The effect of *Salmonella* vaccination on *Salmonella* Dublin blood enzyme-linked immunosorbent assay results

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Abstract

The *Salmonella* Dublin (*S*. Dublin) antibody enzyme-linked immunosorbent assay (ELISA) is used to estimate the prevalence in dairy herds and as the primary diagnostic method in national control plans. Limited evidence is available regarding how U.S. currently licensed *Salmonella* vaccines produce *Salmonella* Dublin antibodies that are detectable by a commercial ELISA. The objective of this study was to determine if any of the commercial *Salmonella* vaccines produce detectable antibodies using this test. Fifty Holstein heifer calves were allocated into 5 groups of 10 and randomly assigned into 1 control (sham vaccinated) and 4 vaccine groups. Calves were vaccinated or received a sham injection twice 2 weeks apart. All were tested using the *S*. Dublin serum ELISA prior to vaccination and again 2 and 5 weeks following the second vaccination. Individual calf ELISA results were reported as percent positivity (%PP) with ≥ 35% considered positive. Percent positivity results were analyzed using a mixed linear regression model in Stata, where percent positivity was the outcome. Fixed effects included the vaccine group, day post-vaccination, and their interaction. Based on the cutoff of ≥ 35%, 5 weeks following second vaccination, the number of positive calves were 1, 4 and 5 for calves administered ENDOVAC-Dairy®, a *Salmonella* Dublin-Typhimurium bacterin (Colorado Serum), and EnterVene®-d, respectively. Calves administered the SRP® vaccine or saline (control) did not have positive ELISA results at any point in the study. This study demonstrates that certain U.S. commercial *Salmonella* vaccines complicate the interpretation of *S*. Dublin ELISA results, further questioning the use in prevalence studies and control programs.

Key words: *Salmonella*, *S*. Dublin, vaccine, ELISA, heifer calves

Introduction

Salmonella enterica subspecies *enterica* is commonly found on farms and many different species can suffer from infections, including humans. This pathogen brings many challenges to farms by causing economic losses and animal welfare concerns.[1,2,3] Further, *Salmonella* spp. cause a significant public health impact as a result of transmission through direct animal contact or their food products, leading to an estimated 1.2 million cases of human illness annually.[1] Additionally, frequent antimicrobial resistance among these zoonotic *Salmonella* pathogens complicates the treatment of human and animal infections.[4]

Cattle farms have significant challenges from *Salmonella* infections frequently due to a host-adapted serovar *Salmonella enterica* subspecies *enterica* serovar Dublin (serogroup D). *Salmonella* Dublin can cause severe infections, outbreaks, economic losses and welfare concerns. Once infected, the bacteria cause an array of disease manifestations including enteritis, pneumonia, septic arthritis and even abortion in adult cows.[2] This host-adapted serovar can also cause asymptomatic carriers which shed the bacteria and pose a moderate risk of transmission to other animals, making it difficult to detect and eradicate from farms.[5] In North America, *S*. Dublin is primarily multidrug resistant further increasing the risk to human and animal health.[6] Due the impact of *S*. Dublin to farms and public health, several countries have implemented control and surveillance programs.[6,7] Additionally, test results are used by producers to make animal movement or purchasing decisions.

Current diagnostic methods for *Salmonella* Dublin include fecal culture, polymerase chain reaction (PCR) and a commercially available enzyme-linked immunosorbent assay (ELISA). Fecal culture is a common approach; however, this method has poor sensitivity, particularly for detecting asymptomatic shedding.[9] Polymerase chain reaction may have a higher sensitivity,[10] but the lack of organism recovery limits serotyping and other strain characterization (e.g., antimicrobial susceptibility).[11] The ELISA measures the level of *S*. Dublin antibodies directed against O-antigens in blood or milk.[12] Unfortunately, this method also has a lower sensitivity at 65% (55 to 75%) at a percent positivity (PP) of ≥ 35%, which is recommended by the manufacturer.[13] On blood, Nielsen et al. found that sensitivity of this ELISA can range depending on the age of calves tested. Authors revealed a sensitivity of 79% (62 to 91%) for calves 100 to 300 days of age and 62% (46-75%) for calves > 300 days of age, using the same cutoff value.[14] Using the bulk milk ELISA for *S*. Dublin, Um et al. estimated a sensitivity of 40.6% (15.6 to 88.8%), using a cutoff of ≥ 15% PP.[15]

Additionally, other potential obstacles with this ELISA can include cross reactions with other antibodies from similar *Salmonella* serovars. The manufacturer of the test warns against potential cross reactions with *Salmonella* Typhimurium specifically. As with any antibody test, the presence of antibodies does not equate to current infection. Similarly, vaccination with commercially available vaccines may cause antibodies to *S*. Dublin in high enough quantities to result in difficulty differentiating infected animals from vaccinated. This lack of differentiation results in difficulty interpretating positive results to the *S*. Dublin antibody ELISA.

Several studies have utilized the *S*. Dublin ELISA to estimate prevalence in dairy herds across several countries.[7,16,17] Even further, several countries have established national control plans for *S*. Dublin utilizing these antibody tests.[8,18,19] Most prevalence estimation methods utilize bulk tank milk antibody testing; however, others also use serum antibody testing

of a subset of animals in a herd.[7,8,16,17,18,19] Although serology is being widely used to estimate *S*. Dublin prevalence and identify potential carriers for animal movement decisions, the common practice of vaccination for *Salmonella* Dublin or other *Salmonella* serovars could complicate the interpretation of serology results. Vaccine types available in the United States include killed whole cell (bacterin), bacterial fractions (subunit) and attenuated modified live.[3] Currently, the only vaccine that is known to induce detectable antibodies with serologic testing is the modified live *Salmonella* Dublin EnterVene®-d vaccine[a].[20] Although several other vaccines are commercially available and commonly used, no other *Salmonella* vaccines have been investigated to see if the antibodies produced result in a positive *S*. Dublin ELISA test.

Knowing whether currently licensed *Salmonella* vaccines generate detectable antibodies with serological testing methods is vital to testing strategies, prevalence studies and national control programs around the world. More research is required before serology testing can be used as a tool to estimate prevalence of *S*. Dublin antibodies. Therefore, the objective of this experimental study was to determine if 4 different currently licensed *Salmonella* vaccines could induce serum antibodies against *Salmonella* Dublin detectable by a commercial *Salmonella* Dublin ELISA[b].

Materials and methods

This experimental trial was conducted from May through July 2023. The procedures of animal handling and sample collection were reviewed and approved by the Institutional Animal Care Use Committee, The Ohio State University (protocol #2023A00000019).

Animals and facilities

In total, 50 Holstein heifers, all born on the same dairy farm in Ohio, were used for the study. No *Salmonella* vaccines were used on this farm in the last 5 years prior to this study. All calves were moved to one of 3 different heifer raising facilities where the first 50 calves > 90 days of age (age range 90 to 114 days) were enrolled into the study. Calves in this age range were used to avoid potential detection of passively transferred maternal antibodies. Calves were originally housed individually in calf hutches and later moved to group housing between 70 and 75 days of age at all locations. Group housing pens were all neighboring each other on each farm and treatment groups were not separated by pens (all housed together). All calves were fed a 17% protein pelleted diet and had ad libitum access to hay and water. Throughout the study, all calves were moved to another heifer raising facility, where they were group housed, at approximately 120 days of age. Calves were not all moved at once; they were slowly moved throughout the study.

Calves were randomly assigned to one of 4 different vaccine groups or control group with 10 calves in each group using a random number generator in Excel[c] while ensuring that each treatment group was well represented on each farm. Thirty-four of the 50 calves were located at one facility with 9 and 7 calves at the other 2 farms due to facility size. All locations had calves represented from each treatment group. Ten calves in each group provided > 80% power to detect at least 1 calf with antibodies, assuming that the mean probability of detectable antibodies following vaccination is at least 15%.[21]

Vaccine administration

Four commercially available *Salmonella* vaccines were used, and the control group received 0.9% saline[d]. The 4 vaccines were: 1) a modified live *Salmonella* Dublin bacterin (EnterVene-d), 2) a killed *Salmonella* Dublin and Typhimurium bacterin (Colorado Serum)[e], 3) a subunit vaccine, *Salmonella* Newport Bacterial Extract (SRP®)[f], and 4) a *Salmonella* Typhimurium bacterin-toxoid (ENDOVAC-Dairy®)[g]. These vaccines were chosen as they were all the licensed *Salmonella* vaccines in the U.S. at the time of this study that contain whole or part of a *Salmonella* bacteria. All vaccines were administered to all calves in the morning on the same day, on the left side of the neck, 2 milliliters each given subcutaneously, except ENDOVAC-Dairy which was administered intramuscularly, following label instructions. All vaccines were kept in a Styrofoam cooler with ice packs and were prepared immediately prior to administration. The control group was given 2 milliliters of normal saline subcutaneously. All vaccines and the control group received a second administration 14-days after the initial dose. The second administration 14-days later was chosen as it fell within the range recommended by all vaccine labels.

Sample collection

Blood samples were obtained just prior to the first vaccine administration and at 2 and 5 weeks following the second vaccine administration. Blood was collected from the jugular vein into a syringe and immediately placed into serum separator blood tubes[h] and placed on ice until they were centrifuged for 10 minutes at 1,534 g later the same day. Serum was then separated into red top tubes[i] and frozen at -4 °F (-20 °C). Samples were shipped frozen, on dry ice, to the Wisconsin Veterinary Diagnostic Laboratory for analysis with the *Salmonella* Dublin antibody ELISA. These results were reported as percent positivity (PP) with ≥ 35% classified as positive for *S*. Dublin antibodies, as recommended by the manufacturer.

Just prior to the first vaccine administration and blood collection, and for the following 2 consecutive days after the first vaccine, a physical exam of each calf was performed by a veterinarian on the research team. This included a rectal temperature, heart rate, respiratory rate, respiratory score and a fecal score. Respiratory scoring was done based on the University of California Davis bovine respiratory disease scoring system for pre-weaned dairy calves.[22] Fecal scoring was performed using the University of Wisconsin Calf Scoring Chart.[23] Physical exams were not conducted per producer request following the second vaccination to avoid the stress from repeated handling.

Environmental sampling

Prior to vaccination, environmental samples (boot swabs[j]) from each farm were collected for detection of *Salmonella* strains in the farm environment that might have resulted in exposure to serogroup D strains prior to vaccination. One swab was taken from the dairy fresh pen, 1 from the calving pens, and 2 swabs were taken from each heifer raising facility. Once in the sampling area, 1 boot swab, previously moistened with buffer peptone water (BPW), was placed over a clean plastic boot cover while wearing gloves. The individual then walked around in a meandering pattern to cover the entire pen, then removed the boot swab and placed into a sterile Whirl-pak® bag[k] when finished. Samples were refrigerated under 39.2 °F (4 °C) until analyzed within 1 to 3 days.

Boot swab samples were subjected to culture and multiplex PCR (m-PCR) assays. The in-house m-PCR included 2 serovar-specific markers, along with the genus-level gene invA. Additionally, standard culture techniques were used for detection and isolation of *S*. Dublin to further support the PCR. Briefly, the environmental boot swabs were pre-enriched in buffered peptone water and incubated at 98.6 °F (37 °C). After 24 hours, aliquots of BPW were taken to undergo manual immunomagnetic separation using Dynabeads™ anti-*Salmonella*[l]. Aliquots from the resultant solution were then transferred to Rappaport-Vassiliadis broth[m] (RV), Selenite Cysteine broth[n] (SC) and Modified Semi-Solid Rappaport-Vassiliadis agar[o] (MSRV) and incubated for 18 to 24 hours at 98.6 °F (37 °C) (SC) or 107.6 °F (42 °C) (RV, MSRV). Aliquots were then streaked onto Xylose- Lysine-Tergitol 4[p] for selective isolation and confirmation. Colonies consistent with *Salmonella* (black colonies) were then serogrouped using the Wellcolex™ Color *Salmonella* Rapid Latex Agglutination Test Kit[q]. Serogroups C1, C2 and E1 were further identified using monovalent antisera.

Statistical analysis

The analysis tested the hypothesis that the U.S. currently licensed *Salmonella* vaccines would result in an increase in percent positivity using the *S*. Dublin ELISA test. Calf data was imported into Stata[r]. The effect of the vaccination on the *S*. Dublin ELISA positivity was investigated using a mixed linear regression model with the percent positivity as the outcome, and the treatment group, day post-second vaccination, and their interaction as fixed effects. The calf ID was used as a random effect with the first-order autoregressive (AR[1]) covariance structure, considering correlations in the outcomes to be highest between adjacent times (day post-vaccination), and decreasing systematically with increasing distance between time points. Post-hoc pairwise comparisons adjusted for multiple tests (Bonferroni correction) were performed to estimate the difference in percent positivity among 4 types of *Salmonella* vaccine and the control group (saline). A *P* value < 0.05 was considered statistically significant.

Results

Each of the 50 enrolled calves had 3 separate blood collections, however, 1 calf died prior to the last testing period. This calf belonged to the EnterVene-d group and died of suspected pneumonia (based on farm records), 4 weeks after the second vaccine administration (this calf was not included in analysis and did not become positive at the 2-week testing period). All 50 calves were categorized as negative on day 1 of the study with low percent positivity (-10.39% +/- 5.95). Two weeks following the second vaccine administration, 1, 2 and 4 calves administered ENDOVAC-Dairy, Colorado Serum and EnterVene-d vaccines were positive on ELISA (PP ≥ 35%) (Figure 1). The EnterVene-d calves had a significantly higher percent positivity than the other 4 groups at 2 weeks following the second vaccine administration ($P < 0.05$).

Five weeks after the second vaccine administration, the number of positive calves were 1, 4 and 5 for calves administered ENDOVAC-Dairy, Colorado Serum and EnterVene-d, respectively (Figure 1). Calves administered the SRP vaccine or saline (control) did not have positive ELISA results at any point in the study. The EnterVene-d and Colorado Serum group each had a significantly higher percent positivity ($P < 0.05$) than the SRP and saline groups at 5 weeks after the second vaccination. It is important to note that 4 calves, that were negative, in the Colorado Serum group, and 3 in the EnterVene-d group, had some increase in percent positivity after the vaccine PP > 0%); however, it was not a strong enough response to be categorized as positive (PP ≥ 35%). Additionally, 2 calves in the Colorado Serum group, and 1 calf in the EnterVene-d group, did not have any increase in PP (PP < 0%) 5 weeks post-second vaccination. The calf that died in the EnterVene-d group had a PP < 0% at 2 weeks following second vaccination. Additionally, the 1 calf in the ENDOVAC-Dairy treatment group that became positive had a higher PP (30.8%) prior to vaccination (Figure 2). Model estimated percent positivity of day and treatment group interaction are shown in Figure 3. Environmental

Figure 1: Number of positive calves within each treatment group at 2 and 5 weeks following the second vaccination in experimental study evaluating the effect of *Salmonella* vaccines on *S*. Dublin blood ELISA. A total of 50 calves were randomly allocated to one of the 5 groups (10 calves/group) using 4 different *Salmonella* vaccines and a control group (saline).

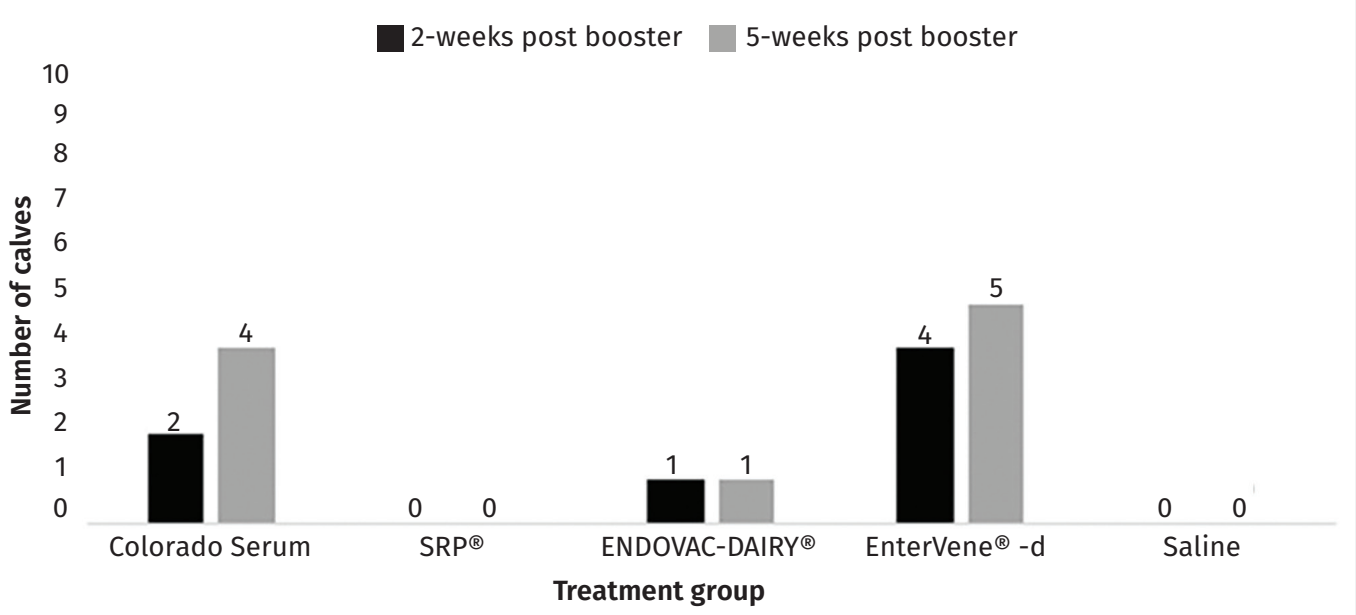
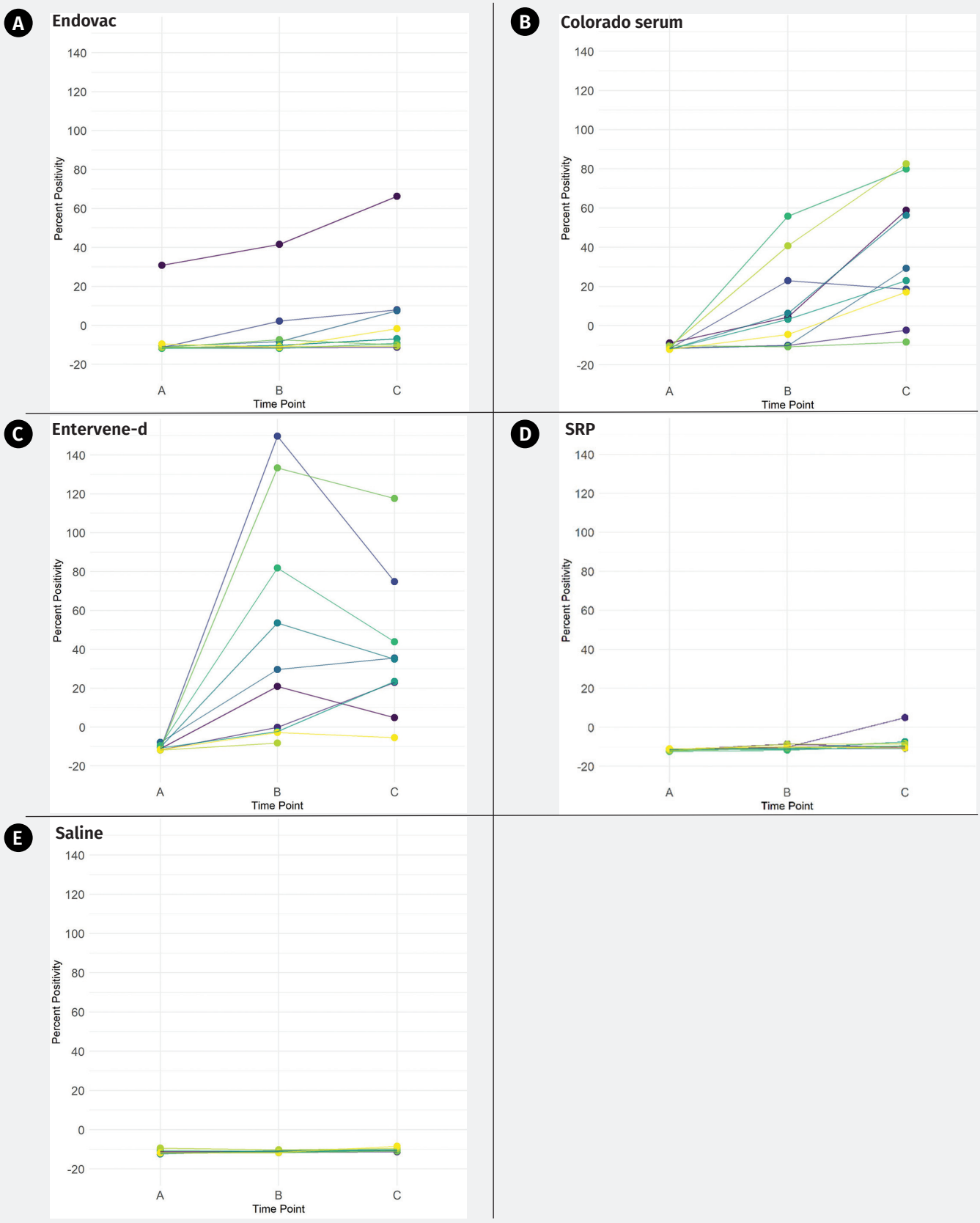


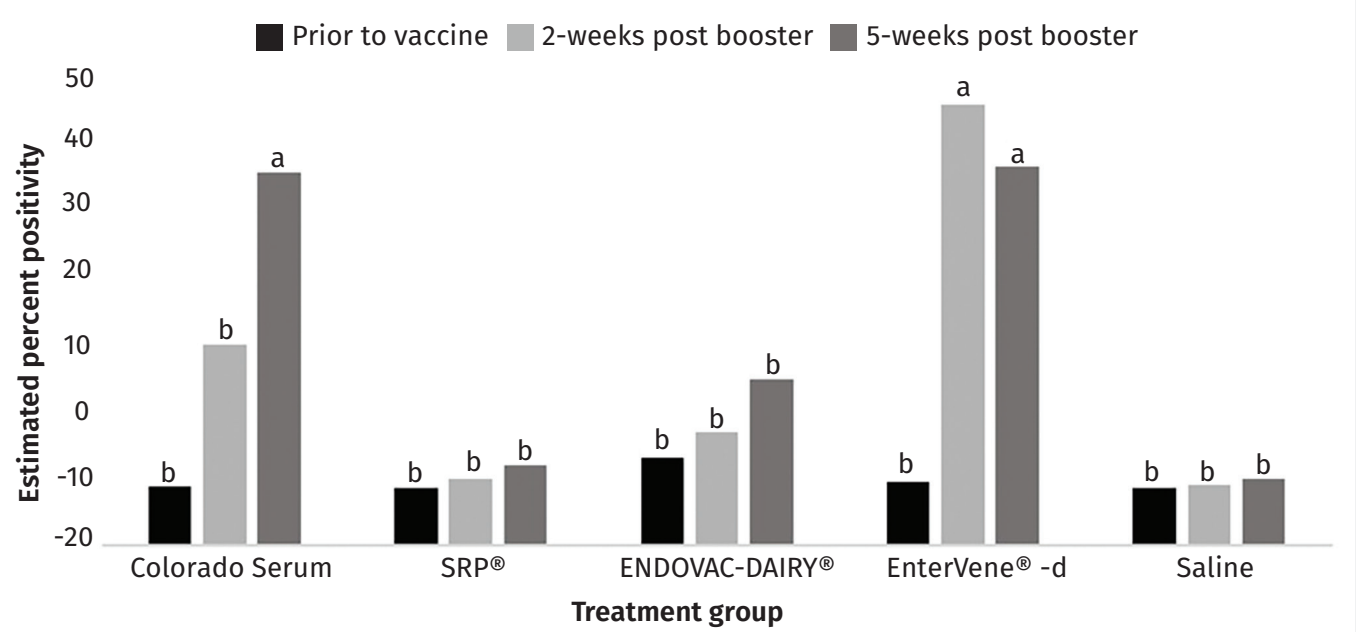
Figure 2: Percent positivity of *S.* Dublin antibodies from each calf in each treatment group, over 3 time periods, in experimental study evaluating the effect of *Salmonella* vaccines on *S.* Dublin blood ELISA. A total of 50 calves were randomly allocated to one of the 5 groups (10 calves/group) using 4 different *Salmonella* vaccines and a control group (saline).



* Time point A is prior to vaccination, B is 2 weeks after second vaccination, and C is 5 weeks after second vaccination.

† Plot A refers to the ENDOVAC-Dairy® vaccine group and B, C, D and E are *Salmonella* Dublin-Typhimurium bacterin (Colorado Serum), EnterVene®-d, SRP® and saline (control), respectively.

Figure 3: Model estimated percent positivity of day and treatment group interaction (95% CI) of each treatment group at 2 and 5 weeks following the second vaccination in experimental study evaluating the effect of *Salmonella* vaccines on *S.* Dublin blood ELISA. A total of 50 calves were randomly allocated to one of the 5 groups (10 calves/group) using 4 different *Salmonella* vaccines and a control group (saline).



Differing letters denote statistical significance.

samples were taken from the source dairy farm and each of the calf grower site locations for *Salmonella* culture and PCR to identify potential exposure to *Salmonella* serogroup D. On culture, 4 out of 6 samples were positive for a *Salmonella* spp. At least 1 boot swab was positive for *Salmonella* spp. within 3 of the 4 locations. All recovered isolates were classified as serogroup C_1, and no serogroup D isolates (consistent with *S*. Dublin) were recovered. On PCR, 5 out of 8 samples were positive for *Salmonella* spp.; however, all samples were negative for *S*. Dublin on the PCR assay specific to *S*. Dublin. Thus, there was no evidence of *S.* Dublin on the premises.

The results of each calf's physical exams on the initial day of vaccine administration as well as the following 2 days are reported in Table 1. No calves had an ear droop, head tilt, ocular discharge or swollen joints at any point. All vaccines, excluding the saline control, produced at least 1 swelling at the injection site. Calves with elevated rectal temperatures (> 103 °F) were found across all days and across all treatment groups. Similar results were seen with nasal discharge, abnormal fecal scores and elevated respiratory rates (> 60 breaths per minute). No calves had a heart rate > 120 beats per minute (bpm) on the first day, but several calves across all treatment groups had high heart rates (> 120 bpm) on both days 2 and 3, consistent with stress from repeated handling.

Discussion

This study demonstrated that 2 commercially available *Salmonella* vaccines, EnterVene-d and Colorado Serum, resulted in detectable antibodies with the *S*. Dublin ELISA, which impacts interpretation of these test results. This study also identified variability in positive results and highlighted limitations in test sensitivity. The modified-live EnterVene-d vaccine was previously shown to produce detectable antibodies in vaccinated cows leading to a positive *S*. Dublin blood ELISA.[20] No further testing had been done for other commercially available *Salmonella* vaccines.

The EnterVene-d vaccine had the highest rate of ELISA positives among the vaccine groups with 50% of the animals positive (5 out of 10). This was similar to another study that also detected ELISA positive animals following EnterVene-d vaccination.[20] The Colorado Serum *Salmonella* Dublin vaccine is a bacterin vaccine that resulted in 4 out of 10 calves being positive at 5 weeks post-booster vaccination. Although positive calves were expected, it was anticipated that more calves in these groups would have become positive. The fewer number of calves that became positive (PP ≥ 35%) within the EnterVene-d and Colorado Serum groups could be explained by normal individual variability to a vaccine; however, another explanation of these results could be due to the lower sensitivity of the test (55 to 75%).[13] Most calves in these 2 groups did show some increase in percent positivity; however, not all those responses were above the cutoff to be considered positive. It is possible that the lower number of positive calves in these groups are from the lower sensitivity of the test rather than a lack of production of antibodies.

Another potential reason for the lower number of positive calves post-vaccination could be explained by calves needing more time to fully reach positivity (PP ≥ 35%). Three additional calves became positive from the first blood test at 2 weeks after the second vaccination and the last test at 5 weeks post-second vaccination. Nielsen et al. found in 2007 that calves that were clinically infected with *S*. Dublin took an average of 36-days to become positive since the onset of shedding.[24] This suggests that it is possible that some calves needed longer

Table 1: Physical exam findings in calves, separated by treatment group and day of exam in experimental study evaluating the effect of *Salmonella* vaccines on *S.* Dublin blood ELISA. Values reflect the number of calves with an abnormal condition.

	***Salmonella* Dublin-Typhimurium bacterin (Colorado Serum) (n = 10)**			**SRP® (n = 10)**			**ENDOVAC-Dairy® (n = 10)**			**EnterVene®-d (n = 10)**			**Saline (Control) (n = 10)**		
	D1	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**
Injection site swelling	N/A	2	3	N/A	1	2	N/A	1	2	N/A	0	1	N/A	0	0
Nasal discharge	4	3	2	0	2	2	2	2	3	1	2	1	1	2	1
Ocular discharge	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	0	0	0	0	3	1	0	3	0	1	2	0	0	4	1
Head tilt/ear droop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swollen joints	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rectal temp > 103.0 °F (39.4 °C)	1	2	1	3	2	8	0	1	1	0	4	6	1	0	3
Abnormal fecal score	1	1	1	0	0	0	1	0	1	0	1	2	1	1	2
Heart rate > 120 bpm	0	7	5	0	9	7	0	9	8	0	9	5	0	3	5
Respiratory rate > 60 bpm	1	1	1	0	1	2	1	2	2	1	2	3	2	1	2

* Fecal scoring was performed using Sheila McGuirk's Calf Health Scoring Chart,[17] a score ≥ 2 was considered abnormal.

† D1 refers to exam findings prior to vaccination, D2 is the day after vaccination, and D3 is the second day after vaccination, resulting in 3 consecutive days.

than 5 weeks from the second vaccination to become positive on the ELISA. Further information is required to know how long each vaccine allows for detectable antibodies within blood or milk without annual revaccination.

The ENDOVAC-Dairy Immune Plus vaccine is a bacterin-toxoid for *Salmonella* Typhimurium. This vaccine resulted in 1 positive calf on blood test in this study. This single positive calf could have been due to a cross reaction to antibodies of *Salmonella* Typhimurium which the test kit company warns that this test may cross-react with this *Salmonella* serotype. However, the calf in the ENDOVAC-Dairy treatment group that became positive (PP ≥35%) had a higher PP (30.8%) prior to vaccination. The increased PP prior to vaccination in this calf could indicate that vaccination had a marginal effect on the test result, and it is possible this specific calf was mounting antibodies for another reason at the time of the study.

The SRP vaccine, a *Salmonella* subunit vaccine, did not result in any positive calves throughout the study, nor did the saline control. This suggests that the SRP vaccine does not induce the antibodies to *Salmonella* Dublin or Typhimurium detectable by this assay. Indeed, this vaccine is not labeled for use in calves and is intended to produce antibodies to the siderophore receptor protein (SRP) found in Gram-negative bacteria, rather than the O antigens detected by the ELISA assay. The ELISA does not include outer membrane proteins, such as the siderophore receptor protein. With the control calves remaining negative and no *S.* Dublin found on any of the environmental samples, it can also be considered that the calves that did become positive (in the other treatment groups) were unlikely to be due to an environmental *Salmonella* Dublin.

From these results, we know that calves vaccinated with EnterVene-d and Colorado Serum *Salmonella* Dublin vaccines generate positive test results on *S.* Dublin ELISA. This information is valuable to prevalence studies and control programs that are using this method for detection. If *Salmonella* vaccines have been used on the farm or on another farm where cows were purchased or raised, this can greatly impact prevalence studies or control program results. For instance, Canada is currently utilizing the *S.* Dublin serum and bulk tank milk ELISA in a surveillance program.[16] Although licensed medications are different in Canada, many calves from the United States are sent over the border and potentially become replacement heifers on dairy farms. The effect of *Salmonella* vaccines on the *S.* Dublin antibody ELISA and its use in the Canadian surveillance program questions the accuracy of the apparent prevalence within the program.

Another potential theory to consider from these results is the ability of calves administered the modified live EnterVene-d vaccine to begin shedding *S.* Dublin bacteria leading to other calves mounting an immune response. Modified live vaccines induce immunity by the weakened bacteria colonizing and multiplying in the host without causing disease. This multiplication leads to shedding of the altered bacteria.[25] It is possible that EnterVene-d vaccinated calves began shedding the bacteria, potentially infecting other calves which could later mount an immune response. This reasoning could be one consideration when reviewing these results, but there is no known evidence of bacterial shedding and subsequent infection from this vaccine. If this was the case, it would also be expected to have positive calves in the SRP vaccine and saline groups since calves from each treatment group were housed together.

Additional research is necessary to further evaluate the timing of positivity on ELISA from vaccine administration and how long positive calves can be detected as the animal matures. Other vaccines licensed in other countries also require evaluation for the same effect as well as similar studies evaluating the *S*. Dublin milk ELISA. Furthermore, additional investigation is required to identify more sensitive testing methods for *S*. Dublin.

Another aspect of this study was to observe physical exam parameters of calves in the first few days after vaccine administration. This study was not sufficiently powered or designed to identify differences in signs or vaccine reactions between study groups; therefore, no statistical analysis was performed for this outcome. All vaccine labels warn against potential anaphylactic reaction, however, none occurred throughout this study. All vaccines produced at least one injection site reaction. On the initial day of the study, calves all had heart rates lower than 120 bpm. On day 2 and 3, several calves within each group had elevated heart rates (> 120 bpm). We believe this is due to the stress the calves underwent while being restrained for exams. Calves were group housed in open pens that lacked headlocks. Calves had to be chased and cornered to be able to place a halter for restraint for each exam. The higher number of calves with an increased rectal temperature (> 103 °F) and/or respiratory rate (> 60 bpm) on day 3 could also be due to stress on the calves during handling. Although physical exams were not conducted after the second vaccine administration, an increase in the number of abnormal physical exam parameters may have occurred due to an increased immune response following the second dose. No calves were observed by farm personnel as ill around the time of the second vaccine administration.

In conclusion, evaluating the current commercially available *Salmonella* vaccines for production of positive *S*. Dublin serum ELISA results revealed EnterVene-d and Colorado Serum both having significantly higher percent positivity 5 weeks post-second vaccination. Therefore, *S*. Dublin antibody ELISA results should be interpreted with caution and in light of the vaccine history of the animal. The use of these *Salmonella* vaccines will interfere with the interpretation of the ELISA and impact prevalence studies or control programs using this test. Additional research is necessary to further evaluate the timing required to become positive on ELISA from vaccine administration and how long this positivity can be detected as the animal matures.

Endnotes

[a] EnterVene®-d, Boehringer Ingelheim Vetmedica, St. Joseph, MO

[b] PrioCHECK™ *Salmonella* Ab Bovine Dublin test, Applied Biosystems, Waltham, MA

[c] Excel, Microsoft Corp., Redmond, WA

[d] Saline, 0.9% sodium chloride Injection USP, Baxter Healthcare Corporation, Deerfield, IL

[e] *Salmonella* Dublin-Typhimurium Bacterin, Colorado Serum Company, Denver CO

[f] *Salmonella* Newport Bacterial Extract, Vaxxon SRP® *Salmonella*, Vaxxinova, Wilmar, MN

[g] *Salmonella* Typhimurium bacterin-toxoid, ENDOVAC-Dairy® Immune Plus, Endovac Animal Healthy LLC, Columbia, MO

[h] Serum Separator Blood Collection Tubes, BD Vacutainer®, Franklin Lakes, NJ

[i] Red Top Blood Collection Tubes, BD Vacutainer®, Franklin Lakes, NJ

[j] Boot Swabs, Envirobootie™, Hardy Diagnostics, Santa Maria, CA

[k] Whirl-Pak® bag, Whirl-Pak Filtration Group, Pleasant Prairie, WI

[l] Dynabeads™ anti-*Salmonella*, Applied Biosystems, Waltham, MA

[m] Rappaport-Vassiliadis broth, Remel, Lenexa, KS

[n] Selenite Cysteine broth, Remel, Lenexa, KS

[o] Modified Semi-Solid Rappaport-Vassiliadis agar, Remel, Lenexa, KS

[p] Xylose-Lysine-Tergitol 5, Remel, Lenexa, KS

[q] Wellcolex™ Color *Salmonella* Rapid Latex Agglutination Test Kit, Thermo Scientific, Kansas City, MO

[r] Stata 18, StataCorp LLC, College Station, TX

Acknowledgements

The authors would like to thank the participating herd for making this project possible and the Wisconsin Veterinary Diagnostic Laboratory for performing the ELISA.

Funding

This work was supported by USDA National Institute of Food & Agriculture.

Conflicts of Interest

The authors have no conflicts of interest to report.

Author contributions

Nogay participated in study conceptualization, acquired and processed samples, performed statistical analysis, and prepared the initial and final drafts of the manuscript. Masterson participated in study conceptualization and edited the initial draft of the manuscript. Locke and Arevalo-Mayorga participated in sample acquisition and processing and edited the initial draft of the manuscript. Cheng provided input on statistical analysis and edited the initial draft of the manuscript. Habing participated in study conceptualization, provided input on statistical analysis and edited the initial and final drafts of the manuscript.

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