zelnate. DNA Immunostimulant: A New Tool to Help Reduce BRD Lung Lesions and Mortality Associated with Mannheimia haemolytica
Summary

USDA has recently licensed Zelnate™ DNA Immunostimulant, a new non-antibiotic immunostimulant for use as an aid in the treatment of Bovine Respiratory Disease (BRD) due to *Mannheimia haemolytica* in cattle 4 months of age or older, when administered at the time of, or within 24 hours after, a perceived stressful event. Zelnate is a new innovation in animal health from Bayer that changes the way veterinarians and cattle producers are able to approach this infectious disease. It is a novel, non-antibiotic technology and first-of-its-kind immunostimulant that enhances an animal’s natural defenses. The stimulation of these defenses helps increase an animal’s ability to fight off this infectious disease. Zelnate was developed in line with Bayer’s pursuit of Science For A Better Life.

Zelnate is the first licensed DNA immunostimulant that aids in the treatment of BRD associated with *M. haemolytica*. It essentially jumpstarts the animal’s own defense system to fight pathogens from the body. The stimulation of the innate immune system has been shown to provide a rapid, potent and broad protective response to infectious agents. Zelnate is administered to cattle at risk of respiratory disease to effectively reduce mortality and lung lesions, which may result in a decreased need for antibiotics. This new non-antibiotic innovation from Bayer was developed based on the company’s long history and expertise in BRD treatment.

The studies detailed in this report show Zelnate reduces the amount of lung lesions resulting from BRD and deaths related to BRD associated with *M. haemolytica*, and thus demonstrate why Zelnate can be an integral part in a BRD control program.

Introduction

BRD is a costly disease for the beef industry across the United States. The costs associated with the disease include the costs of time, labor, facilities, medication, decreased performance and mortality. Lung lesions (lung tissue consolidation) are one result of this disease and the associated inflammation. At slaughter these lesions are found in calves that showed outward signs of BRD and are also seen in some calves that were never recognized to be suffering from BRD. Bottom line is when lung lesions are present, they are associated with decreased performance of the animal as measured by decreased average daily gain (Figure A). Death of an animal is an obvious loss with no recuperation of all the investment put into the animal.

Currently many products and processes are used to try to minimize the frequency and the impact of BRD. In addition to environmental and management strategies that are employed, vaccinations and antimicrobial drugs are used to help mitigate the impact of BRD. Despite all of the currently available tools, BRD continues to be a huge problem for producers.

Zelnate is not an antibiotic. Nor is it a vaccine. Zelnate is a next generation immunostimulant licensed for use in animal health. It contains a unique formulation of DNA and a liposome carrier that has been shown to rapidly trigger innate immunity in cattle to counter the multifactorial complex known as BRD associated with *M. haemolytica*. Zelnate offers stability, efficacy and safety, as proven in challenge models and field studies involving large populations. Zelnate can help address current challenges in infectious disease management, complementing current approaches for BRD.

The *M. haemolytica* challenge studies detailed in this report demonstrate how the appropriate dose and timing of administration of Zelnate were determined. The studies also show Zelnate reduces lung lesion scores, which are associated with decreased calf performance, plus Zelnate has demonstrated reduced mortality rates in feeder cattle.
The first study in this report is actually a compilation of three pilot studies designed to provide a proof of concept for Zelnate™ DNA Immunostimulant and the ability of a challenge model using *Mannheimia haemolytica* to induce BRD. *M. haemolytica* is the primary bacterial pathogen associated with BRD. The next two studies used this challenge model to establish a minimum protective dose and timing of administration for Zelnate by measuring lung lesions (a measure of morbidity) and mortality rates.

### Lung Lesion Scoring

BRD is a serious disease that causes clinical symptoms such as elevated body temperature, cough, depression and difficult respiration. The inflammatory reaction and the disease process occurring within the lungs often cause long-term damage or consolidation of lung tissue. It is also possible this damage occurs in animals in which we do not recognize any outward symptoms. We refer to the damage done to the lung tissue as lung lesions. These lung lesions again are related to decreased performance as measured by decreased average daily gains in calves. Lung lesions are scored to express the relative degree of damage suffered by the lung.

Lung lesion scores are expressed as a percentage of the total lung that is consolidated as a result of BRD. The bovine lung consists of 8 lobes. Each lobe is a certain percentage of the overall lung volume. To score lung lesions of a calf’s lung, an investigator estimates the amount of each lobe that is consolidated and multiplies that by the percentage of total lung accounted for by that particular lobe. This is repeated for each lobe. The figures for each of the 8 lobes are added together to get the final number (Figure B).

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<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
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<th>Overall Findings</th>
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<tr>
<td>Wittum, et al.</td>
<td>1996</td>
<td><em>JAVMA</em></td>
<td>Lung lesions at slaughter = 1 ADG</td>
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<td>2014</td>
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The sum of these 8 individual lobe estimates provide one overall estimate of lung consolidation for each calf.
Summary
This is a compilation of three pilot studies used to provide a proof of concept for both Zelnate™ DNA Immunostimulant and an M. haemolytica challenge model. Calves entering the study were assigned to treatment or control groups receiving either Zelnate or control solution at various dosages ranging from 0.25x to 10x label dose at various times from day -2 to day 1 relative to the M. haemolytica challenge. It was determined the M. haemolytica challenge was successful in inducing BRD, and Zelnate administered at dosages equal to or greater than the label dose showed consistent efficacy in reducing lung lesions.

Cattle
The calves in these studies were mixed sex (bulls, steers and heifers) Holstein calves of weaning age or older (weaning defined as being on a milk free diet) with a weight range of 250–1,000 lbs. The calves were farm-sourced from Indiana. They were required to be in good health showing no signs of BRD such as depression, nasal discharge, cough or abnormal respiratory character. They were also required to be free of any signs of other infectious disease such as pinkeye or foot rot, be BVDV PI negative, have an Mannheimia haemolytica titer of ≤ 1:1024, and be free of non-infectious disease including bloat or injury.

Study Groups
As stated earlier, this study is actually a compilation of three individual pilot studies. Calves were assigned to groups receiving Zelnate or a negative control at various dates (before, at the time of and after an M. haemolytica challenge) and various doses ranging from 0.25x to 10x the label dose.

All groups received an intratracheal M. haemolytica challenge on day 0 of the study (Figure C).

M. haemolytica challenge
An M. haemolytica inoculum was produced at the study site within 48 hours of the challenge. The inoculum contained 10^6 – 10^8 CFU/mL of M. haemolytica.
On day 0, each animal was individually restrained and 60 mLs of the inoculum was administered intratracheally at the tracheal bifurcation via endoscope. The person administering the challenge was blinded.

Observation
The animals were observed from day 0 through day 5. Signs of BRD were recorded. Animals that died received a post-mortem exam. On day 5, all animals were euthanized and received a post-mortem exam including lung lesion scoring.

Figure C: Disease Model Study Timeframe

Results
By the end of the 5-day observation period, all dosages except the 0.25x and the 0.5x had shown consistent efficacy in reducing lung lesions (Figure D).
**Study 2**

**Pivotal Minimum Protective Dose (MPD)**

**Summary**
The goal of this study was to determine the minimum protective dose of Zelnate™ DNA Immunostimulant. Calves were assigned to three groups. Each group received either 1x Zelnate (label dose), 4x Zelnate or a negative control. Calves in all groups received the *Mannheimia haemolytica* challenge on day 0. Results showed calves in both Zelnate groups had statistically significant reduced lung lesions at necropsy compared to the control group.

**Cattle**
The calves in this study were 96 Holstein steers between the ages of 3–4 months with an average weight of 300 lbs. The calves were farm-sourced from Indiana. They were required to be in good health showing no signs of BRD such as depression, nasal discharge, cough or abnormal respiratory character. They were also required to be free of any signs of other infectious disease such as pinkeye or foot rot, be BVDV PI negative, have an *M. haemolytica* titer of ≤ 1:1024, and be free of non-infectious disease including bloat or injury.

**Study Groups**
Included in the study were a total of 96 calves. They were randomly assigned to the following groups:
- **Group 1** (n=32): received Zelnate at 1x (label dose) in 2 mL volume injected intramuscularly
- **Group 2** (n=32): received Zelnate at 4x label dose in 2 mL volume injected intramuscularly
- **Group 3** (n=32): received negative control in 2 mL volume injected intramuscularly

All groups received their treatment or control injections and an intratracheal *M. haemolytica* challenge on day 0 of the study (Figure E).

**M. haemolytica challenge**
An *M. haemolytica* inoculum was produced at the study site within 48 hours of the challenge. The inoculum contained 10^7 CFU/mL of *M. haemolytica*. On day 0, each animal was individually restrained and 60 mLs of the inoculum was administered intratracheally at the tracheal bifurcation via endoscope. The person administering the challenge was blinded.

**Results**
The *M. haemolytica* challenge again proved to be successful. By day 5 of the observation period, BRD morbidity rates reached 96.3% and mortality rate was 1.3% (n=2). No statistically significant difference was seen between the groups for BRD morbidity or mortality. However lung lesion scores were significantly reduced between the Zelnate treatment groups and the negative control group [12.12% in the negative control group; 6.28% (P=0.0273) in the 1x dose treatment group; 6.20% (P=0.0109) in the 4x dose treatment group]. No statistical difference was seen between the 1x (label dose) and the 4x treatment groups (Figure F).

**Observation**
The animals were observed from day 0 through day 5. Signs of BRD were recorded. Animals that died received a post-mortem exam. On day 5, all animals were euthanized and received a post-mortem exam including lung lesion scoring.

**Figure E: Pivotal MPD Study Timeframe**

**Figure F: Average Lung Lesions**

Average lung lesion scores between calves receiving either Zelnate or a negative control at the same time as an intratracheal *M. haemolytica* challenge. Lung lesion scores reflect those observed on day 5 post-challenge.

Zelnate significantly (P<0.05) reduced lung lesions compared to the control.

*Statistically significant reduction (P<0.05)
Study 3  Pivotal Timing of Administration (TOA)

Summary
The minimum protective dose (MPD) study had demonstrated reduction in lung lesion scores when Zelnate™ was administered on day 0 of a Mannheimia haemolytica challenge. The objective of this study was to determine if the MPD showed efficacy when administered 24 hours before and 24 hours after an M. haemolytica challenge. In the study, calves were divided into groups receiving the Zelnate MPD or negative control at day -1 or day +1 relative to M. haemolytica challenge. Results showed the group administered Zelnate at day +1 had numerically reduced lung lesion scores and statistically significant less mortality.

Cattle
The calves in this study were 160 Holstein steers between the ages of 3–4 months with an average weight of 222.6 lbs. The calves were farm sourced from Indiana. They were required to be in good health showing no signs of BRD such as depression, nasal discharge, cough or abnormal respiratory character. They were also required to be free of any signs of other infectious disease such as pinkeye or foot rot, be BVDV PI negative, have an M. haemolytica titer of ≤ 1:1024, and be free of non-infectious disease including bloat or injury.

Study Groups
Included in the study were a total of 160 calves. They were randomly assigned to the following 4 groups:
- **Group 1 (n=40):** received Zelnate at 1x (label dose) in 2 mL volume injected intramuscularly on day -1
- **Group 2 (n=40):** received negative control in 2 mL volume injected intramuscularly on day -1
- **Group 3 (n=40):** received Zelnate at 1x (label dose) 2 mL volume injected intramuscularly on day +1
- **Group 4 (n=40):** received negative control in 2 mL volume injected intramuscularly on day +1

All groups received an intratracheal M. haemolytica challenge on day 0 of the study (Figure G).

M. haemolytica challenge
An M. haemolytica inoculum was produced at the study site within 48 hours of the challenge. The inoculum contained 10^8 CFU/mL of M. haemolytica. On day 0, each animal was individually restrained and 60 mLs of the inoculum was administered intratracheally at the tracheal bifurcation via endoscope. The person administering the challenge was blinded.

Observation
The animals were observed from day 0 through day 5. Signs of BRD were recorded. Animals that died received a post-mortem exam, including lung lesion scoring. On day 5, all animals were euthanized and received a post-mortem exam, including lung lesion scoring.

Figure G: Pivotal TOA Timeframe

![Figure G: Pivotal TOA Timeframe](image)

Results
The M. haemolytica challenge proved to be a strong one as BRD morbidity rates reached 67.5% on day 1 of the observation period, and by day 5 there was a 98% morbidity rate and a mortality rate of 11% (n=17). No significant differences were seen between the group receiving Zelnate on day -1 and the negative control group. On necropsy exam, lung lesion scores for the group administered Zelnate on day +1 were numerically reduced (Figure H). Mortality rates showed a statistically significant reduction from 20% in the day +1 negative control group to 2.5% in the day +1 group administered Zelnate (Figure I). This means Zelnate was able to reduce mortality even in the face of clinically active BRD.
Conclusion

BRD continues to be a major issue facing cattle producers despite advancements in vaccine technology and introduction of new antimicrobials into the marketplace. Vaccines have the potential to stimulate the calf’s immune system to produce antibodies to specific microbes. This increase in antibody production is a product of the acquired immune system and takes several days to weeks to reach its maximum effect. Antimicrobials attack bacteria, either killing the organism or slowing its reproduction, which helps the animal’s immune system to eventually clear the infection barring any decreased susceptibility of the bacteria to the antimicrobial product.

Zelnate is a new and different approach to fighting BRD. Zelnate attacks BRD by engaging the host rather than the pathogen. It attacks BRD from a different angle by helping stimulate the animal’s own innate immune system to fight off the infection. The innate immune system is quick to respond to infection. In doing so, Zelnate has been shown to affect two of the measurements of the impact of BRD: lung lesions and mortality. Lung lesions are associated with BRD and are correlated with decreased ADG in production animals (Figure A) and mortality is the ultimate measure of loss due to BRD. Zelnate as a stand-alone therapy has been shown by the studies above to:

- Significantly reduce lung lesion scores associated with BRD when administered in the face of disease challenge (Study 2)
- Significantly reduce the risk of mortality when administered in the face of clinical BRD (Study 3)

Zelnate can be a new management option to aid in the treatment of BRD due to *Mannheimia haemolytica.*
This product is based on technology developed by Juvaris BioTherapeutics and is patent protected. Animal health applications are being exclusively developed by Bayer Animal Health and are the subject of Bayer patent applications.