PEER REVIEWED

Efficacy of a DNA immunostimulant for on-arrival control of bovine respiratory disease in fall-placed feedlot calves

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Abstract

A randomized complete block design trial was conducted in a commercial finishing feedlot in southern Alberta, Canada using auction-market origin fall-placed steer calves (n = 5430; initial body weight 618 ± 22 lb; 280.9 ± 10.0 kg) to evaluate the comparative efficacy of on-arrival treatment with a DNA immunostimulant and tulathromycin versus tulathromycin alone for prevention of bovine respiratory disease (BRD). The addition of the DNA immunostimulant reduced first-pull treatment rates for BRD (P=0.02), case fatality rate for BRD (P=0.08), mortality rate for BRD (P=0.03), mortality rate for BRD and histophilosis (P=0.09), average daily gain ($P \leq 0.01$) with dead weights included, and increased dry matter conversion ($P \leq 0.01$) with dead weights included.

Key words: bovine respiratory disease, BRD, feedlot, Zelnate

Résumé

Un essai utilisant un plan avec blocs aléatoires complets a été mené dans un parc d'engraissement de finition dans le sud de l'Alberta (Canada) avec des jeunes bouvillons (n = 5430; poids corporel initial 618 ± 22 lb; 280.9 ± 10.0 kg) provenant d'encans et arrivés en automne pour évaluer l'efficacité relative d'un traitement à l'arrivée avec un immunostimulant à l'ADN en conjonction avec de la tulathromycine par rapport à un traitement incluant seulement de la tulathromycine pour prévenir le complexe respiratoire bovin (CRB). L'addition de l'immunostimulant à l'ADN a permis une réduction du taux de premier traitement pour le CRB (P = 0.02), du taux de létalité (P = 0.08), du taux de mortalité relié au CRB (P = 0.03), du taux de mortalité relié au CRB avec Histophilose (P = 0.09), du gain moyen quotidien ($P \leq 0.01$) incluant le poids des morts et une augmentation du taux de conversion alimentaire ($P \leq 0.01$) avec le poids des morts inclus.

Introduction

Various metaphylactic antimicrobials, such as long-acting oxytetracycline, tilmicosin, ceftiofur crystalline free acid, tildipirosin, gamithromycin, and tulathromcycin are used upon arrival in fall-placed feedlot calves to reduce morbidity and mortality from bovine respiratory disease (BRD).[1-3,5-8,10-14] While these antimicrobials reduce BRD disease rates, BRD losses from treatment and labor costs, mortality, and reduced performance from disease continue to be costly to the North American feedlot industry. A new DNA immunostimulant[a] was recently developed to aid in the treatment of BRD due to *Mannheimia haemolytica* when administered at the time of, or within 24 hours after a perceived stressful event.[4,9]

Zelnate®[a] is a bacterial produced plasmid DNA with a liposome carrier designed to stimulate the innate immune system in cattle.[4] With increased societal pressure on the feedlot industry to identify strategies to reduce overall antimicrobial usage, veterinarians are looking for new technologies and/or management practices to prevent, treat, and control BRD. Zelnate® is a new immunostimulant product recently available to veterinarians in Canada and the US, but there is little peer-reviewed published scientific data on its efficacy in feedlots to reduce BRD losses.[9]

The purpose of this controlled commercial field trial was to evaluate the effectiveness of a DNA immunostimulant when administered on arrival to fall-placed backgrounded calves in reducing morbidity and mortality due to naturally occurring BRD in a commercial feedlot. Secondary objectives were to measure feedlot performance (average daily gain and dry matter conversion).

Materials and Methods

Study facility

This trial was conducted at a commercial feedlot in southern Alberta, Canada with a 1-time feeding capacity of 15,000 head. The animals were housed in open dirt-floor

pens with a heated automatic waterer and a concrete feed bunk within the fence line facing a common feed alley. Each pen held 250 to 300 animals. The hospital and treatment area of this feedlot was used to administer treatments and weigh animals. The hospital had a roof and concrete floor and was equipped with a hydraulically operated squeeze chute with weigh scale and chute-side computer and health data management system.[b] Body temperatures were taken with an electronic thermometer.[c]

Cattle were fed rations consisting of barley grain, barley or corn silage, corn dried distiller grains with solubles, and supplement formulated to meet nutritional requirements of feedlot cattle, consistent with normal feeding protocols in the feedlot. Monensin sodium (33 ppm, 100% dry-matter basis) was included in the ration throughout the feeding period to improve performance and control bloat and coccidiosis. Tylosin phosphate (11 ppm, 100% dry-matter basis) was included in the ration throughout the feeding period to reduce liver abscesses. All pens were fed their rations 3 times daily on an *ad libitum* basis using truck-mounted mixers on load cells. Feed intake was recorded by pen, with feed from sick and chronic pens prorated back to the original lot of cattle. The dry-matter content of the ration varied from starter rations (approximately 55% DM) to finishing rations (approximately 77% DM).

Study animals

A total of 5,430 crossbred steer calves approximately 6 to 8 months of age with an average induction weight of 618 lb (281 kg) were used in this study. All calves had been recently purchased through the auction market system from western Canada and northwestern USA and shipped to the feedlot. These calves were fall-placed and recently weaned from the ranch. The history of the calves was not known since that information is not typically provided to feedlots in Alberta.

Upon arrival at the finishing feedlot, calves were given a modified-live IBR, PI3, BRSV, and BVD type 1 & 2 vaccine,[d] 8-way clostridial bacterin,[e] *Histophilus somni* bacterin,[e] *Mannheimia haemolytica* leukotoxoid vaccine,[d] ivermectin pour-on[f] or injectable,[g] anabolic implant,[h] and tulathromycin.[i] On-arrival treatment for tulathromycin was dosed according to the average weight of animals in each processing group. The weight range within processing groups was typically 100 lb (45.4 kg). If it was raining or wet snow was falling, the animals within a processing group were treated with an injectable ivermectin rather than the pour-on ivermectin. All animals were uniquely identified with a numbered feedlot eartag and CCIA (Canadian Cattle Identification Agency) tag. Animals were enrolled in the study within 48 hours after arrival at the feedlot.

Experimental design

A randomized block design was used. Each block consisted of 2 treatment-paired pens as they were filled. A total of 20 pens or 10 blocks with 250 to 300 calves per pen were created. The sample size used here is typical for commercial feedlot trials when assessing metaphylactic drugs or feed additives, and the pen is the unit of analysis.[10-14]

The 2 treatments were: 1) Zelnate® 2 mL IM and 2) control. The DNA immunostimulant was administered at arrival regardless of body temperature. Given that Zelnate®[a] is licensed in Canada and being used as per label directions, with the feedlot operating as per its normal management practices, there was no requirement for any government approval to conduct the study.

Animals, regardless of treatment group, were treated according to the feedlot's standard treatment protocol for BRD. The post-metaphylactic interval (PMI) for tulathromycin was 10 days following on-arrival treatment. This was the standard PMI used for tulathromycin at this feedlot and it was the same for all trial animals.

Animals relapsing a third time with BRD were considered chronics; thus, no further treatment was given and they were placed in a chronic pen. Therapeutic drugs were used at label dose with label withdrawals adhered to. Treatment dosages were based on the individual body weight of the sick animal.

Animal allotment

Experimental animals were selected from groups of animals arriving at the feedlot from October 24 to December 8, 2016. As new cattle were presented for processing, the calves within each arrival processing group were randomly assigned to 1 of 2 treatment groups using systematic randomization in groups of 5 head. All on-arrival calves within these arrival processing groups were eligible for inclusion in the study. A coin was flipped to determine which of the feeding pens was the DNA immunostimulant pen and which was the control pen. Then a coin was flipped to determine if the first calf through the chute for a new block of pens went into the DNA immunostimulant or control group. Every group of 5 animals through the chute went into the same treatment group. For example, if the coin flip was heads and heads was set for DNA immunostimulant, then the first 5 calves through the chute received DNA immunostimulant, the second 5 calves through the chute received nothing, the next 5 calves through the chute received DNA immunostimulant, and so on until the 2 pens were filled. Calves were processed and individually weighed in the processing chute. The scale in the processing chute was verified with a standard weight of 1000 lb (454 kg) and calibrated as necessary prior to processing. After every 20 head, the scale was tared to zero. Calves from the 2 treatment groups were penned separately. Once 2 pens were full (approximately 250 to 300 animals in each pen), 2 new pens were filled until 20 pens were placed on trial. Each pen was an experimental unit and each group of 2 treatment-paired pens represented a block. Animals were moved to their home pen and maintained as a unit for the duration of the trial, which was from induction processing until administration of the terminal implant and terminal weight sorting (approximately

30 to 40 days before slaughter). Feedlot personnel who processed the cattle were different from feedlot personnel who checked the cattle daily for illness.

Observations

Any animals appearing "sick" based on subjective parameters such as general appearance and attitude, gauntness, reluctance to move, separation from group, and signs of respiratory disease, such as nasal discharge, ocular discharge, abnormal respiration, and coughing, were moved to the hospital area of the feedlot for closer observation. Upon presentation at the hospital facility, the rectal temperature of the "sick" calf was taken with an electronic thermometer and its identification was entered into the chute-side computer.[b]

A diagnosis of the initial case of UF (undifferentiated fever) was made on an animal if the following criteria were satisfied: 1) the case abstract, which appeared on the computer screen, indicated no previous treatment history for BRD (UF or NF); 2) there was an absence of clinical signs attributable to organ systems other than the respiratory tract as described above; and 3) animals met the temperature criteria (≥ 104.0°F; 40°C). If all these criteria were met, then the animal was treated and designated as UF. Animals with clinical signs of pneumonia not meeting the febrile rectal temperature criteria above were treated and designated as NF (no-fever). All BRD treated animals (UF and NF) were returned to their home pen the same day of treatment unless they were severely compromised. Cattle that were on a daily treatment regime or animals administered a long-acting antimicrobial that were unable physically to return to their home pen due to severe illness or weakness were housed in the hospital pen until they could be returned to their home pen.

A diagnosis of a relapse case of BRD (UF or NF) was made on the individual if the following criteria were satisfied: 1) the case abstract indicated previous treatment for BRD (UF or NF) and 2) there was an absence of clinical signs attributable to organ systems other than the respiratory tract. An animal was considered a relapse for BRD if it was repulled for BRD at any time while on feed, regardless of the time interval from previous treatment. Animals that relapsed were treated according to the feedlot's standard treatment protocol for UF or NF.

A calf was defined as a chronic if it had been pulled as a third relapse. Such individuals were sent to the chronic pen. If the calves were moribund at any time, they were humanely euthanized. Calves that were gaining weight, but could not be returned to their home pen because they could not compete for feed/water with their peers, were sent to a railer pen for fattening prior to slaughter. Feed from these cattle was prorated back to their home pen. Animals that died during the trial period were necropsied by feedlot veterinarians to determine the cause of death. The mortality diagnosis was based on gross morphologic findings.

Statistical analysis

Equations used to calculate morbidity and mortality rates have been previously defined.[13] Bovine respiratory disease (BRD) cases included both UF and NF. Individual body weights at processing and terminal sort were imported into a spreadsheet program[j] and an average weight was calculated for each pen. From the computerized animal health data, disease rates for UF, NF, BRD (UF and NF), and crude, BRD, and BRDHS mortality were calculated for each pen.

Terminal sort weight, days-on-feed (DOF), daily dry-matter intake (DDMI), average daily gain (ADG), and dry matter conversion (DMC) were calculated for each pen. Terminal sort weights were pencil shrunk 4%, which is a common industry standard. Average DOF per pen was calculated as the total head days divided by the number of head inducted. Average daily gain per pen was calculated as the total terminal sort weight subtracted from the total weight inducted divided by the total head days. Daily DMI per pen was calculated as the total pounds of feed fed divided by the total head days. Dry matter conversion per pen was calculated as the total pounds of feed fed divided by the total live weight gain. Feedlot performance was calculated with and without the weight of dead animals excluded from the total terminal sort weight. Dead weight was based on computerized calculated body weights in FeedIT® based on last known measured weight and ADG of pen.

Data were analyzed using an analytical software program.[k] A randomized block design was used to compare outcomes between experimental groups. Mixed linear regression models were used to evaluate continuous outcomes and mixed logistic regression models were used to compare proportional outcomes such as morbidity and mortality risk. Replicate (block) was a random effect in all models. *P* value for statistical significance was set at 0.05.

Results and Discussion

When administered at feedlot processing within 48 hours of arrival, the DNA immunostimulant Zelnate® significantly reduced first treatments for BRD (P=0.02), BRD mortality rates (P=0.03), and tended to reduce BRD case fatality rates (P=0.08), and BRD and histophilosis mortality rates (P=0.09) (Table 1). Insufficient sample size and low disease rates, type 2 error, may explain why the last 3 variables did not approach typical statistical significance at P<0.05. In another feedlot study,[9] the DNA immunostimulant administered on arrival did not significantly reduce first treatments for BRD, although it tended to reduce third treatment rates for BRD. Similar to our study in feedlot steers, the DNA immunostimulant when administered on arrival to feedlot heifers reduced BRD case fatality rate and BRD mortality. In the heifer study, overall mortality[8] was also reduced. There was no significant difference in overall mortality rate with the DNA immunostimulant in the steer calves in the current study, possibly due to a 2% lower mortality rate than the previous

Table 1. Efficacy of Zelnate®* on morbidity and mortality in feedlot steer calves at moderate risk of developing respiratory disease.

Health variable	Experimental group		RR (95% CI)	*P*-value
	Zelnate	Control		
No. of pens	10	10		
No. of animals	2,715	2,715		
First UF† treatment, %	8.9	10.6	0.83 (0.70-0.97)	0.02
First UF relapse, %	15.1	15.2	0.96 (0.67-1.52)	0.87
Second UF relapse, %	12.6	17.2	0.85 (0.28-1.61)	0.73
Third UF relapse, %	0	0	---	---
First NF‡ treatment, %	4.3	3.9	1.09 (0.84-1.41)	0.49
First NF relapse, %	11.8	9.1	1.08 (0.48-2.00)	0.84
Second NF relapse, %	10.0	5.0	2.0 (0.17-2.06)	0.54
Third NF relapse, %	0	0	---	---
First BRD, %	13.2	14.5	0.90 (0.78-1.03)	0.12
First BRD relapse, %	13.5	13.6	0.96 (0.69-1.43)	0.85
Second BRD relapse, %	13.5	16.9	0.85 (0.33-1.53)	0.69
Third BRD relapse, %	0	0		---
BRD CFR, %	0.44	3.07	0.33 (0.03-1.03)	0.08
Crude mortality, %	1.04	1.23	0.84 (0.51-1.40)	0.52
BRD mortality,§ %	0.08	0.42	0.18 (0.04-0.82)	0.03
BRDHS mortality,‖ %	0.35	0.69	0.50 (0.22-1.11)	0.09
Removals, %	3.4	2.1	1.6 (1.15-2.19)	<0.01

* Zelnate®, Bayer, Shawnee Mission, KS, USA

† UF = undifferentiated fever

‡ NF = no fever

§ BRD = bovine respiratory mortality from fibrinous and/or bronchopneumonia

‖ BRDHS = bovine respiratory disease and *Histophilus somni* mortality from fibrinous and/or bronchopneumonia, pleuritis, myocarditis, pericarditis, arthritis

study, making it more difficult to identify a treatment effect when mortality rate is low.

The ADG and DMC with dead weight included was less in the calves given Zelnate (Table 2). It is possible that the immunostimulant in the absence of disease challenge was metabolically demanding and may have caused a transient reduction in performance. When dead weight was not included in the performance variables, ADG and DMC were not statistically different between the 2 treatment groups, similar to the heifer study,[9] where the DNA immunostimulant had no effect on feedlot performance or carcass data.

The removal rate for railers, i.e., animals sent to slaughter prior to the rest of the pen, was higher in the Zelnate group than in the control group. The most common cause for removal was founder, followed by buller, bloat, injury, and chronic footrot. The removal categories with the higher removal rates in the Zelnate vs control group were for founder and injuries/footrot. It is not known why the removal rate would differ between the 2 treatment groups. This could be a statistical error caused by multiple 2-by-2 comparisons.

Additional research should be conducted in different BRD risk calves to determine the reliability of the findings here and evaluate the efficacy and cost effectiveness of Zelnate® when administered at feedlot arrival or other times of stress. As well, alternative methods of using this DNA immunostimulant with and without different metaphylactic or treatment drugs should be evaluated.[9]

Conclusion

A DNA immunostimulant (Zelnate®) administered at arrival processing reduced first treatments for BRD (P=0.02), mortality from BRD (P=0.03), and tended to reduce BRD case fatality rates (P=0.08) and BRD and histophilosis mortality (P=0.09). Further research is needed to determine the value and return on investment of this DNA immunostimulant.

Endnotes

[a] Zelnate®, Bayer, Shawnee Mission, KS

[b] FeedIT, ITS Global, Okotoks, Alberta

[c] M750 thermometer, GLA Agricultural Electronics, San Luis Obispo, CA

[d] Bovishield Gold One Shot, Zoetis Canada Inc., Kirkland, QC

[e] Vision® 8 Somnus with Spur®, Merck Animal Health, Intervet Canada Corp, Kirkland, QC

[f] Bimectin™ Pour-On, Bimedia-MTC Animal Health Inc., Cambridge, ON

[g] Bimectin® Injection, Bimedia-MTC Animal Health Inc., Cambridge, ON

[h] Revalor G®, Merck Animal Health, Intervet Canada Corp, Kirkland, QC

[i] Draxxin®, Zoetis Canada Inc., Kirkland, QC

[j] Microsoft Office Excel 2013, Redmond, WA

[k] Stata 11, Stata Corp, College Station, TX

Table 2. Effect of Zelnate®* on feedlot performance of steer calves at moderate risk of developing bovine respiratory disease.

Performance variable	Experimental group		SEM	*P*-value
	Zelnate	Control		
No. head/pen	271.5	271.5	11.16	1.00
Avg in wt, lb	617	619	1.00	0.18
Avg terminal sort wt, lb	1,350	1,352	4.44	0.62
Avg wt gain, lb	733	733	4.14	0.84
DOF§ – terminal sort	203	203	0.10	0.32
DDMI‖ – terminal sort, lb	18.4	18.3	0.06	0.48
ADG¶,† – terminal sort, lb/day	3.29	3.30	0.02	0.76
ADG‡ – terminal sort, lb/day	3.15	3.24	0.03	<0.01
DMC#,† – terminal sort, lb/lb	5.61	5.57	0.04	0.33
DMC‡ – terminal sort, lb/lb	5.85	5.68	0.06	<0.01

* Zelnate®, Bayer, Shawnee Mission, KS, USA
† weight of dead animals removed
‡ weight of dead animals added
§ DOF = days-on-feed from arrival to terminal weight sort
‖ DDMI = daily dry matter intake, from arrival to terminal weight sort
¶ ADG = average daily gain, from arrival to terminal weight sort
# DMC = dry matter conversion, from arrival to terminal weight sort

Acknowledgements

We would like to thank the participating feedlot owner and staff, as well as research technician Leo Camacho for his participation in this study.

This research was supported by Bayer Animal Health.

References

1. Abell KM, Theurer ME, Larson RL, White BJ, Apley M. A mixed treatment comparison meta-analysis of metaphylaxis treatments for bovine respiratory disease in cattle. *J Anim Sci* 2017; 95:626-635.
2. Booker CW, Abutarbush SM, Schunicht OC, Jim GK, Perrett T, Wildman BK, Guichon PT, Pittman TJ, Jones C, Pollock CM. Evaluation of the efficacy of tulathromycin as a metaphylactic antimicrobial in feedlot calves. *Vet Ther* 2007; 8:183-200.
3. Compiani R, Gianluca B, Bonfanti M, Fucci D, Pisoni G, Jottini S, Torres S. Comparison of tildipirosin and tulathromycin for control of bovine respiratory disease in high-risk beef heifers. *Bov Pract* 2014; 48:114-119.
4. Ilg T. Investigations on the molecular mode of action of the novel immunostimulator Zelnate: Activation of the cGAS-STING pathway in mammalian cells. *Molecular Immunology* 2017; 90:182-189.
5. Lechtenberg K, Daniel CS, Royer GC, Bechtol DT, Chester ST, Blair J, Tessman RK. Field efficacy study of gamithromycin for the control of bovine respiratory disease in cattle at high risk of developing the disease. *Intern J Appl Res Vet Med* 2011; 9:189-197.
6. Miller JF, Hubbert ME, Reinhardt D, Loest CA, Schwandt EF, Thomson DU. Comparison of tulathromycin, tilmicosin, and gamithromycin for metaphylactic treatment of high-risk calves for control of bovine respiratory disease. *Bov Pract* 2016; 50:175-179.
7. Nickell JS, White BJ. Metaphylactic antimicrobial therapy for bovine respiratory disease in stocker and feedlot cattle. *Vet Clin North Am Food Anim Pract* 2010; 26:285-301.
8. Nickell JS, White BJ, Larson RL, Blasi D, Renter D. Comparison of short-term health and performance effects related to prophylactic administration of tulathromycin versus tilmicosin in long-hauled, highly stressed beef stocker calves. *Vet Ther* 2008; 9:147-156.
9. Rogers KC, Miles DG, Renter DG, Sears JG, Woodruff JL. Effects of delayed respiratory viral vaccine and/or inclusion of an immunostimulant on feedlot health, performance, and carcass merits of auction market-derived feedlot heifers. *Bov Pract* 2016; 50:154-162.
10. Van Donkersgoed J. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. *Can Vet J* 1992; 33:786-795.
11. Van Donkersgoed J, Merrill JK. A comparison of tilmicosin to gamithromycin for on-arrival treatment of bovine respiratory disease in feeder heifers. *Bov Pract* 2012; 46:46-51.
12. Van Donkersgoed J, Merrill JK. Efficacy of tilmicosin for on-arrival treatment of bovine respiratory disease in backgrounded winter-placed feedlot calves. *Bov Pract* 2013; 47:7-12.
13. Van Donkersgoed J, Merrill JK, Hendrick S. Comparative efficacy of tilmicosin versus tulathromycin as a metaphylactic antimicrobial in feedlot calves at moderate risk for respiratory disease. *Vet Ther* 2008; 9:241-247.
14. Van Donkersgoed J, Hendrick S, Nickel T. Comparison of gamithromycin and tildipirosin for metaphylaxis treatment of winter-placed feedlot calves for control of bovine respiratory disease. *Bov Pract* 2017; 51:184-189.