Effects of tilmicosin treatment on *Pasteurella haemolytica* organisms in nasal secretion specimens of calves with respiratory tract disease

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Objective—To determine the effect of tilmicosin treatment on number of *Pasteurella haemolytica* (PH) organisms in nasal secretion specimens of calves with respiratory tract disease.

Animals—206 British mixed-breed beef calves, 2 to 5 months old.

Procedure—In 2 separate studies of outbreaks, calves (study 1, n = 101; study 2, n = 105) that developed respiratory tract disease after transport to a feedlot were treated with tilmicosin. Nasal secretion specimens were examined for PH organisms to determine the status of colonization.

Results—In both studies, PH serotypes A1 and A6 were isolated. In study 1, tilmicosin treatment eliminated or markedly reduced the number of PH organisms in calves on days 1, 4, and 5 after treatment. In study 2, tilmicosin treatment eliminated PH organisms in calves on days 1, 2, 5, and 6 after treatment.

Conclusions and Clinical Relevance—Overall, tilmicosin treatment increased the number of culture-positive calves that became culture-negative and decreased the number of culture-negative calves that became culture-positive for up to 6 days after treatment. Tilmicosin treatment decreased the number of PH organisms in nasal secretion specimens, which indicated that fewer PH organisms were available to infect the lungs or to infect other calves. By reducing colonization, prophylactic use of tilmicosin before transport or at the time of arrival at a feedlot is likely to reduce the incidence of acute respiratory tract disease in calves for the initial several days after arrival, which is the period when they are most susceptible to infectious organisms. (*Am J Vet Res* 2000;61: 525–529)

Pasteurella haemolytica (PH) inhabits the tonsils and nasal passages of healthy cattle, representing a small portion of the normal bacterial flora.[1-5] After transport or during viral-induced illnesses, PH serotype A1 can undergo rapid, selective growth in the nasopharynx. This selective and profound population increase is a likely prerequisite for the onset of pneumonic pasteurellosis.[6,7]

During 2 separate field studies, an outbreak of respiratory tract disease was documented when calves arrived at a feedlot.[8] Calves with clinical signs of respiratory tract disease were treated with tilmicosin,[a] a macrolide antibiotic that attains therapeutic concentrations in pulmonary tissues.[9,b] It is effective against most PH isolates[10-13] and for use in treating undifferentiated respiratory tract disease in cattle.[10,14-19] In the study reported here, nasal secretion specimens obtained from transported calves were examined to determine the number of PH organisms after tilmicosin treatment and, thus, evaluate the effect of tilmicosin treatment on PH colonization of the nasopharynx.

Materials and Methods

Animals—For study 1, 101 British mixed-breed beef calves, 2 to 5 months old, were obtained from 4 farms.[8] Study 2 involved 105 British mixed-breed steer and bull beef calves, 2 to 5 months old. The calves were purchased at several regional sales barns, then congregated at a common order-buyer barn.

Experimental design

Study 1—Calves were transported approximately 50 km to a local order-buyer barn (days 0 to 2). Nasal secretions were collected. On day 6, calves were transported 1,600 km by truck to a feedlot at the Conservation and Production Research Laboratory (USDA, Agriculture Research Service) in Bushland, Tex; they arrived on day 7. The day after arrival (day 8), nasal secretions were collected (first feedlot specimen). Rectal temperatures were recorded daily. Calves with rectal temperature ≥ 40 C for 2 consecutive days were treated by administration of a single dose of tilmicosin phosphate (10 mg/kg of body weight, SC) on the second day on which they had the high rectal temperature. Nasal swab specimens were collected daily from all calves treated with tilmicosin but were collected from only 4 untreated calves. Nasal secretions were obtained from all calves again on day 14 (second feedlot specimen).

Study 2—Calves were congregated at the order-buyer barn, and nasal secretions were collected (days 0 to 2). On day 3, the calves were transported 1,600 km by truck to the same feedlot as in study 1; they arrived on day 4. The next day (day 5), nasal secretions were obtained (first feedlot specimen). Rectal temperatures were recorded daily. Calves with rectal temperature ≥ 40 C for 2 consecutive days were treated by administration of a single dose of tilmicosin phosphate (10 mg/kg of body weight, SC) on the second day on which they had the high rectal temperature. Nasal swab specimens were collected from treated and untreated calves on days 6, 7, and 8. Nasal secretions were obtained from all calves on day 12 (second feedlot specimen).

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Specimens—Nasal secretion specimens were collected by direct aspiration or by nasal swab. Specimens obtained by each method were processed and assayed by different means. Nasal secretions were aspirated from each ventral nasal meatus through a modified pipette into a trapping tube.[2,3] Immediately after collection, specimens of nasal secretions were supplemented with glycerol to a final concentration of 15% and stored on dry ice. After arrival at our laboratory, specimens of nasal secretions were stored at –70 C until analyzed. Nasal swab specimens were collected by inserting 2 cotton-tipped swabs into the nostrils. Swabs were placed into dry tubes, sealed, and stored on dry ice until transport to our laboratory for analysis.

Bacterial culture—In study 1, nasal secretions collected by aspiration were thawed and briefly sonicated, and serial 10-fold dilutions were prepared. A 0.1-ml aliquot of each dilution was applied to each of 2 blood agar base[c] plates containing 5% bovine blood. In study 2, specimens of nasal secretions collected by aspiration were treated in the same manner as the nasal swab specimens.

Nasal swab specimens were incubated overnight at 37 C. The PH colonies then were identified and serotyped.[20,21] In studies 1 and 2, nasal swab specimens were applied to blood agar base plates containing 5% bovine blood, using a standard procedure to form zones of decreasing growth that could be used for determining relative amount of PH organisms. Nasal swab specimens were thawed then streaked onto half of a blood agar base plate containing 5% bovine blood (zone 1). The plate was rotated 90°, and half of the plate was streaked from side to side with a sterile swab proceeding downward to include half of zone 1 and an adjacent one-fourth of the uninoculated plate (zone 2). The remaining fourth of the plate was streaked for colony isolation on 2 consecutive areas with a sterile loop (zones 3 and 4).

Determination of the effect of tilmicosin on colonization

Study 1—Initial PH colonization was determined by examination of bacterial cultures of the first feedlot nasal secretion specimens. Effect of tilmicosin treatment on colonization was determined by examination of bacterial cultures of nasal swab specimens and nasal secretions. A positive effect of tilmicosin was determined on the basis of a lack or reduction in the amount of PH organisms cultured from nasal swab specimens and nasal secretions collected after treatment. Reduction in the amount of PH organisms was defined as PH colonies in zones 2, 3, or 4 of the blood agar plates before treatment, but PH colonies only in zone 1 on 2 or more consecutive days after treatment.

Study 2—Effect of tilmicosin treatment on colonization was determined on the basis of the detection of PH organisms, or lack thereof, in bacterial cultures of each of the nasal secretion and nasal swab specimens collected before and after tilmicosin treatment.

Statistical analyses—Colonization status between groups of calves were compared by use of a χ^2 test. The Yates correction for continuity was used when necessary. The Fisher exact test was used when there was a small number of calves in a group. A value of $P < 0.05$ was considered significant.

Results

Respiratory tract disease at the feedlot

Study 1—Within 3 days after arrival at the feedlot, 70 calves were febrile, lethargic, and had clinical signs of respiratory tract disease. Sixty-seven calves were treated with tilmicosin on days 9 or 10, and 66 survived. One calf was treated on day 9, but it died as a result of pneumonic pasteurellosis on day 10. Four other calves became febrile and were treated on days 11 to 18. Of the 70 treated calves, 61 responded to treatment within 24 hours, as evidenced by a reduction in rectal temperature.

Study 2—After arrival at the feedlot, 8 calves died as a result of pneumonic pasteurellosis before they were treated (days 5 and 6). Two days after arrival (day 6), 53 calves had respiratory tract disease and were treated. On days 7 and 8, 5 and 4 additional calves, respectively, were treated. Of the 62 treated calves, 58 responded to treatment within 24 hours, as evidenced by a decrease in rectal temperature. Two untreated calves died as a result of pneumonic pasteurellosis on days 7 and 8. For studies 1 and 2, necropsies were conducted and diag-

Table 1—Effect of a single treatment of tilmicosin on *Pasteurella haemolytica* colonization of the nasopharynx of calves in a feedlot (study 1)

		Day of study*					
Day of treatment	**Colonization status†**	**8**	**9**	**10**	**11**	**12**	**14**
9 (n = 34)	+	25	27	7[a]	12	1	19[b]
	—	8	7	26‡	21§	31	14
	ND	0	0	0	0	1	0
10 (n = 33)	+	22	4	24[a]	8	1	17[c]
	—	11	2	9	25‡	32	16
	ND	0	27	0	0	0	0
Not treated (n = 31)	+	27	4‖	4‖	4‖	2‖	28[b, c]
	—	4	0	0	0	2	3
	ND	0	27	27	27	27	0

*Calves arrived at the feedlot on day 7. The first feedlot specimen was collected on day 8, and the second feedlot specimen was collected on day 14. †Colonization of nasopharynx inferred on the basis of results of bacterial culture of nasal secretions and nasal swab specimens. ‡Includes 6 calves with reduced numbers of *P haemolytica*. §Includes 3 calves with reduced numbers of *P haemolytica*. ‖Nasal swab specimens were collected from only 4 untreated calves on days 9 to 12.

+ = *P haemolytica* detected. — = *P haemolytica* not detected. ND = Not done.

[a]Values with the same superscript letter differed significantly ($P < 0.001$). [b]Values with the same superscript letter differed significantly ($P = 0.007$). [c]Values with the same superscript letter differed significantly ($P = 0.002$).

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Table 2—Effect of a single treatment of tilmicosin on *P haemolytica* colonization of the nasopharynx of calves in a feedlot (study 2)

Day of treatment	Colonization status†	Day of study* 5	6	7[a]	8[b]	12[c]
6 (n = 53)	+	47	48	7	2	9
	—	6	5	46	51	44
Not treated (n = 42)	+	33	34	28[a]	23[b]	16[c]
	—	8	8	14	12	13

*Calves arrived at the feedlot on day 4. The first feedlot specimen was collected on day 5, and the second feedlot specimen was collected on day 12. †Colonization of nasopharynx inferred on the basis of results of bacterial culture of nasal secretions and nasal swab specimens.

+ = *P haemolytica* detected. — = *P haemolytica* not detected.

[a-c]Values differed significantly ($P < 0.001$) between treated and not treated groups.

Table 3—Effect of a single treatment of tilmicosin on *P haemolytica* colonization of the nasopharynx of calves in a feedlot (study 2)

Day of treatment	No. of days between treatment and collection of second feedlot specimen	Colonization status at day 12* +	0	*P* value†
Not treated (n = 22)	NA	15	7	NA
6 (n = 48)	6	9	39	< 0.001
7 (n = 7)	5	1	6	0.026
8 (n = 3)	4	0	3	0.052
9 (n = 1)	3	0	1	0.348
10 (n = 10)	2	0	10	0.003
11 (n = 4)	1	0	4	0.022

*Day 12 = second feedlot specimen collected. †Fisher exact test to detect difference from calves that were not treated.

NA = Not applicable.

Table 4—Effect of tilmicosin treatment to cause culture-positive calves to become culture-negative and culture-negative calves to become culture-positive for *P haemolytica* (study 2)

Group	Day of study	Colonization status of nasopharynx before treatment	Day of subsequent sample collection	No. of calves	Colonization status of nasopharynx after treatment +	—	*P* value*
Treated	6	+	8	48	2	46	< 0.001
Not treated	6	+	8	34	20	14	
Treated	6	—	8	5	0	5	0.231
Not treated	6	—	8	8	3	5	
Treated	7	+	8	5	0	5	0.001
Not treated	7	+	8	23	19	4	
Treated	7	—	8	2	0	2	1.00
Not treated	7	—	8	12	4	8	
Treated	8–11	+	12	9	0	9	0.001
Not treated	8–11	+	12	16	11	5	
Treated	8–11	—	12	9	0	9	0.029
Not treated	8–11	—	12	8	4	4	

*Comparison of difference between treated and untreated calves that changed from culture-positive to culture-negative, or vice-versa. Overall, significantly ($P = 0.003$) fewer culture-negative calves given treatment became culture-positive, compared with culture-negative calves that were not treated.

noses established by the Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, Tex.

Serotypes of PH isolates—Two serotypes of PH, A1 and A6, were isolated from cultures of nasal secretion specimens and nasal secretions. On each day of culture, serotype A1 isolates were most numerous (study 1, 57 to 85%; study 2, 76 to 96%). Cultures containing both serotypes were collected from calves in study 1 (2 to 9%), but only in 2 samples from calves in study 2. During the course of the studies, some calves became culture-negative for PH, others became culture-positive, some lost a serotype, and others gained a serotype. Because PH organisms of both serotypes responded to tilmicosin treatment in the same manner, the serotypes were not addressed separately.

Isolation of *P haemolytica* after tilmicosin treatment

Study 1—The PH colonization status of 67 calves treated on days 9 and 10 and of 31 untreated calves were recorded during days 8 to 14 (**Table 1**). After treatment with tilmicosin on day 9, the number of calves that were culture-positive on days 10, 11, and 12 was significantly less than the number of culture-positive calves on day 9. Similarly, after treatment with tilmicosin on day 10, the number of calves that were culture-positive on days 11 and 12 was significantly less than the number of culture-positive calves on day 10. However, because there were only 4 untreated calves for comparison with all treated calves on days 10 or 11 and 12, the amount of natural cessation of PH colonization that would have resulted without treatment could not be determined. Therefore, comparisons were made between calves treated on day 9 with those treated on day 10 (effect for 1 day after treatment), calves treated on day 10 with untreated calves on day 14 (effect for 4 days after treatment), and calves treated on day 9 with untreated calves on day 14 (effect for 5 days after treatment). Tilmicosin treatment had a significant positive effect (lack or reduction of PH) on the

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PH colonization status of calves on days 1, 4, and 5 after treatment.

Study 2—The PH colonization status of all treated calves and all untreated calves were compared for each day during days 5 through 8 and day 12. Tilmicosin treatment had a significant ($P < 0.001$) positive effect (lack of PH) on the PH colonization status of calves for days 1, 2, and 6 after treatment (**Table 2**).

The effect of interval after tilmicosin treatment on the colonization status of all calves was determined on samples collected on day 12. Tilmicosin treatment had a significant positive effect (lack of PH) on the PH colonization status of calves 6 days ($P < 0.001$), 5 days ($P = 0.026$), 2 days ($P < 0.001$), and 1 day ($P = 0.022$) after treatment. The number of calves 4 and 3 days after treatment were too small for statistical analysis (**Table 3**).

Overall, tilmicosin treatment influenced the likelihood that a calf would become culture-positive or culture-negative for PH in nasal secretion specimens for up to 6 days after treatment. Treatment caused significantly more culture-positive calves to become culture-negative, whereas significantly fewer culture-negative calves became culture-positive (**Table 4**).

Discussion

In the 2 separate studies reported here, nasal secretion specimens were examined for PH organisms as a method for determining colonization status of the nasopharynx in feedlot calves. Tilmicosin treatment eliminated or reduced detectable colonization by PH for up to 6 days in transported calves with respiratory tract disease. Treatment increased the number of culture-positive calves that became culture-negative and decreased the number of culture-negative calves that became culture-positive.

Tilmicosin is effective for use in treating cattle with respiratory tract disease[9,10,14-18,b] and is used as a prophylactic treatment to prevent or delay the onset of respiratory tract disease.[9,22-24] Most PH isolates tested have been susceptible to tilmicosin.[10-13] Minimal inhibitory concentrations of tilmicosin persist in plasma for at least 24 hours after treatment, whereas concentrations in pulmonary tissues are approximately 10-fold that of plasma and persist for several days.[9,19,b] Thus, the success of prophylactic treatment with tilmicosin in reducing or delaying the onset of respiratory tract disease has been attributed to control of PH in the lungs. On the basis of the findings reported here, the prophylactic effect of tilmicosin possibly could be extended to include clearing or reducing PH in the nasopharynx. However, tilmicosin reportedly does not achieve minimal inhibitory concentrations in nasal secretions, but minimal inhibitory concentrations are achieved in tracheal mucosa and the palatine[d] and pharyngeal[e] tonsils. It is conceivable that inhibitory concentrations of tilmicosin are achieved in critical areas of the mucosal surfaces of the nasopharynx.

Effect of antibiotic treatment on the elimination of PH serotype A2 from the nasopharynx of healthy calves has been studied. Long-acting oxytetracycline reduced the number of isolations from a treated group of calves by 31% during the first week after treatment but did not eliminate PH from those calves.[25]

Although tilmicosin can prevent or delay the onset of respiratory tract disease, prophylactic treatment in situations in which the time of onset of respiratory tract disease cannot be predicted, such as in housed calves, has met with limited success.[26] However, when calves are assembled and transported, outbreaks of acute respiratory tract disease are most likely to occur immediately after arrival. Treatment with tilmicosin at the time of arrival resulted in reduction of the incidence of respiratory tract disease during the initial 28 to 30 days in the feedlot[22-24] and extended the mean interval until cattle required initial treatment for pneumonia.[23]

Treatment with tilmicosin immediately before cattle are transported also should be beneficial in reducing the incidence of respiratory tract disease. Transport stress often triggers increased shedding of PH organisms. We have consistently isolated PH from a few calves before transport and from many of the same calves after transport.[4,7,8,27] On the basis of the study reported here, we believe tilmicosin treatment reduces the number of PH in the nasopharynx, leaving fewer organisms to reach the lungs or to be transmitted to other calves. Reduction of colonization by prophylactic use of tilmicosin before transport or at the time of arrival in a feedlot is likely to reduce the incidence of acute respiratory tract disease at the feedlot for the initial several days, the period when transported calves are most susceptible to infectious disease.

[a]Micotil, Elanco Animal Health, Indianapolis, Ind.

[b]Thomson TD, Matsuoka T. Correlation of lung concentrations of a new veterinary antimicrobial with efficacy against *Pasteurella haemolytica* pneumonia in feedlot cattle (abstr), in *Proceedings*. 70th Conf Res Workers Anim Dis 1989;199.

[c]Bacto Blood Agar Base, Difco Laboratories, Detroit, Mich.

[d]Fossler SC, Moran JW, Thomson TD. Pharmacologic mechanism for tilmicosin in the control of calf pneumonia (abstr), in *Proceedings*. 79th Conf Res Workers Anim Dis 1998;P82.

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