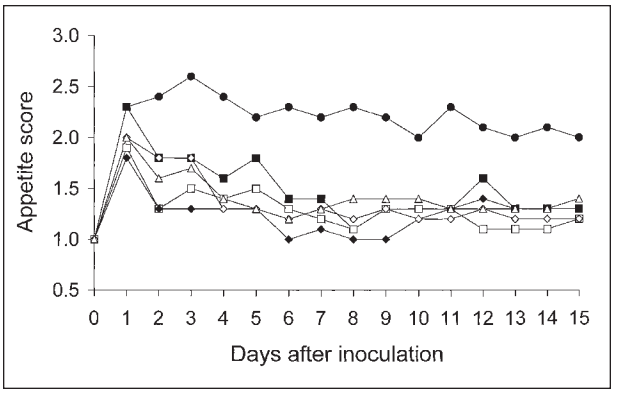

Figure 3—Mean appetite scores of calves with experimentally induced pneumonic pasteurellosis treated with difloxacin or enrofloxacin. *See* Figure 1 for key.

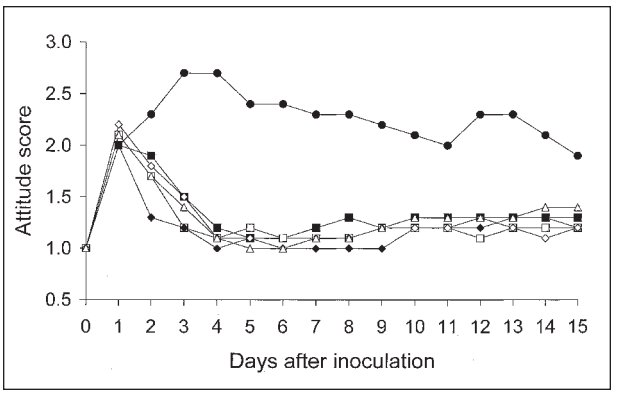

Figure 4—Mean attitude scores of calves with experimentally induced pneumonic pasteurellosis treated with difloxacin or enrofloxacin. *See* Figure 1 for key.

ly higher than mortality rate for the treatment groups. Average daily gain for the control calves was significantly less than average daily gain for calves in the treatment groups.

Rectal temperatures of all calves were > 40 C on day 1. Rectal temperatures decreased rapidly during the first 24 hours in calves treated with difloxacin or enrofloxacin but remained > 39.4 C until day 7 in control calves (**Fig 1**). Mean rectal temperatures for calves in the treatment groups were significantly lower than mean rectal temperatures for calves in the control group from day 2 through day 7.

For all calves, respiratory score on day 1 was ≥ 2 (**Fig 2**). Mean respiratory scores for calves in the treatment groups were significantly lower than mean score for calves in the control group from day 2 through day 14. Similarly, mean appetite scores for treatment groups were significantly lower than mean score for calves in the control group from day 2 through day 12 (**Fig 3**), except that mean score for calves treated with difloxacin 10% at a dosage of 2.5 mg/kg was not significantly different from mean score for the control group on days 5 and 12. For all calves, attitude scores on day 1 were ≥ 2. Mean attitude scores for the treatment groups were significantly lower (less depression) than the mean score for calves in the control group from day 2 through day 14 (**Fig 4**), except that mean scores for calves treated with difloxacin 10% at a dosage of 2.5 mg/kg were not significantly different from mean scores for the control group on day 2. Cure rate for the control group was significantly lower than cure rate for the treatment groups (Table 1). Mean percentage of diseased lung for the control group was significantly greater than mean percentages for the treatment groups. *Mannheimia haemolytica* was isolated from the lungs of 10 control calves and from at least 2 calves in each of the treatment groups.

Discussion

In the present study, calves inoculated with *M haemolytica* developed severe pneumonic pasteurellosis, and clinical signs and gross pathologic findings in these calves were similar to findings in a previous study.[9] One drawback to this protocol for experimental induction of pasteurellosis is the rapid onset of severe disease, which could become refractory to treatment within as short a time as a few hours.[10] The effect of this, however, would be to underestimate the efficacy of the drugs used, compared with use in a field situation in which calves with a wider spectrum of disease severity would likely be encountered. One other important drawback was that we were able to evaluate only clinical efficacy, whereas in a field situation such as a feedlot, the economic benefit of treatment would be equally, if not more, important. It is conceivable, for instance, that a calf treated with difloxacin or enrofloxacin would survive, but the cost of the drug, the labor to administer the drug and observe the calf, the increase in days to market, and other factors would make treatment impractical economically.

Difloxacin and enrofloxacin were found to be equally effective for treatment of calves with experimentally induced pneumonic pasteurellosis in this study and resulted in substantial improvements in clinical signs, average daily gain, and percentage of diseased lung. In addition, use of 10 versus 5% difloxacin and use of a low (2.5 mg/kg, q 24 h) versus high (5 mg/kg, q 24 h) dosage of difloxacin did not seem to have any effect on efficacy. However, *M haemolytica* was recovered from the lungs of several treated calves, indicating that treatment of infected calves with difloxacin or enrofloxacin may not eliminate the organism.

[a]Formula 516990, OH Kruse, Tulare, Calif.
[b]California Veterinary Diagnostic Laboratory, Davis, Calif.
[c]Microsoft Excel for Windows 95, version 5.0, Microsoft Corp, Redmond, Wash.
[d]Difloxacin 10% injectable solution, Fort Dodge Animal Health, Princeton, NJ.
[e]Difloxacin 5% injectable solution, Fort Dodge Animal Health, Princeton, NJ.
[f]Baytril 5%, Bayer AG, Leverkusen, Germany.

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Appendix

Scoring systems for determining severity of clinical signs of disease in calves with experimentally induced pneumonic pasteurellosis

Score	Clinical signs
Respiratory score	
1	< 50 breaths/min; no coughing
2	50 to 70 breaths/min, with or without mucoid nasal discharge
3	> 70 breaths/min, with or without purulent nasal discharge
Attitude score	
1	Bright, alert, and responsive
2	Slightly lethargic; standing, but not exploring its environment
3	Moderately lethargic; recumbent, but will rise with stimulation
4	Severely lethargic; recumbent and will not rise
Appetite score	
1	Consumes at least two-thirds of food offered over 24 hours
2	Consumes one- to two-thirds of food offered over 24 hours
3	Consumes less than one-third of food offered over 24 hours