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Serological Response to Administration of *Brucella abortus* Strain RB51 Vaccine in Beef and Dairy Heifers, using Needle-Free and Standard Needle-Based Injection Systems

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Abstract

More than four million doses of *Brucella abortus* strain RB51 (SRB51) are administered in the United States every year. Accidental autoinoculation of humans has the potential to result in local and systemic symptoms; therefore, investigation of alternative delivery methods is warranted. The objective of this study was to compare the immunologic responses of four to eight-month-old beef and dairy heifers to vaccination with 10^{10} colony-forming units of SRB51, delivered either by standard needle-and-syringe system or needle-free injection system. There was no difference in the 30-day SRB51 antibody response to the two vaccination methods, and no difference in response between beef and dairy heifers. These data demonstrate that the humoral immune response of heifers to vaccination with SRB51 using a needle-free injection system is similar to the response obtained with a standard needle system. Further studies to define the cellular immune response and protection against infection are recommended before this administration method is considered for routine use.

Keywords: bovine, vaccination, brucella, needle-free injection

Résumé

Il y a plus de quatre millions de doses de la souche RB51 (SRB51) de *Brucella abortus* administrées chaque année aux États-Unis. L'auto-inoculation accidentelle chez l'humain peut causer des symptômes locaux et systémiques de sorte que la recherche de méthodes alternatives d'administration est justifiée. L'objectif de cette étude était de comparer, chez des génisses laitières et de boucherie de 4 à 8 mois d'âge, la réponse immunologique à la vaccination avec 10^{10} unités formatrices de colonies de SRB51 administrées soit avec le système standard d'aiguille et de seringue ou soit avec un système d'injection sans aiguille. Il n'y avait pas de différence au niveau de la production d'anticorps contre SRB51 à 30 jours entre les deux méthodes de vaccination pas plus qu'entre la réponse des taures laitières ou de boucherie. Ces données démontrent que la réponse immunitaire humorale des taures à la vaccination avec SRB51 ne diffère pas selon que le vaccin soit administré avec aiguille ou avec le système d'injection sans aiguille. Plus d'études sont nécessaires afin d'éclaircir la réponse immunitaire cellulaire et la protection contre l'infection avant que cette méthode d'administration sans aiguille ne soit utilisable de façon routinière.

Introduction

Brucellosis is a zoonotic bacterial disease with important public and animal health implications. In the United States, the Cooperative State - Federal Brucellosis Eradication Program targets the eradication of the disease in cattle with a major effort to vaccinate heifers against *Brucella abortus*,[18,26] most recently using a

modified-live vaccine utilizing *B. abortus* strain RB51 (SRB51).[1] While the US Brucellosis Vaccination Program has decreased the prevalence of cattle infected with *B. abortus*, and consequently the risk of transmission of this zoonotic agent to humans, the use of SRB51 carries an important health risk to veterinarians who administer the vaccine.[2-4] Accidental autoinoculation of humans with SRB51 vaccine is associated with local and systemic events. These reactions may include one or more of the following symptoms: erythema or induration at the site of injection, myalgia, fever, arthralgia, headaches, fatigue, sweats, chills, vomiting or diarrhea.[2] Technology to reduce this risk would be welcome in both the public health and food system veterinary communities.

Needle-free injection devices have been used in the delivery of vaccines and drugs in human and veterinary medicine.[9,11-15] In veterinary medicine, the use of needle-free injection devices has the potential to reduce disease spread (especially of blood-borne diseases, compared to using a single needle repeatedly), eliminate broken needles in carcasses, reduce stress in vaccinated animals, reduce medical waste, and improve safety with increased accuracy of product administration.[12-14,17,25] Use of a needle-free injection device will reduce risk to humans, due to a reduction in employee injuries related to unintentional injections.[11-14] The important question is whether vaccine delivered using the needle-free system results in an effective immune response in the vaccinated animals. Given that no studies have been performed with a needle-free injection device using SRB51 vaccination, the present study was undertaken to evaluate the serologic and cell-mediated (based on interferon production) immune response to SRB51 in heifers vaccinated with a needle-free injection device, compared to a standard needle-based injection system.

Materials and Methods

The study was designed as a randomized controlled clinical trial with two primary treatment groups: a needle-free (treatment) and conventional needle vaccine delivery system (control). Within each treatment group, a sham vaccine (saline solution) was included to evaluate any potential effects of the delivery systems on the study outcomes. Animals were randomly allocated to needle-free or standard-needle injection and then, within each treatment, randomly allocated to vaccination and sham groups in a ratio of five vaccinated to one sham-vaccinated animal.

A total of 135 heifers were included in the study. Heifers were blocked by breed and allocated into the study groups. Animals came from two locations: four to eight-month-old Angus or Angus-cross heifers from the University of California Sierra Foothills Research and Extension Center, Brown's Valley, California, and four to seven-month-old Holstein heifers from a commercial dairy in the Central Valley of California. Ages (in days) of animals in the study were obtained from herd records.

The vaccine used in the trial was the commercial lyophilized *Brucella abortus* SRB51 vaccine,[a] which was diluted with sterile diluent according to the manufacturer's instructions to a final concentration of 0.5 x 10^{10} colony-forming units (CFU) of *Brucella abortus* SRB51 per mL. Animals in the vaccine treatment groups received a 2 mL dose (approximately 10^{10} CFU) of SRB51 vaccine, administered subcutaneously either by needle-free injection device or by standard 18G x 1" needle. Sham vaccines (2 mL saline) were administered with the needle-free injection device or with a standard 18G x 1" needle.

A commercial needle-free system[b] was used to deliver vaccine in the treatment group. The system consisted of a pneumatic amplifier (CO_2) that delivered a pressure of 75 pounds per square inch using a standard nozzle orifice of 0.36 mm. The delivery of vaccine to the control groups was done using a single-use disposable syringe with an 18-gauge needle. All injections were administered subcutaneously in the right cervical region. Calves were monitored daily for signs of adverse reactions to the injections.

At day 0 (pre-vaccination) and day 30 after vaccination, a physical examination of all study heifers was performed and blood was collected into serum separator tubes.[c] The serum was divided into 1 mL aliquots, frozen, and stored at -94°F (-70°C). The serum samples were sent to Agricultural Research Service (ARS-USDA), Ames, Iowa, to assess the presence of *Brucella abortus* antibody.

Brucella abortus antibody was detected by enzyme-linked immunosorbent assay (ELISA). A stock solution of *Brucella abortus* SRB51 was spectrophotometrically adjusted to 10^8 CFU per mL in 0.1M carbonate-bicarbonate buffer (pH 9.6). To each well in a polystyrene microtiter plate,[d] 0.1 mL of the adjusted bacterial suspension was added and incubated at 39.2°F (4°C) overnight to coat each well with 10^7 CFU of SRB51. Plates were blocked with 300 µL of 0.02M phosphate-buffered saline (pH 7.2) containing 0.25% fish gelatin (PBS-FG). After incubation at room temperature for two hours, plates were rinsed three times with 300 µL of 0.02M phosphate-buffered saline containing 0.05% Tween 20 (PBS-Tween). Serum samples were diluted 1:800 in PBS-FG and 100 µl was added in triplicate to wells in the microtiter plate. After incubation at room temperature for two hours, plates were rinsed three times with 300 µl of PBS-Tween. The secondary antibody, rabbit anti-bovine IgG (heavy and light chain specific), was diluted 1:2500 in PBS-FG. A 100 µl aliquot of the secondary antibody was added to each well and plates were incubated at room temperature for two hours. Microtiter plates were then washed

three times with 300 µl of PBS-Tween. A 100-µl aliquot of substrate (0.18M 3,3',5,5' tetramethylbenzidine and 0.015% H_2O_2 in 0.1M citrate buffer (pH 4.0)) was added to each well. After incubation in the dark at room temperature for 30 minutes, the color reaction was stopped by addition of 100 µl of 0.18M sulfuric acid to each well. The optical density (OD) of wells in microtiter plates was read on an ELISA plate reader[e] at 450/550 nm. Optical density has a strong linear correlation to antibody titer due to the measurement of conversion of substrate by the peroxidase on the secondary antibody.

At day 90, blood samples were collected by jugular puncture from a random sample of 21 sham-vaccinated animals (eight needle-free and 13 standard-needle injection), 45 needle-free vaccinates, and 54 standard-needle vaccinates and placed into acid-citrate dextrose tubes.[f] Samples were kept at room temperature and shipped overnight to the ARS-USDA laboratory, arriving within 24 hours of collection. Peripheral blood mononuclear cells were purified using density gradient centrifugation, and numbers of viable cells per mL determined by Trypan blue exclusion. Fifty µl of each cell suspension, containing 5×10^5 cells, was added to each of two separate flat-bottom wells of 96-well microtiter plates[d] containing 100 µl of RPMI 1640 medium only, or 1640 medium containing γ-irradiated Brucella abortus strain RB51 (108 bacteria per well). Plates were incubated at 98.6°F (37°C) in 5% CO_2. Aliquots (100 µl) of supernatants from wells, with or without RB51 antigens, were obtained at 24 hours after initiation of culture. Concentrations of γ-IFN in control and RB51-stimulated wells were determined using a commercially available kit[g] for bovine gamma interferon, performed in accordance with the manufacturer's instructions. To assess background γ-IFN production, each plate contained wells without SRB51 antigen. Known concentrations of γ-IFN were included on each plate and used to calculate a standard curve. Net interferon production was calculated as the difference between the γ-IFN concentration in the wells with antigen and the wells without antigen. The OD of kit standards and test samples were read at 450 nm using an ELISA plate reader.[e] Negative (serum from non-vaccinated cattle) and positive (negative serum spiked with recombinant bovine interferon) controls were included in the assay. Linear regression analysis was performed on standards and used to calculate the IFN concentrations for samples.

Statistical Analysis

Brucella abortus antibodies were measured by OD at day 0 and day 30. Four values for each animal were recorded; the mean of the quadruplicates was calculated and used as the final data for statistical analysis. In order to normalize these data, a natural logarithmic transformation of OD was used. The difference of natural logarithmic of OD between day 0 (pre-vaccination) and day 30 (post-vaccination) was calculated and defined as the outcome variable ($\Delta \log OD$).

The *B. abortus* antibody response to vaccination was analyzed using mixed model procedure of a commercially available statistical program.[h] The model used to investigate the difference in antibody response between treatment groups and control groups was as follows:

$$\Delta \log OD_{ijkl} = \mu + G_i + B_j + A_k + \gamma_l + \varepsilon_{ijkl}$$

Where $\Delta \log OD_{ijkl}$ is the dependent variable, µ is the overall mean, G_i is the effect of the treatment group i, B_j is the effect of breed j, A_k is the effect of age, γ_l is the two-way interaction terms, and ε_{ijk} is the residual error.

Median values for γ-IFN for each group were compared using the nonparametric Kruskal-Wallis test.[h] Results for all tests were considered to be statistically significant if the *P*-value was < 0.05.

Results and Discussion

We observed that calves tolerated the needle-free injections very well, as no adverse reactions such as injection site swelling, fever, or allergic reactions were noticed during the 12 weeks post-vaccination follow-up. During the vaccination neither group of calves showed significant signs of stress, such as jumping or vocalizing; this observation was also reported in vaccination of sheep using needle-free injection.[17]

The needle-free injection system has some disadvantages, such as the time required for setting up and subsequently cleaning the entire system, making it inefficient to use when working with small groups of animals. In addition, movement of calves when not well restrained can increase risk of aerosol production, and consequently the risk to the operator of infection via conjunctiva or open wounds with strain RB51. Personal protective equipment, including goggles and gloves, should always be worn when vaccinating against brucellosis.

The median OD from the needle-free and standard-needle vaccinated groups and sham (saline)-vaccinated groups at day 0 (pre-vaccination) were similar (Figure 1), indicating that antibody levels were equivalent prior to vaccination. The effect of group treatment was significant. Heifers vaccinated with SRB51 using either the needle-free or standard-needle delivery system had greater $\Delta \log OD$ (thus greater antibody levels) when compared with sham-vaccinated heifers ($P<0.001$) (Figure 1).

The main interest of the study was comparing the serological response to SRB51 between the standard-needle and needle-free injection. Thus, the saline-vaccinated heifers were not included in subsequent analysis. Serological response ($\Delta \log OD$) of needle-free vaccin-

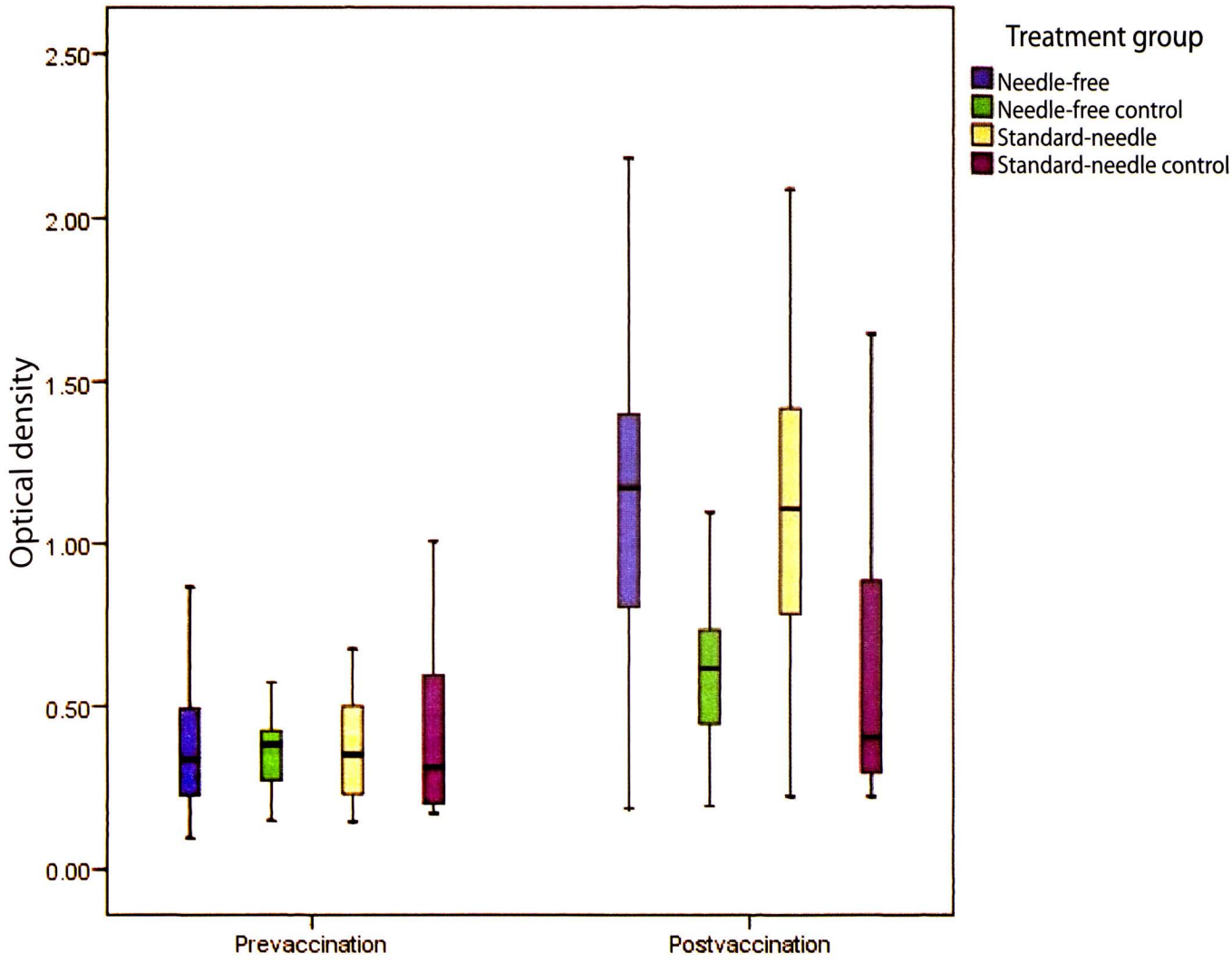

Figure 1. Boxplot of ELISA optical density (OD) values for serum of saline and SRB51-vaccinated heifers. Heifers were vaccinated with either a 2 mL volume containing 10^{10} CFU colonies of SRB51 using a needle-free and standard-needle vaccine delivery system or 2 mL saline solution using a needle-free and standard-needle system. Responses are presented as prevaccination values and 30 days post-vaccination.

ated heifers was not significantly different from the standard-needle vaccinated heifers, when the beef and dairy groups were analyzed together (Table 1). Serological response of beef heifers vaccinated with SRB51 did not differ from those of dairy heifers. These findings agree with previous reports in vaccination studies evaluating *Mannheimia haemolytica* (MH), *Leptospira pomona* (LP), and infectious bovine rhinotracheitis (IBR) in dairy heifers.[13] In previous studies using the needle-free system, steers developed higher serologic responses to MH and IBR compared to those using needle-and-syringe injection system.[12,13]

Needle-free injection systems have been used in human medicine for delivery of vaccines and medications since the 1940s. Reports in the literature on immunologic responses to needle-free vaccination have primarily evaluated responses to viral or DNA vaccines. In the human literature, most studies comparing jet injection to delivery via needle and syringe have found equivalent or enhanced immune responses to vaccination.[9] Similar results have been reported for needle-free vaccination of swine and cattle.[13,14] At the present time, data on needle-free inoculation with live bacterial vaccines is lacking in the literature.

The effect of age was not significant (P=0.723) when needle-free and standard-needle vaccinated groups were evaluated. This is not surprising, given the variety of results published related to the effect of age on immune response to *Brucella* vaccination. It has been shown that vaccination age did not affect protection against infection

Table 1. Results of a mixed model multivariate analysis of $\Delta \log OD$ comparing antibody response to SRB51 vaccination using either a needle-free or standard-needle vaccine delivery system.

Variable	Level	Estimate	95% Confidence Interval	*P*-value
Group	Needle-free SRB51	0.036	(-0.124 , 0.96)	0.655
	Standard-needle SRB51	Reference		
Breed	Beef	0.099	(-0.242, 0.439)	0.566
	Dairy	Reference		
Age	Days	0.001	(-0.004; 0.006)	0.723

and abortion,[5] while other studies have observed that the humoral immune response is affected by animal age at the time of vaccination or infection.[8,10,22,24] Thus, it seems apparent that the effect of age on immune response is inconsistent and further study is warranted.

The amount of γ-interferon produced for the interferon test by saline groups and vaccinated groups with needle-free injection and standard needle did not differ at day 90 ($P>0.05$). By sampling at only one time point, the peak production of gamma interferon may have been missed,[7,27] and therefore a significant difference was not detected. Previous studies with bison showed statistically significant higher interferon production at 8, 12, and 20 weeks after vaccination with hydrogel ballistic delivery, compared with non-vaccinated bison.[24] Previous research demonstrated that using a needle-free vaccine delivery device for administration of SRB51 vaccine in bison has at least equivalent cell-mediated immune response as conventional needle and syringe administration.[21] Further studies to quantify specific T-lymphocyte cell subpopulations would better define the response to needle-free administered antigens.[19,23]

Needle-free injection devices have been used extensively in human medicine. Other researchers have shown that the method improves safety and is easier and faster than traditional syringe-and-needle injection.[15,16] To the authors' knowledge, this is the first study to examine serologic response to SRB51 using a needle-free injection system in cattle, although SRB51 vaccination using vaccine delivery devices other than a standard needle-and-syringe has been evaluated in bison.[6,20] These bison studies demonstrated that the immunologic response using ballistic delivery of SRB51 via photopolymerized hydrogel is similar to that of subcutaneous vaccination.[21]

Conclusions

The use of a needle-free injection system in veterinary medicine offers an alternative for vaccination and treatment of cattle when the aim is to reduce the potential for accidental needle sticks in humans, decrease carcass damage due to broken needles, and prevent transmission of blood-borne diseases in animals.[17,25] The use of needle-free injection systems to administer SRB51 is not currently approved by USDA-APHIS. If needle-free administration of SRB51 is to become an approved administration method and its use becomes widespread, it has the potential to reduce human exposure to SRB51, as there is virtually no risk of self-injection of vaccine using the needle-free device. However, added care must be exercised to avoid aerosol exposure, as it is possible that pressurized systems may be more likely to produce aerosols than needle-and-syringe administration. Since antibody responses do not correlate with protective immunity against brucellosis, and cellular immunologic responses (based on interferon production) could not be established with certainty in the current study, it would suggest that additional studies, including challenge studies, will be necessary in order to evaluate the efficacy of needle-free vaccines in preventing infection with *Brucella abortus*.

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Endnotes

[a]RB51, Colorado Serum Company, Denver, CO
[b]Pulse 250™ Micro Dose Injection System, Pulse Needle-Free Systems Inc., Lenexa, KS
[c]Corvac™ Serum Separator Tubes, Covidien, Mansfield, MA
[d]Immulux™, DYNEX Technologies Inc., Chantilly, VA
[e]Molecular Devices Corp., Sunnyvale, CA
[f]BD Vacutainer®, BD, Franklin Lakes, NJ
[g]BioSource™, Invitrogen Corp., Carlsbad, CA
[h]SPSS version 16 for Windows, General Linear Model (GLM) procedure, SPSS Inc., Chicago, IL

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