PEER REVIEWED

Metaphylaxis with tildipirosin did not inhibit effectiveness of an experimental monovalent vaccine of live, attenuated *Mannheimia haemolytica* administered intranasally to dairy calves

Scott Nordstrom,[1] DVM; **Michael Bolton,**[1] DVM, MS; **Kevin Hill,**[1] DVM; **Tim Parks,**[1] DVM; **Mani Vaidyanathan,**[1] DVM, PhD; **Geert Vertenten,**[1] DVM, PhD; **Kelly Lechtenberg,**[2] DVM, PhD
[1] Merck Animal Health, 35500 W. 91st St., DeSoto, KS 66018
[2] Midwest Veterinary Services, Inc., Oakland, NE 68045
Corresponding author: Dr. Scott Nordstrom, scott.nordstrom@merck.com

Abstract

The objective of this study was to evaluate the effect of tildipirosin on the efficacy of a concurrently administered experimental vaccine against *Mannheimia haemolytica*. The experimental vaccine was developed for intranasal (IN) administration and contained the same live, attenuated, streptomycin-dependent *Mannheimia haemolytica*, at the same titer as in commercial product(s). Eighty-eight 14-week-old Holstein or Holstein-cross male calves from a single source were randomly assigned (using a 2 x 2 factorial design) to 1 of 4 treatment groups (22 head per group) as follows: 1) experimental vaccine (VAX) (IN); 2) VAX (IN) + tildipirosin subcutaneously (SC); 3) placebo vaccine (PLBO) (IN) as negative control; and 4) PLBO (IN) + tildipirosin (SC). Seventy days after enrollment, 84 calves were challenged with virulent *M. haemolytica.* Clinical assessment of each calf was recorded daily after challenge. There was no significant ($P > 0.05$) effect of any experimental treatment on clinical signs. Seven days after challenge, the calves were euthanized, a percent lung lesion score (LLS) was assigned to each, and samples of lung were submitted for isolation of *M. haemolytica*. There was no significant ($P = 0.51$) effect of tildipirosin on LLS for calves vaccinated with VAX or those vaccinated with PLBO; however, VAX resulted in significantly ($P = 0.046$) lower LLS. Under the conditions of this study, concurrent administration of tildipirosin and IN live, attenuated, streptomycin-dependent *M. haemolytica* vaccine with the same antigen as used in this study did not interfere with the efficacy of the vaccine.

Key words: bacterial vaccine, Mannheimia, tildipirosin, interference, immunization, lung lesions, bovine respiratory disease model

Introduction

Processing protocols utilized to in-process stocker and feeder cattle are designed to provide protection against bovine respiratory disease (BRD). For calves at high risk of developing BRD, protocols may include concurrent administration of a vaccine that contains a live, attenuated bacterial antigen to stimulate acquired immunity, and an antimicrobial to provide control (metaphylaxis) of targeted bacterial pathogen(s). The authors could find only 1 reference regarding the effect of an antimicrobial on the efficacy of such a vaccine. That study found no significant effect on serum antibodies produced following concurrent subcutaneous (SC) administration of tilmicosin and an attenuated, live bacterial vaccine that contained *Mannheimia haemolytica* and *Pasteurella multocida* administered intramuscularly (IM);[3] however, no bacterial challenge was used in that study.

Menge et al[5] reported pharmacokinetic values following a single injection of tildipirosin at 1.8 mg/lb (4 mg/kg) of body weight (BW), SC. Tildipirosin was rapidly and extensively distributed to lung tissue and bronchial fluid (BF).[5] Concentrations peaked in lung tissue (14.768 ± 2.135 µg/g of lung) at 24 h, and the ratio of concentration in lung:plasma peaked at 214.5 on d 10 after administration. Concentrations in BF remained relatively steady at about 3. 4 µg tildipirosin/g of BF between 24 and 72 h after administration. The ratio of concentration in BF:plasma at 10 h was 5.2, and at 21 d was 56. The minimum inhibitory concentration of 90% of isolates (MIC_{90}) is 1 to 4 µg tildipirosin/mL. Based on that, mean concentrations of tildipirosin in lung exceeded the MIC of isolates of *Histophilus somni* for 16 days, and of *M. haemolytica* and *P. multocida* for at least 16 d. Mean concentrations of tildipirosin in bronchial fluid exceeded the MIC_{90} of *M. haemolytica* and *P. multocida* for 21 d, and approximated the MIC_{90} of *H. somni* for about 3 d. Concentrations of tildipirosin in nasal secre-

tions are not known, and speculation based on the previous data should be avoided. Given those data for distribution of tildipirosin, and for the susceptibility *in vitro* of those bacterial pathogens associated with BRD, interference with a live, attenuated bacterial antigen administered intranasally (IN) was a realistic hypothesis.

The objective of this study was to evaluate the effect of tildipirosin on the efficacy of an experimental vaccine when concurrently administered. Specifically the primary outcome variable, lung lesion scores (LLS) resulting from a virulent challenge, were used as a measure of the influence of tildipirosin when administered SC concurrently with an experimental, live, attenuated, monovalent vaccine (VAX) administered IN that contained streptomycin-dependent *M. haemolytica*. Clinical signs (attitude scores, respiratory scores, and rectal temperature) and isolation of *M. haemolytica* from samples of lung were evaluated as secondary outcome variables. The working hypothesis was that concurrent administration of tildipirosin and the experimental vaccine would not change the efficacy of the vaccine, as indicated by comparing/contrasting primary or secondary outcome variables among the treatment groups.

Materials and Methods

Prior to initiation of the study, the Institutional Animal Care and Use Committee of the Veterinary and Biomedical Research Center, Inc. (VBRC) reviewed, approved, and assigned number VAC19019B to the protocol and procedures applicable to handling, feeding, care, and management of the calves involved in this study.

Animals/Housing/Feed

Eighty-eight 14-week-old Holstein or Holstein-cross male calves from a dairy in Nebraska were transported to a contract research facility in St. George, Kansas. Consumption of colostrum provided to each calf at birth was not known for calves enrolled in this study. During the day that calves were delivered, they were randomly assigned an identification number from 1 to 88 using VAXRANDNUMB,[a] and then sorted by VAXRANDNUMB in ascending order. One of 4 experimental treatments was then assigned by repeatedly using the sequence Group 1, Group 2, Group 3, and Group 4 (see descriptions of treatments below in section *Design/analysis*). On the day of enrollment (d 0) average BW of the calves was 206.1 lb (93.5 kg) (range 132 to 272 lb; 59.9 to 123.4 kg). On the day of challenge (d 70) the average BW of the calves was 287.3 lb (130.3 kg) (range 178 to 378 lb; 80.7 to 171.5 kg). At the end of the study (d 77), average weight of the calves was 288.8 lb (131 kg) (range 198 to 388 lb; 89.8 to 176 kg).

Calves were housed in non-adjacent pens (22 calves per pen) that housed only individuals assigned to the same treatment. Pens were constructed in a dry lot with dirt floors using portable panels. An empty pen was maintained between pens that contained calves to prevent nose-to-nose contact with other treatment groups or any other cattle. On d 40, calves were moved to grass pastures so that natural shade could be provided because of unexpected, extreme ambient heat at the study site. The protocol was amended, and calves were combined from 4 groups into 2 groups as follows: calves vaccinated with VAX were commingled and moved into a small, fenced, grass pasture; calves vaccinated with PLBO were commingled and moved into a non-adjacent, fenced grass pasture. Blinding was maintained throughout the process of commingling of the groups of calves. Two days (d 68) prior to challenge (d 70), the remaining calves were all placed into a common pen such that all calves from all 4 treatment groups were housed together during the challenge phase of the study.

Calves had access *ad libitum* to fresh water, and once daily throughout all phases of the study, were fed a total mixed non-medicated diet containing brome hay (39%), wet distillers' grain (59%), and mineral supplement (2%). No products that could affect the vaccine or the virulent challenge organism were added to the water or the feed. All calves were observed daily by trained, blinded personnel at approximately the same time of day, for health and wellbeing throughout the duration of the trial. For each individual calf, a respiratory score, attitude score, and rectal temperature were recorded daily after challenge. Security and safety of the pens, and function of equipment were observed following the schedule. Proper personal protective equipment was used by personnel whether handling the experimental products, handling the calves, performing necropsies, handling samples of lung, or disposing of animal remains.

Calves were acclimated for 5 d after arrival and were 14 weeks old (+/- 5 d) at the time of vaccination/enrollment. Criteria for a calf to be included were the following: seronegative for antibody (microagglutination titer ≤ 256) to *M. haemolytica* leukotoxin;[b] negative for persistent infection (PI) with bovine virus diarrhea virus (BVDV) using immunohistochemistry staining of a sample of skin;[c] not previously vaccinated; and healthy on the day of vaccination/enrollment (d 0). After calves arrived and during the acclimation period, they were observed by trained personnel daily for clinical abnormalities. Abnormalities or clinical signs of disease observed for any individual calf were recorded and reported to the study director for appropriate action. On d -1 and 0 prior to vaccination, rectal temperature and respiratory rate were recorded for each calf. Confounding factors related to genetics, colostral intake, or individual animal exposure were mitigated to the extent possible through randomization.

At any time after arrival at the research facility, a calf that exhibited the following clinical signs, alone or in combination, was considered as a candidate for euthanasia to be determined and performed by the attending veterinarian: 1) were not observed to eat and/or drink for 48 hours; 2) severe depression and rectal temperature < 99° F (37.4° C); 3) severe respiratory distress with deep, labored breathing for more

than 6 h; or 4) moribund and unable to move. Additionally, any calf could be euthanized if the attending veterinarian determined that euthanasia would prevent needless suffering from any cause.

After vaccination and prior to challenge, 2 calves in Group 1 and 2 calves in Group 3 were removed from the study. One calf from Group 1 was removed because of pre-existing respiratory disease. Three calves (2 from Group 3 and 1 from Group 1) died. Necropsy revealed the cause of death for these 3 calves was respiratory disease unrelated to the experimental procedures because bacterial challenge had not been administered. Each of these calves was treated according to written protocol with medication approved for treatment of cattle with BRD.

Eighty-four of the 88 calves enrolled were challenged on d 70 (Table 1). Two calves in Group 3 died before the end of the study. Necropsies were performed, LLS were recorded for those calves, and were included in the analysis. All other calves remained through the observation period (d 77).

Products

Treatments were: 1) an experimental monovalent, live, attenuated vaccine (VAX) that contained a proprietary seed stock of streptomycin-dependent *M. haemolytica*; 2) a placebo vaccine (PLBO) was prepared to contain the same medium and filler in a lyophilized pellet as the experimental vaccine but without the bacterial antigen; and, 3) commercially available tildipirosin.[d] The experimental vaccine was prepared using the same seed culture of *M. haemolytica*, and at the same titer (proprietary information) licensed for commercial product(s) of Merck Animal Health, Desoto, KS. Quality control, and regulatory measures were applied for preparation of the experimental vaccine and the placebo as for preparation of commercial vaccine. Tildipirosin was selected for use in this study because it is approved for treatment and control of BRD in calves at high risk of developing BRD caused by *M. haemolytica.*

Table 1. Experimental treatment groups and number of calves per group.

		Experimental Vaccine	
		Yes (VAX)*	**No (PLBO)****
Tildipirosin†	No	22 head enrolled **Group 1** 20 challenged	22 head enrolled **Group 3** 20 challenged
	Yes	22 head enrolled **Group 2** 22 challenged	22 head enrolled **Group 4** 22 challenged

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine

** PLBO = placebo IN vaccine

† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

Design/Analysis

A completely randomized, 2 x 2 factorial design (Table 1; Figure 1) was used; individual calf was the experimental unit. Based on previous experience with similar trials approved by the United States Department of Agriculture (USDA) and utilized in efficacy and duration of immunity for licensure of commercially available products containing the same *M. haemolytica* antigen, and using related power calculations, 22 replicates (calves) were used per treatment group. Each calf was randomly assigned to 1 of 4 treatment groups:

1) VAX (2 mL, IN)
2) VAX (2 mL, IN) + tildipirosin (1.8 mg/lb = 4 mg/kg; SC side of neck)
3) PLBO (2 mL, IN) negative control
4) PLBO (2 mL, IN) + tildipirosin (1.8 mg/lb = 4 mg/kg; SC side of neck).

After treatments were administered on d 0, calves were housed in pens that contained only individuals from a treatment group. Experimental bacterial challenge was administered by percutaneous transtracheal injection on d 70, and consisted of a suspension of 6.63×10^8 colony forming units (CFU) of virulent *M. haemolytica* in 40 mL Tryptic Soy Broth (TSB). After experimental bacterial challenge, all calves were observed daily, at approximately the same time each day. Rectal temperature, respiratory score, and attitude score were recorded daily for each individual calf for 7 d after challenge. Calves that died before the end of the study were submitted for necropsy by a veterinarian at the research facility, lung lesions were scored by trained, experienced personnel designated by the sponsor, and samples of lung were submitted[e] for isolation of *M. haemolytica* used for the challenge. Clinical signs (respiratory scores described in Table 4, attitude scores described in Table 5, rectal temperature) of all calves were recorded daily. On d 77, the calves were euthanized (sedation with ketamine followed by stunning with penetrating captive bolt device, and exsanguination); lesions in the lungs were scored;[4] and samples (10 to 20 gm) of lung, taken from the interface of the healthy and the diseased lung, were submitted to the diagnostic laboratory[e] (within 48 hours of collection) for isolation of the *M. haemolytica* strain used for the challenge as described above. The primary outcome variable

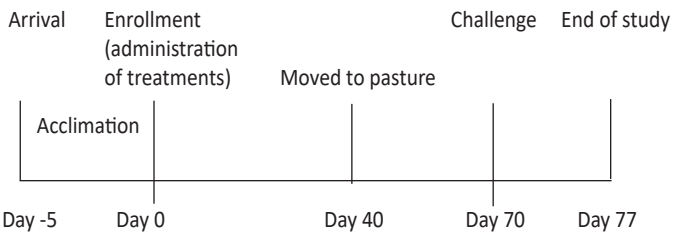

Figure 1. Timeline of major events for this study. Clinical signs (respiratory score, attitude score, rectal temperature) were recorded daily throughout the study.

was the total lung lesion score (LLS) that was calculated and recorded as a percent (%) based on a weighted sum of the estimated portion of each lung lobe that was abnormal.[4] The formula used was as follows: (left cranial x 0.05) + (left middle x 0.06) + (left caudal x 0.32) + (right cranial x 0.06) + (right posterior cranial x 0.05) + (right middle x 0.07) + (right caudal x 0.35) + (accessory x 0.04) = Total Lung Lesion Score (%). Lesions in lungs were scored independently by 2 individuals, and the average of those 2 scores was used for analyses. Personnel recording clinical scores, administering the challenge, scoring lung lesions, or performing bacterial isolation procedures were blinded to the treatment group to which the animal was assigned.

Secondary outcome variables were isolation of *M. haemolytica* from samples of lung, and clinical signs (respiratory score, attitude score, and rectal temperature as described above). In all statistical analyses, fixed effects of vaccine (yes = VAX; or, no = PLBO), tildipirosin (yes/no) and their interaction were evaluated given the 2 x 2 design of the study.[f] Statistical significance was set at $P \leq 0.05$. A linear model analyzing the distribution of the LLS did not meet the normality assumption. Thus, original observations were ranked (with ties) prior to analysis by a nonparametric approach using ranks. In addition, *M. haemolytica* recovery (yes/no) was evaluated using a logistic regression model (binomial distribution and logit link). Attitude and respiratory scores, as well as rectal temperatures, were analyzed in linear mixed models for categorical and continuous data, respectively, with a random effect to account for repeated measures on calves over time (day post-challenge).

Results

During the study, no adverse events were recorded.

Primary Response Variable

There was no significant ($P = 0.39$) effect of (vaccination x tildipirosin) interaction on LLS. The effect of tildipirosin on LLS was not significant ($P = 0.51$; Table 2; Table 3, Figure 2); however, VAX resulted in significantly ($P = 0.046$) lower LLS (median LLS = 1.48%; mean LLS = 3.22%; standard deviation [SD] = 4.11%) than did PLBO (median LLS = 3.25%; mean LLS = 11.24%; SD = 17.79%).

Secondary Response Variables

There was no significant ($P = 0.39$) effect of interaction (vaccination [VAX / PLBO] x tildipirosin [yes / no]), or of vaccination ($P = 0.84$) on isolation of *M. haemolytica*

Table 2. Descriptive statistics for lung lesion score (LLS) by treatment group. (N = number of calves; Standard deviation = SD). There was no significant ($P = 0.39$) effect of interaction (vaccination x tildipirosin).

	Treatment group			LLS		
Number	Vaccination	Tildipirosin†	N	Median	Mean	SD
1	VAX*	No	20	1.74	2.94	3.95
2		Yes	22	1.34	3.49	4.32
		Subtotal	42	1.48	3.22	4.11
3	PLBO**	No	20	4.29	13.35	19.35
4		Yes	22	2.57	9.31	16.46
		Subtotal	42	3.25	11.24	17.79
	P = 0.046	P = 0.51	84			

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine

** PLBO = placebo IN vaccine

† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

Table 3. Quartile analysis for LLS by treatment group. There was no significant ($P = 0.39$) effect of interaction (vaccination x tildipirosin).

	Treatment group		LLS					
No.	Vaccination	Tildipirosin†	Minimum	Maximum	Median	Mean	Lower quartile	Upper quartile
1	VAX*	No	0	16.54	1.74	2.94	0.47	2.86
2		Yes	0	13.31	1.34	3.49	0.61	5.65
3	PLBO**	No	0.13	65.56	4.29	13.35	1.02	19.77
4		Yes	0	70.39	2.57	9.31	0.27	14.07

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine

** PLBO = placebo IN vaccine

† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

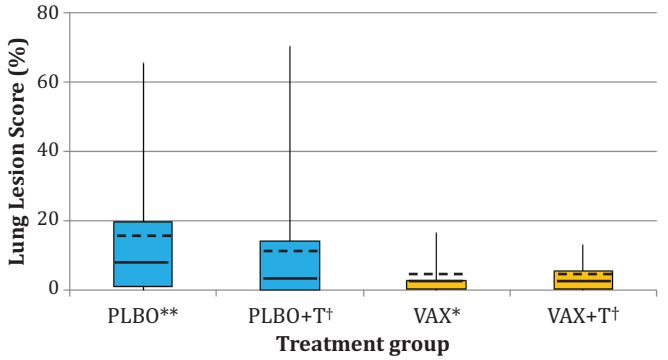

Figure 2. Quartile summary of LLS by treatment group. Mean = -----; Median = ——. Upper and lower margins of the boxes represent values for the upper (75%) and lower (25%) quartile, respectively. Ends of the bars above and below the boxes represent the maximum and minimum values, respectively.

*VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine

**PLBO = placebo IN vaccine

† T = tildipirosin; Zuprevo, Merck Animal Health, Madison, NJ

from samples of lung. If *M. haemolytica* was isolated from a sample of lung from any individual calf, that individual calf was recorded as "positive" for recovery of *M. haemolytica.* The proportion of calves from which *M. haemolytica* was isolated was significantly (P = 0.02) greater for calves that were concurrently treated with tildipirosin (43.05%, ± 7.54% SEM) than for those that were not concurrently treated with tildipirosin (17.36%, ± 6.02% SEM). Other pathogens associated with BRD were not isolated.

Calves in all groups developed clinical signs of respiratory disease (measured as respiratory scores, attitude scores, and rectal temperature) that were analyzed as stated above. Among the 4 categories for respiratory scores across all treatment groups, there was no significant (P-values rounded to 1.00) effect of day, vaccination, tildipirosin, or vaccination x tildipirosin interaction. Treatment x day interaction was not significant (P = 0.75; Table 4, Figure 3).

Among the 4 categories for attitude scores, there was no significant (P-values rounded to 1.00) effect of vaccine, tildipirosin, or vaccine x tildipirosin interaction; and, treatment x day interaction was not significant (P = 0.77). There was no significant (P-values rounded to 1.00) effect of vaccination, tildipirosin, or vaccination x tildipirosin interaction, or of treatment x day interaction (P = 0.58). However, across all treatment groups abnormal attitude score, as defined in Table 5 below, was significantly (P < 0.01) affected by day (Table 5; Figure 4).

For rectal temperature, there was no significant effect of vaccination (P = 0.18), tildipirosin (P = 0.49), vaccination x tildipirosin interaction (P = 0.10), or treatment x day interaction (P = 0.18) (Table 6). However, mean temperatures across all treatment groups were significantly (P < 0.01) affected by Day (Figure 5).

Discussion

This study was conceptualized and designed to mimic a scenario in which cattle that are at high risk of developing BRD would be vaccinated IN with a live, attenuated vaccine against a bacterial pathogen associated with BRD, and medicated with an injectable (SC) antimicrobial to control bacterial pathogens associated with BRD (metaphylaxis). The complexity of naturally occurring BRD led us to choose this experimental challenge model because it has historically and consistently produced pulmonary lesions, and because the authors had experience with it. The model allowed control of factors that could have confounded the results. Calves were from a single source, had not been vaccinated, and were not intentionally deprived of colostrum. The age and size of calves were appropriate for the predictable reproducibility of the pulmonary lesions with the experimental model. Clinical signs were used to monitor general health and wellbeing of the calves.

Table 4. Respiratory scores* were not significantly (P-values rounded to 1.00) affected by treatment group or by interaction of the treatment groups.

Treatment group				Respiratory Score* (%)			
Number	Vaccination	Tildipirosin†	N	0	1	2	3
1	VAX*	No	20	76.88	20.63	2.50	0.00
2		Yes	22	65.34	31.82	2.84	0.00
3	PLBO**	No	20	64.47	31.58	3.29	0.66
4		Yes	22	72.16	25.00	1.70	1.14

*0 = Normal: no abnormal respiratory signs; rate and effort appropriate for the environment
1 = Mild respiratory distress: serous nasal or ocular discharge, and/or cough
2 = Moderate respiratory distress: mucous or mucopurulent nasal or ocular discharge, and/or increased respiratory rate or effort
3 = Severe respiratory distress: marked increased respiratory rate or effort, with 1 or more of the following: open-mouthed breathing; abdominal breathing; and/or, extended head and neck

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine

** PLBO = placebo IN vaccine

† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

For this study, 14-week-old dairy calves were selected because of the genetic uniformity of that population of calves, and because of experience with the challenge model. For these calves, consumption of colostrum at birth was not known, and transfer of colostral antibodies was not evaluated given the age of the calves when enrolled in the study.

A placebo was prepared to mimic the experimental vaccine, but without *M. haemolytica*. Quality control and regulatory measures were applied for preparation of the experimental vaccine and placebo as for preparation of commercial vaccines. Tildipirosin was selected for use in this study because it is approved for treatment and control of BRD in calves at high risk of developing BRD caused by *M. haemolytica*.

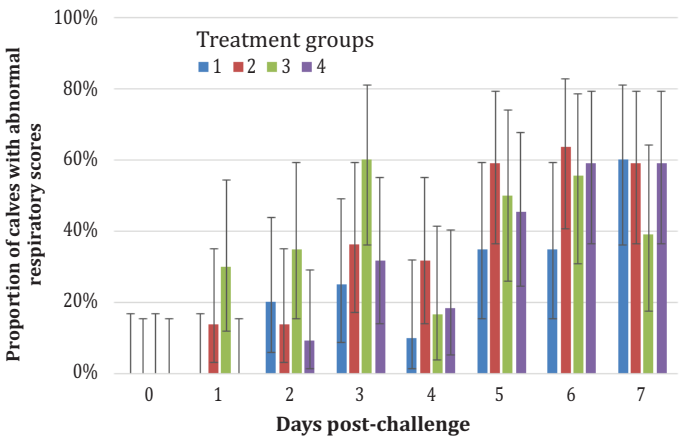

Figure 3. Proportion (%) of calves with abnormal respiratory scores (score > 0) by day post-challenge and by treatment group. Error bars represent 95% exact confidence intervals. Interaction of treatment x day was not significant (P = 0.75), but respiratory scores were significantly (P < 0.01) affected by day.

1 = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine
2 = tildipirosin (Zuprevo, Merck Animal Health, Madison, NJ)
3 = placebo IN vaccine
4 = placebo IN vaccine + tildipirosin

Outcomes were not considered to have been affected by the housing adjustment made to help optimize animal care. All calves were treated equally, beginning the study in groups of 22 head, combined into 2 groups (by vaccination status) then finally, combined again for the challenge phase of the study.

Clinical signs (respiratory scores, attitude scores, and rectal temperature) differed significantly by day (day-to-day changes) across all treatment groups (Figures 3, 4, and 5). Clinical assessment did not provide a measurement that was sensitive or specific enough to distinguish an effect of treatment on LLS associated with BRD. That finding is expected with this model, and has been reported by other investigators using experimental models and for cattle with naturally occurring BRD.[1,7,8,10] Assessment of pulmonary lesions using transthoracic ultrasound, radiography, or computer-assisted auscultation may have provided a means whereby research and clinical care could have been evaluated with greater specificity and sensitivity.[2,6,9]

Isolation of the organism from lungs at the end of the study was strongly influenced by sampling technique (the technicians subjectively choose samples at the apparent interface of healthy and actively diseased lung) and was primarily used for qualitative evaluation of the model (i.e. fulfillment of Koch's postulates, consistency of infection, no complicating co-morbidity, and to assure that there was no breech to the model by other unexpected pathogens). The effect of tildipirosin on recovery of *M. haemolytica* was not expected, and was not explained by results of the study. To the knowledge of the authors this is the first study of the effects of a macrolide administered 70 d prior to a virulent bacte-

Table 5. Attitude scores* were not significantly (*P*-values rounded to 1.00) affected by treatment group or by vaccination x tildipirosin interaction.

Treatment Group				Attitude Score* (%)			
	Vaccination	Tildipirosin†	N	0	1	2	3
1	VAX*	No	20	71.88	22.50	5.63	0.00
2		Yes	22	64.20	31.25	4.55	0.00
3	PLBO**	No	20	63.82	30.92	4.61	0.66
4		Yes	22	66.48	28.98	3.41	1.14

*0 = Normal: bright, alert, responsive
1 = Mildly depressed: may stand isolated with head down, ears drooping, but responsive to stimulation.
2 = Moderately depressed: may remain recumbent or stand isolated with head down, may show signs of muscle weakness (standing cross-legged, knuckling or swaying when walking), depression obvious when stimulated.
3 = Severely depressed: may be recumbent and reluctant to rise, or if standing, is isolated and reluctant to move; when moving may show ataxia, knuckling or swaying evident; head carried low with ears drooping; eyes dull, possible excess salivation/lacrimation, obvious gauntness.
4 = Moribund: unable to stand; approaching death; highly unlikely to respond to any antimicrobial treatment.

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine
** PLBO = placebo IN vaccine
† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

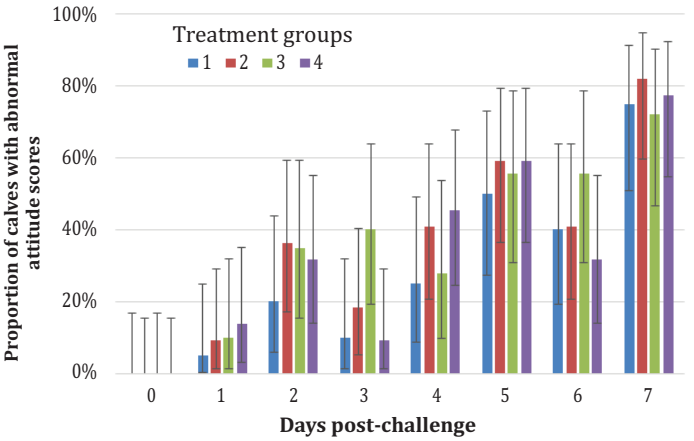

Figure 4. Proportion (%) of calves with abnormal attitude scores (score > 0) by day post-challenge and by treatment group. Error bars represent 95% exact confidence intervals. Interaction of treatment x day was not significant (P = 0.77), but attitude scores were significantly (P < 0.01) affected by day.
1 = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine
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3 = placebo IN vaccine
4 = placebo IN vaccine + tildipirosin

rial challenge. Therefore, no precedent has been established on which to formulate a testable hypothesis to explain the finding. It is speculative to draw conclusions given the time between administration of tildipirosin and the challenge, as well as the subjectivity of collection of samples. Isolation/recovery of the organism used for challenge was intended to be an indicator of adequacy of the challenge.

Calves were challenged intratracheally with virulent *M. haemolytica* 70 d after vaccination, and LLS were evaluated 7 d post-challenge. Given the pre-slaughter withdrawal time of 21 d, it is realistic to expect no clinically relevant residual effect of the dose of tildipirosin administered 70 d prior to challenge. Based on results of this study, concurrent SC administration of tildipirosin, and IN administration of a surrogate, experimental, live, attenuated vaccine with *M. haemolytica* did not interfere with the efficacy of the vaccine. The primary effect on LLS was from the experimental, monovalent, live, attenuated vaccine.

Conclusions

Under conditions of this study, 1) the experimental vaccine was efficacious; 2) tildipirosin administered SC concurrently with the vaccine IN did not interfere with the efficacy of the vaccine; and 3) LLS were consistently lower and less variable for calves vaccinated with the experimental vaccine. We conclude that tildipirosin administered SC to control BRD poses minimal risk of interfering with a vaccine administered IN that contains the live, attenuated, bacterial antigen as used in this study. We further conclude that proper immunization with an intranasal *M. haemolytica* vaccine provides more consistent control of lung lesions caused by *M. haemolytica* associated with BRD, than does no immunization. These findings do not necessarily apply to other antimicrobial medications or to other vaccines that contain live, attenuated bacterial antigens.

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Endnotes

[a] Microsoft® Excel RAND function
[b] Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX
[c] Institute of Agriculture and Natural Resources, School of Veterinary Medicine and Biomedical Sciences, Veterinary Diagnostic Center, University of Nebraska, Lincoln, NE
[d] Zuprevo® -18%, Merck Animal Health, Madison, NJ
[e] Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS
[f] SAS 9.4, SAS Institute, Inc., Cary, NC

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Table 6. Rectal temperatures, recorded daily during the 8-day observation period post-challenge, were not significantly affected by vaccination (P = 0.18), tildipirosin (P = 0.49), vaccination x tildipirosin interaction (P = 0.10), or treatment x day interaction (P = 0.18). However, mean temperatures, of all treatment groups, were significantly affected by day (P < 0.01).

Treatment Group				Rectal Temperature (°F)			
	Vaccination	Tildipirosin†	N	Mean	Median	Minimum	Maximum
1	VAX*	No	20	101.88	101.9	100.6	103.7
2		Yes	22	102.06	102.0	100.3	104.9
3	PLBO**	No	20	102.10	102.0	100.7	105.8
4		Yes	22	102.04	102.0	100.0	105.6

* VAX = experimental intranasal live, attenuated streptomycin-dependent *Mannheimia haemolytica* vaccine
** PLBO = placebo IN vaccine
† tildipirosin = Zuprevo, Merck Animal Health, Madison, NJ

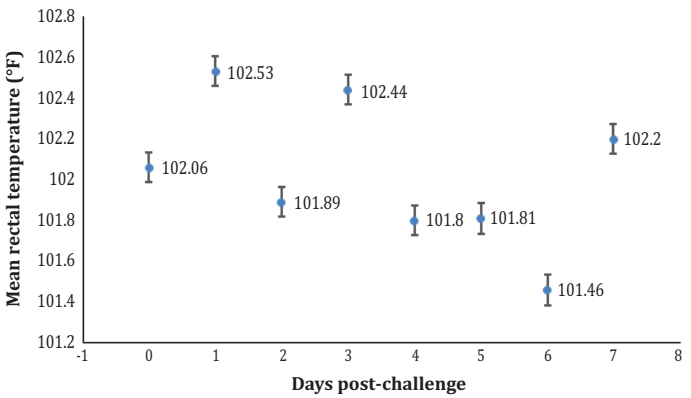

Figure 5. Model-adjusted mean rectal temperature (° F) by day. Temperatures for all treatment groups were combined by day, and error bars represent standard errors of the means (SEM). Mean temperatures of all treatment groups were significantly (P < 0.01) affected by day.

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