PEER REVIEWED

A Comparison of Serological Responses when Modified-live Infectious Bovine Rhinotracheitis Virus Vaccine, *Mannheimia haemolytica* Bacterin-Toxoid and *Leptospira pomona* Bacterin are Administered with Needle-free versus Conventional Needle-based Injection in Holstein Dairy Calves

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Abstract

A total of 104, five- to ten-month old Holstein heifers and steers were blocked by age within sex-group, randomized to treatment and vaccinated with 5-way modified-live virus vaccine, *Mannheimia haemolytica* bacterin-toxoid and 5-way Leptospira bacterin utilizing either needle-free or conventional needle-and-syringe injection techniques. Blood samples were collected from all animals at the time of vaccination and 21 days later, and the serum analyzed for antibody titers to infectious bovine rhinotracheitis (IBR) virus as the indicator of serological response to the 5-way viral vaccine, *Mannheimia haemolytica* (MH) leukotoxin, and *Leptospira pomona* (LP) as the indicator of serological response to the 5-way Leptospira bacterin. On day 21 the serological response of heifers to the IBR fraction of the 5-way viral vaccine, MH bacterin and LP fraction of the 5-way Leptospira bacterin were not significantly different between routes of administration. On day 21 the serological response of steers to the IBR fraction of the 5-way viral vaccine and MH bacterin was significantly higher for the needle-free route of administration, while the serological response to the LP fraction was not significantly different between routes of administration.

Résumé

Un total de 104 taures et bouvillons de race Holstein, âgés entre 5 et 10 mois, ont été regroupés en fonction de l'âge et alloués aléatoirement à un traitement. Les animaux ont été vaccinés avec un vaccin à virus modifiés vivants pentavalent, une bactérine-toxoïde de *Mannheimia haemolytica* (MH) et une bactérine de *Leptospira* pentavalente, tous administrés avec la technique sans aiguille ou avec la technique d'injection conventionnelle avec aiguille et seringue. Des échantillons de sang ont été recueillis chez tous les animaux au moment de la vaccination et 21 jours plus tard. Le sérum a été analysé pour les titres d'anticorps contre le virus de la rhinotrachéite infectieuse bovine (IBR) en tant qu'indice d'une réponse sérologique au vaccin pentavalent et pour la leucotoxine MH et pour les anticorps contre *Leptospira pomona* (LP) en tant qu'indice d'une réponse sérologique à la bactérine de *Leptospira* pentavalente. Au jour 21, il n'y avait pas d'effet de la voie de vaccination sur la réponse sérologique des taures à la fraction IBR du vaccin viral pentavalent, à la bactérine MH et à la fraction LP de la bactérine *Leptospira* pentavalente. Au jour 21, la réponse sérologique des bouvillons à la fraction IBR du vaccin viral pentavalent et à la bactérine MH était plus élevée chez les individus recevant l'injection sans aiguille alors que la réponse sérologique à la fraction LP ne variait pas selon la voie d'administration.

Introduction

Beef and dairy beef quality assurance (BQA) guidelines recognize that inadequate animal restraint or use

of small diameter needles may result in needle breakage, which poses a hazard to those who handle or eat the meat. Furthermore, blood-borne infectious diseases, such as bovine leukosis or anaplasmosis, may be transmitted animal-to-animal when a single needle is utilized to inject multiple animals.[6,7] One technology that potentially minimizes these problems is a pneumatically-powered, needle-free injection device that utilizes air pressure to drive the vaccine through the skin and into the underlying subcutis or muscle.[12]

Needle-free technology traces its roots to industrial accidents in the 19th century when French workmen using pressurized grease guns in factories inadvertently injected themselves. This concept was developed into "jet injectors" which were adopted for use by the US military to vaccinate draftees/recruits in the mid-1950s, and to administer smallpox vaccine in the early 1960s.[10] Needle-free injection devices have been used extensively since that time in human and veterinary medicine to deliver both vaccines and drugs.[2,4,8,9,11] Immunogenicity studies in humans and animals have shown no significant decrease, and an occasional increase, in vaccine efficacy when vaccines were delivered with needle-free delivery systems versus conventional needle systems.[1,3,5,8] The purpose of this study was to compare the serological response when an IBR-containing modified-live virus vaccine, a *Mannheimia haemolytica* (MH) bacterin-toxoid and a *Leptospira pomona* (LP)-containing bacterin were injected into Holstein heifers and steers utilizing either needle-free injection[a] or conventional needle-and-syringe injection methods.

Materials and Methods

Animal Background

Fifty-four Holstein replacement heifers and 50 Holstein steers from the Kansas State University dairy herd were utilized. All animals were five to 10 months of age and had been individually identified with uniquely numbered ear tags at birth. All animals had been vaccinated at birth with an oral modified-live rotavirus-coronavirus vaccine[b] and an intranasal infectious bovine rhinotracheitis-parainfluenza 3 vaccine.[c] At five weeks of age, all animals were vaccinated with a modified-live infectious bovine rhinotracheitis (IBR)-bovine viral diarrhea (BVD)-parainfluenza 3 (PI_3)-bovine respiratory syncytial virus (BRSV) vaccine,[d] *Mannheimia haemolytica* bacterin-toxoid[e] and 7-way clostridial bacterin-toxoid.[f] All calves were revaccinated at 4-5 months of age with a modified-live IBR-BVD-PI_3-BRSV vaccine[d] and 7-way clostridial bacterin-toxoid.[f] In addition, all heifers received a 5-way *Leptospira canicola-grippotyphosa-hardjo-icterohaemorrhagiae-pomona* bacterin[g] and *Leptospira hardjo-bovis* bacterin[h] at 4-5 months of age. All older heifers had been calfhood vaccinated with RB-51 *Brucella abortus* vaccine prior to 10 months of age. Animals of similar age and the same sex were housed in groups of 4-5 animals per pen. All management procedures were applied uniformly to all animals in each pen. Water and feed were available *ad libitum*.

Randomization

Animals were blocked into pairs by age within each sex group, and the route of administration of products was randomly allocated to each animal of each pair in each age block. A random number generator was utilized to assign the treatment to each animal of each pair; the animal with the lower random number was assigned to Treatment 1, and the animal with the higher random number was assigned to Treatment 2.

Treatments

All treatments were administered on day 0. Treatments were administered in a fixed pattern, with modified-live virus vaccine administered in the right side of the neck, and *Mannheimia haemolytica* and Leptospira injections administered in the left side of the neck. Treatment 1 (T1) consisted of a 2mL dose of 5-way modified-live virus vaccine[i] administered by needle-free (NF) intramuscular (IM) injection in the right side of the neck (Figures 1 and 2); a 2 mL dose of *Mannheimia haemolytica* bacterin-toxoid[e] administered subcutaneously (SC) in the left side of the neck utilizing a disposable 3 mL syringe and 18-gauge x 1-inch needle (syringe/needle; S/N); and a 2 mL dose of 5-way Leptospira bacterin[h] administered IM in the left side of the neck utilizing S/N. Needles were discarded following each S/N injection. The needle-free injector pressure was set

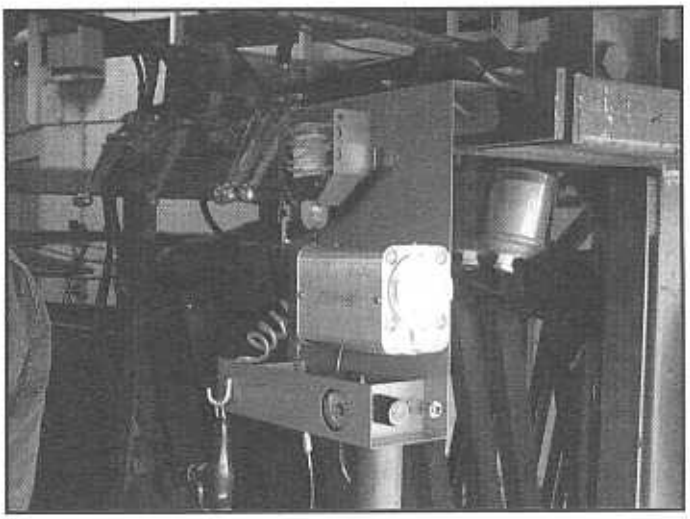

Figure 1. Felton pneumatic system with variable dose and pressure settings.

Figure 2. Felton needle-free injector.

at 85 pounds per square inch (psi) to ensure IM injection of the modified-live virus vaccine (DL Cook, personal communication, 2004).

Treatment 2 (T2) consisted of a 2 mL dose of 5-way modified-live virus vaccine administered IM in the right side of the neck utilizing S/N, a 2 mL dose of MH bacterin-toxoid administered SC in the left side of the neck by NF injection, and a 2 mL dose of 5-way Leptospira bacterin administered IM in the left side of the neck by NF injection. Needles were discarded following each S/N injection. The needle-free injector pressure was set to 75 psi to ensure SC injection of the MH bacterin-toxoid, and 85 psi to ensure IM injection of the Leptospira bacterin (DL Cook, personal communication, 2004). Additionally, all animals were vaccinated with a 7-way clostridial bacterin-toxoid[f] by IM injection. Each vaccine was given in a consistent injection site. Four-inch (10.2 cm) spacing was maintained between injection sites.

Animal Management

Animals were housed in their original pens and groupings throughout the 21-day study. Water and feed were available *ad libitum* according to established protocol. Animals were observed daily for signs of disease. Ten animals from T1 and 12 animals from T2 exhibited transient swelling at the site of the MH bacterin-toxoid injection. No animals required treatment during the study.

Sample Collection and Evaluation

Blood samples were collected from each animal on day 0 and day 21. All blood samples were chilled and forwarded to the Kansas State University Veterinary Diagnostic Laboratory for serological evaluation. Routine $\log^2$ serum neutralization evaluation for the presence of antibody to IBR virus was performed as an indicator of the serological response to the modified-live virus vaccine. Enzyme-linked immunosorbent assays (ELISA) using whole cell and supernatant antigens of *M. haemolytica* were used to estimate serum antibody responses to the MH bacterin-toxoid. Microscopic agglutination evaluation for the presence of antibody to LP was performed as an indicator of the serological response to the Leptospira bacterin.

Data Management and Analysis

Statistical analyses for titer levels were performed with the Mixed Procedure of SAS (SAS, 2000; SAS Institute, Inc, Cary, NC). A split-plot analysis was conducted to account for repeated measurements that included the fixed effects of treatment and day of bleeding as the repeated measure. Satterthwaite adjustment was used for the degrees of freedom. All treatment means were separated ($P < .05$) using the Least Significance Difference procedure when the respective F-tests were significant ($P < .05$) unless otherwise stated.

Results and Discussion

Treatment Least-Squares Means of IBR virus, *Mannheimia haemolytica* and *Leptospira pomona* serological responses on day 0 and day 21 for heifers and steers are shown in Tables 1 and 2, respectively. There was no pre-existing statistical difference in IBR, MH or LP titer means for heifers on day 0, and no significant difference in IBR, MH or LP treatment means on day 21.

In the steer population, there was no pre-existing statistical difference in IBR, MH or LP titer means on day 0. On day 21, there were significant differences in IBR and MH treatment means, with higher mean titers associated with needle-free administration; however, there was no significant difference in LP treatment means.

An attempt was made to pool heifer and steer means, but a significant sex by treatment interaction with MH prevented pooling of the data.

Although a 5-antigen multivalent modified-live virus vaccine was used in this study, IBR titers were selected as the single indicator of serological response to the multivalent vaccine. Similarly, while a 5-antigen multivalent Leptospira bacterin was used, LP titers were selected as the single indicator of serological response to this bacterin.

All heifers had been previously vaccinated with products containing IBR, MH and LP antigens. Steers had been previously vaccinated with IBR and MH anti-

Table 1. Treatment Least-Squares Means of IBR, *Mannheimia haemolytica* and *Leptospira pomona* serological responses in dairy heifers.

Administration method / antigen	Day 0 titer	SE	Day 21 titer	SE
T1 Needle-free / IBR	2.00	0.71	12.30	4.7
T2 Needle / IBR	0.52	0.20	6.52	1.6
[a] P value	0.68		0.11	
T1 Needle / *M. haemolytica*	0.271	0.02	0.348	0.02
T2 Needle-free / *M. haemolytica*	0.262	0.02	0.326	0.02
[a] P value	0.76		0.51	
T1 Needle / *L. pomona*	0.0	0	177.8	81
T2 Needle-free / *L. pomona*	0.0	0	70.4	25
[a] P value	1.00		0.08	

[a] P value for comparisons within antigen and day.

Table 2. Treatment Least-Squares Means of IBR, *Mannheimia haemolytica* and *Leptospira pomona* serological responses in dairy steers.

Administration method / antigen	Day 0 titer	SE	Day 21 titer	SE
T1 Needle-free / IBR	1.44	0.27	9.84	3.4
T2 Needle / IBR	1.12	0.35	3.20	0.9
[a] P value	0.89		0.01	
T1 Needle / *M. haemolytica*	0.177	0.01	0.247	0.01
T2 Needle-free / *M. haemolytica*	0.210	0.01	0.290	0.01
[a] P value	0.08		0.02	
T1 Needle / *L. pomona*	0.0	0	24.0	10.4
T2 Needle-free / *L. pomona*	0.0	0	16.0	7.5
[a] P value	1.00		0.38	

[a] P value for comparisons within antigen and day.

gens at the participating dairy, but not a Leptospira bacterin. As a result, it was anticipated that pre-existing titers to IBR and MH antigens would be present at the day 0 blood sampling in both heifers and steers. The absence of pre-existing titers to LP in steers was expected since they had not been previously vaccinated. The lack of pre-existing titers to LP in previously vaccinated heifers was somewhat of a surprise. Furthermore, 21-day post-treatment LP titers were detected in only 20 of 54 heifers and 10 of 50 steers; the serologic response did not differ by route of administration among heifers or steers, but we anticipated that a greater proportion of heifers would have responded to vaccination with the LP antigen.

The findings of this study indicate that use of a needle-free injection system to vaccinate dairy heifers and steers can produce IBR, MH and LP serological responses at least equivalent to those obtained with conventional needle-and-syringe injection systems. Use of the Felton Pulse™ 250 system offers a viable option for vaccinating dairy calves when it is desirable to reduce potential for needle injury to animal handlers, prevent losing broken needles in tissue, or possibly prevent transfer of blood-borne disease.

Further research is needed to define the cell-mediated immune response to vaccination, and to determine if differences in tissue reaction exist when vaccinating cattle with a needle-free injection system as compared to traditional needle-based injections.

Conclusion

These results suggest that use of a needle-free injection system to vaccinate dairy heifers and steers can

result in similar or sometimes greater serological responses to vaccination than when using conventional syringe-and-needle injection systems.

Footnotes

[a] Felton Pulse™ 250 Needle-Free Injector System, Felton International, Lenexa, KS
[b] Calf-Guard® (modified-live bovine rota-coronavirus vaccine) Pfizer Animal Health, New York, NY
[c] TSV-2® (modified-live bovine rhinotracheitis-parainfluenza3 vaccine) Pfizer Animal Health, New York, NY
[d] Bovi-Shield® 4 (modified-live bovine rhinotracheitis-virus diarrhea-parainfluenza3-respiratory syncytial virus vaccine), Pfizer Animal Health, New York, NY
[e] One Shot® (*Pasteurella haemolytica* bacterin-toxoid), Pfizer Animal Health, New York, NY
[f] Fortress® 7 (*Clostridium chauvoei-septicum-novyi-sordellii-perfringens* Types C & D bacterin-toxoid) Pfizer Animal Health, New York, NY
[g] Leptoferm-5® (*Leptospira canicola-grippotyphosa-hardjo-icterohaemorrhagiae-pomona* bacterin) Pfizer Animal Health, New York, NY
[h] Spirovac® (*Leptospira hardjo-bovis* bacterin) Pfizer Animal Health, New York, NY
[i] Bovi-Shield® Gold 5 (modified-live bovine rhinotracheitis-virus diarrhea-parainfluenza3-respiratory syncytial virus vaccine), Pfizer Animal Health, New York, NY

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