PEER REVIEWED

Characterization of Specific Passive Immunity Stimulated by Vaccination of Beef Cows Grazing Native Range with *E. coli* O157:H7-SRP®

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Abstract

A total of 20 four-year-old, mixed breed cows were selected from the Kansas State University commercial cow-calf herd for this study. Cows were assigned randomly to one of two treatments: placebo, or *E. coli* O157:H7 SRP® (Epitopix LLC, Wilmar, MN) vaccination prior to calving. Serum total protein (TP) and serum *Escherichia coli* O157:H7 SRP® antibody levels (SRP) were measured pre-suckle and at set intervals post-suckle in calves. All 20 cows were negative for fecal shedding of *E. coli* O157:H7 throughout the study. Vaccination of cows had no effect on calf serum TP level ($P > 0.05$) compared to controls, however, length of time post-birth had a significant effect on calf serum TP levels ($P < 0.001$). Failure of passive transfer was recognized as early as six hours post-birth. A vaccine treatment by time post-birth interaction was observed for calf serum SRP® antibody levels ($P < 0.01$). Calves born to dams vaccinated with *E. coli* O157:H7 SRP® vaccine had elevated serum SRP antibody titers over time compared to placebo vaccinated calves. This study demonstrated that successful *E. coli* O157:H7 SRP® antibody passive transfer can occur in beef calves under natural range conditions. This could be the first step toward understanding life cycle immunization strategies against *E. coli* O157:H7 in cattle, and its effects on shedding of the organism by the animal at the time of harvest.

Key words: *E. coli* O157:H7, SRP, passive immunity, food safety

Résumé

Un total de 20 vaches de quatre ans et de race mélangée ont été sélectionnées d'un troupeau vache-veau commercial de la *Kansas State University* pour cette étude. Les vaches ont été allouées au hasard à l'un des deux traitements suivants : un placebo ou une vaccination avec *E. coli* O157:H7 SRP® (Epitopix LLC, Wilmar, MN) avant le vêlage. La concentration sérique des protéines totales et le niveau sérique d'anticorps contre *E. coli* O157:H7 SRP® ont été mesurés chez les veaux avant l'allaitement et à intervalle régulier suivant l'allaitement. Aucune des vaches n'a excrété *E. coli* O157:H7 dans ses fèces durant toute la durée de l'étude. La vaccination des vaches n'avait pas d'effet sur la concentration sérique des protéines totales chez les veaux ($P > 0.05$) par rapport au groupe témoin ; toutefois, la concentration sérique des protéines totales variait significativement en fonction de la longueur de l'intervalle depuis la naissance ($P < 0.001$). L'insuffisance du transfert passif a été reconnue moins de six heures suivant la naissance. Il y avait une interaction entre le traitement et l'intervalle de temps depuis la naissance pour le niveau sérique d'anticorps contre *E. coli* O157:H7 SRP® ($P < 0.01$) chez les veaux. Les veaux dont la mère avait été vaccinée avec le vaccin contenant *E. coli* O157:H7 SRP® montraient des titres sériques d'anticorps plus élevés en fonction du temps que les veaux du traitement placebo. Cette étude a démontré qu'un transfert passif d'anticorps contre *E. coli* O157:H7 SRP® peut avoir lieu chez des veaux de boucherie en condition d'élevage sur le terrain. Ceci représenterait une première étape afin de mieux comprendre les stratégies d'immunisation contre *E. coli* O157:H7 basées sur le cycle de vie et leurs effets sur l'excrétion du micro-organisme chez les bovins à la fin de l'élevage.

Introduction

Calves are born agammaglobulinemic due to the separation of fetal and maternal blood flow that pre-

vents transfer of immunoglobulins from cow to fetus in utero.[2] Following parturition, the calf is reliant upon immunoglobulins contained in the colostrum for protection from disease challenge during the first two to four weeks of life.[3] Colostrum feeding has been reported as one of the most important management practices to prevent mortality during the first 21 days of a calf's life.[22] Calves that fail to consume adequate colostrum within the first 12-24 hours after birth are at a higher risk for disease, death, and decreased performance.[23,24] A number of studies have been performed examining the passive transfer of specific antibodies to beef calves, but none have examined *E. coli* O157:H7.[7,10,14,20]

The epidemiology of *E. coli* O157 is not well understood and the prevalence of *E. coli* O157:H7 in cow-calf herds has not been thoroughly studied. Some researchers have estimated the beef cattle herd-level prevalence of *E. coli* O157:H7 to be 80-100%.[8,11,15,17] However, individual animal-level prevalence appears much lower, estimated to range from 2-18%.[8,11,15,16] Knowledge of environmental risks and best management practices that impact infection/colonization of an animal with *E. coli* O157:H7 is necessary to design effective *E. coli* O157:H7 control strategies. Currently, most control strategies for *E. coli* O157:H7 are aimed at reduction of pathogen load at the feedyard level or post-harvest.[12,16] Two studies utilizing beef cattle have suggested that initial infection of beef calves with *E. coli* O157:H7 may occur from their dams during the immediate postnatal period.[8,11] Therefore, current control strategies that target the feedlot and harvest phases of the production system are combating a post-colonization issue. Methods to greatly decrease or eliminate the initial colonization of young beef calves would be a better food safety solution.

A new *E. coli* O157:H7 SRP® vaccine[a] has been issued conditional licensure for use in feedlot cattle in the United States.[5,19] SRP is an acronym for siderophore receptor and porin proteins. It is assumed that most circulating immunoglobulins will be concentrated in the colostrum of cattle.[9,13,21] Therefore, the use of this vaccine with its unique epitopes and mode of action warrants further investigation into its ability to achieve passive transfer to possibly eliminate or decrease colonization of *E. coli* O157:H7 in beef cattle. The objective of this study was to determine whether *E. coli* O157:H7 SRP® specific antibodies could be detected in the serum of calves that consumed colostrum from dams that were vaccinated before parturition with *E. coli* O157:H7 SRP® vaccine.

Materials and Methods

General overview

In January 2009, 20 cows from the Kansas State University commercial cow-calf herd were utilized to study the efficacy of passive transfer of specific *E. coli* O157:H7 SRP® antibodies in beef calves. Four-year-old cows were selected from the herd to control for parity and age, as well as predicted calving dates and balance across pastures. Cows were maintained on native, dormant bluestem pasture and supplemented six days per week with a soybean meal-based supplement. Cows were assigned randomly to one of two treatments: placebo or *E. coli* O157:H7 SRP® vaccination.

Cows were vaccinated with their assigned vaccine treatments at 60 and 30 days prior to the projected start of the calving season. Blood samples were collected from cows prior to the initial vaccination to ensure they were *E. coli* O157:H7 SRP® antibody-free. Sera were tested for presence of *E. coli* O157:H7 SRP® antibodies using enzyme-linked immunosorbent assay (ELISA). Laboratory personnel were blinded to treatment assignments.

At the time of calving, fecal, blood, and colostrum samples were obtained from each cow and a pre-suckle blood sample was obtained from each calf. Blood samples were obtained from calves at 6, 12, and 24 hours and at 7, 14, and 21 days postpartum. Serum total protein and *E. coli* O157:H7 SRP® antibody concentrations were measured in all calves.

Serology

ELISA and colostrum assay. Immulon-2 ELISA plates[b] were coated with *E. coli* O157:H7 SRP® antigen diluted in coating buffer (1.59 g/L Na_2CO_3, 2.93 g/L $NaHCO_3$, pH 9.65). One-hundred (100) µl of the antigen solution was added to each well of the plate and allowed to incubate overnight at 39.2°F (4°C). The coating antigen was then removed from the plate and replaced with 200 µl blocking buffer (10g/L Poly Vinyl Alcohol, 1L 1xPBS). The plates were then covered and incubated at 98.6°F (37°C) for one to two hours, after which the blocking buffer was removed. Serum from a calf hyper-immunized with the *E. coli* O157:H7 SRP® vaccine was used as a positive control. Samples and control sera were then diluted to 1:100 in blocking buffer. Test sera were added to the first and twelfth well of the plate in duplicate and diluted 4-fold moving towards the center of the plate, so that the last dilution achieved was 1:102,400. The plates were then covered and incubated at 98.6°F for one hour. Plates were then washed three times with 0.05% Tween-PBS. The conjugate (sheep anti-bovine IgG H&L HRP)[c] was then diluted to 1:1,600 in the blocking buffer plus 1% sheep sera. One hundred (100) µl of the conjugate solution was added to each well, then the plate was covered and incubated at 98.6°F for one hour. The plate was then washed three times with 0.05% Tween-PBS. Pre-warmed, two-component ABTS[d] was combined, and 100 µl was added to each well of the plate. The plate was incubated at room temperature until the optical density of the positive control wells was between 0.8 and 1.2. Plates were then mixed and placed into the plate reader[e] and read at 405/490nm. The cut-off value for each series was calculated by multiplying the

positive control by 0.5 and plotting it against the sample dilution curves. Sample titers were calculated based on the intersection of the cut-off line and the sample curves. Sample titers were then reported as the reciprocal of the dilution at which the cut-off crossed the sample curve.

Total protein

Blood was obtained from the jugular vein of each calf at each time point using 9 mL vacuum serum tubes.[f] The blood was then allowed to clot while being refrigerated for 12-24 hours. Samples were then centrifuged at 3,500 RPM for 10 minutes to achieve serum separation from the clot. Serum was placed onto the refraction crystal of a commercially available, temperature compensated refractometer.[g] Total protein was read and recorded as g/dL.

Fecal culture

Fecal samples were obtained directly from the rectum of each animal as it was restrained at each handling time point. Samples were placed into collection vials, placed on ice, labeled with sequential numbers to blind treatment assignments, and sent overnight to the testing laboratory.[h] Upon arrival at the laboratory, fecal samples were processed for isolation of *E. coli* O157 by immunomagnetic separation. Samples were weighed and approximately two grams of each fecal specimen was placed into a Whirl-pak™ filter bag.[i] Gram-negative broth containing cefixime (0.05 mg/L), cefsulodin (10 mg/L), and vancomycin (8 mg/L) (GNccv) was used to get the fecal specimen in a liquid state for sample processing. Samples were normalized by weight so each sample was present at a ratio of one gram of feces per 10 mL of GNccv broth. Samples were incubated at 98.6°F overnight for enrichment of *E. coli* O157. Following enrichment, 1 mL of each sample was added to a 96-well plate containing 20 µl of magnetic Anti-O157 Dynabeads.[j] Enriched cultures were allowed to incubate with the magnetic beads on a shaker at room temperature for at least 15 minutes as per the manufacturer's directions. Magnetic particles were recovered and washed using an 8-channel magnetic PickPen.[k] After the final wash, the particles were released into 100 µl of wash buffer (PBS containing 0.05% Tween 20) in a 96-well plate for plating of *E. coli* O157 that was bound to the magnetic particles. For plating, 50 µl was plated onto a Sorbitol MacConkey agar plate containing cefixime and tellurite (CT-SMAC)[l] and 50 µl was plated onto a Chromagar-O157 plate.[m] The inoculum was spread onto each agar plate and the plates were incubated at 98.6°F overnight. Plates were observed for suspect *E. coli* O157 colonies, which were then tested for O157 agglutination using an O157 test kit from Remel[n] per the manufacturer's instructions. Positive samples were sub-cultured to Chromagar-O157 or CT-SMAC to acquire a pure culture of *E. coli* O157.

Vaccine preparation

The commercially available *E. coli* O157:H7 SRP® vaccine was prepared in the same manner as reported previously.[6,18,19] The placebo vaccine contained phosphate-buffered saline emulsified with the same commercial adjuvant[o] used in the vaccine. Vaccine and placebo bottles were marked A or B to blind the vaccine administrator to the treatments. Each cow received 2 mL of the corresponding vaccine subcutaneously in the neck following Beef Quality Assurance guidelines.[4]

Data analysis

Data were recorded and summarized using the Excel[p] program. ELISA results were transformed using a log base 2 function prior to using the data for statistical analysis. The individual calf served as the experimental unit. Data were analyzed using the wsanova procedure in STATA®[q] for repeated measures of an individual. Cow vaccination treatment, pasture, cow ELISA at the time of calving, calf ELISA, time, and their interactions were all presented as possible variables for the model. Colostrum endpoint ELISA data were analyzed using a generalized linear model using a Gaussian distribution and identity link function. P-values ≤ 0.05 were considered significant.

Results and Discussion

No cows required assistance during parturition, and all calves nursed within two hours after birth without assistance. Vaccination treatment had no effect on the calf serum total protein (TP) level ($P > 0.05$). However, the length of time post-birth had a significant effect on calf serum TP levels ($P < 0.001$; Figure 1). This was an expected finding, given that calves are born agammaglobulinemic until absorption of maternal antibodies from colostrum.[2] One calf in the study was classified as having failure of passive transfer (TP level < 5.5 g/

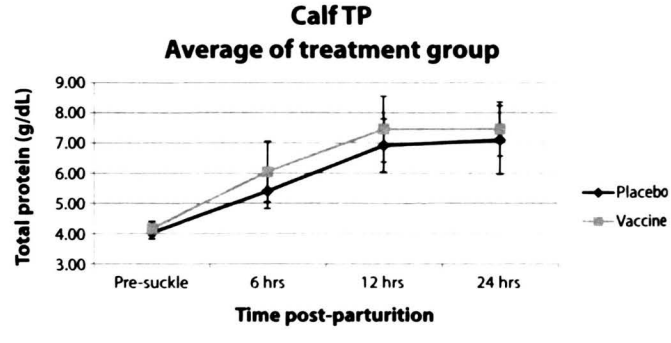

Figure 1. Average total protein (TP) of calves in the vaccination (squares) and placebo (diamonds) treatment groups at pre-suckle, and 6, 12, and 24 hours post-suckle. Error bars signify the standard deviation for the measurements at the given time point.

dL at 24 hours). This resulted in a 5% failure of passive transfer prevalence, which is consistent with other prevalence estimates in beef cattle, yet well below that of many dairy estimates.[13,21] The calf which exhibited failure of passive transfer was born to a cow that had very little udder development and milk production at the time of parturition, which was likely the main factor for failure of passive transfer.

A vaccine treatment by time post-birth interaction was observed for calf serum *E. coli* O157:H7 SRP® antibody levels ($P < 0.01$; Figure 2). This interaction was explained by no vaccine treatment difference in calf serum *E. coli* O157:H7 SRP® antibody levels pre-suckle, but a significant increase in calf *E. coli* O157:H7 SRP post-suckle titers in the calves born to SRP®-vaccinated cows compared to calves born to cows that received the placebo vaccination.

Of the 20 cows enrolled in the study, only 19 colostrum samples were collected and analyzed. The average endpoint *E. coli* O157:H7 SRP® antibody titers differed ($P < 0.001$) between treatments, 150 and 16,835 for placebo and vaccinated cows, respectively (Figure 3). Natural range assignment was not a significant factor for antibody titer level of either treatment ($P > 0.70$). The marked difference in antibody titers between vaccinated and placebo cows' colostrum is in agreement with earlier work which examined *E. coli* K99 vaccine efficacy and protection in neonatal calves.[1]

Another observation from this study was the length of time post-parturition in which calves achieved adequate passive transfer. The majority of the calves had adequate passive transfer (TP = 5.5 g/dL) by six hours post-parturition (Figure 1). These TP levels were achieved in spite of increased handling of cows and calves immediately after parturition that was imposed by the protocol. These data suggest that the passive transfer status of neonatal calves may be accurately assessed prior to partial gut closure at 12 hours, and full closure at 24 hours.[21] This may be a useful strategy for producers to employ when undertaking an aggressive colostrum management program.

All 20 cows were negative for fecal shedding of *E. coli* O157:H7 at all three sampling times. These data do not correspond to previous findings for fecal prevalence of beef cow-calf *E. coli* O157:H7 which reported individual animal level prevalence ranging from 2 to 18% and a herd-level prevalence ranging from 87 to 100%.[8,11,15,17] The current study did not employ a true random sample of the herd required to truly establish herd prevalence. Rather, the focus of the current study was colostrum quality and composition, and *E. coli* O157:H7 prevalence was secondary. Therefore, investigators controlled parity to decrease variation in colostrum quality between cows of different parity. Selection bias could have been inadvertently introduced to this study if four-year-old cows are at a lower risk for shedding *E. coli* O157:H7 than other cows in the herd. Previous studies reported a greater chance of isolating *E. coli* O157:H7 through repeated sampling.[17] Some researchers have reported that beef cows may not shed detectable levels of *E. coli* O157:H7 prior to parturition, but will shed this foodborne pathogen within one week after parturition.[8] The third and final sampling of cows in this study occurred immediately after parturition, yet no cows were found to be shedding *E. coli* O157:H7. The exact onset of shedding and the factors related to the induction of shedding warrant further investigation.

This study documented the speed at which passive transfer can occur in beef cattle following parturition. Calves were observed to have successful passive transfer in the first six hours after birth. This information could be used by producers in situations where colostrum intake is unknown or the calf has high value. A producer or veterinarian could identify passive transfer failure prior to significant gut closure, and intervene with colostrum support.

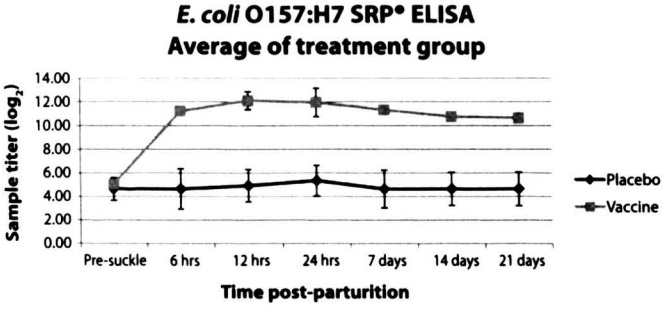

Figure 2. Average *E. coli* O157:H7 SRP® antibody of calves in the vaccination (squares) and placebo (diamonds) treatment groups at pre-suckle, and 6, 12, and 24 hours, and 7, 14, and 21 days post-suckle. Error bars signify the standard deviation for the measurements at the given time point.

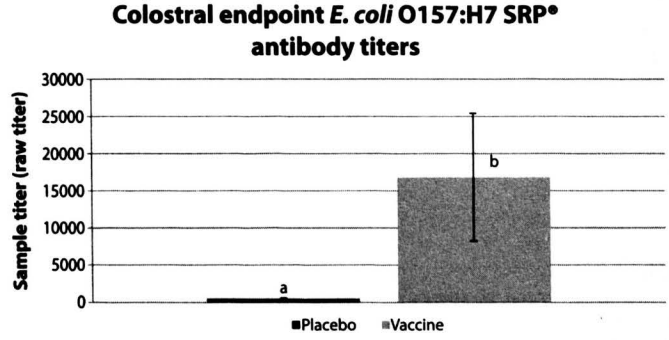

Figure 3. Average endpoint *E. coli* O157:H7 SRP® antibody titers of colostral samples by vaccination and placebo treatment groups. Error bars represent the standard deviation.

Conclusions

This is the first report of successful *E. coli* O157:H7 SRP® antibody passive transfer in beef calves under natural range conditions. These data provide information for further study into possible cross protection of this vaccine against neonatal *E. coli* diarrhea strains, such as K99. This study is the first step of understanding life cycle immunization strategies against *E. coli* O157:H7 in cattle, and its effects on shedding of the organism by the animal at the point of harvest.

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Endnotes

[a]*E. coli* O157:H7 SRP® vaccine, SRP® is a trademark of Epitopix LLC, Willmar, MN. SRP stands for siderophore receptor and porin proteins
[b]Immulon-2 ELISA plates, Dynatech Laboratories, Chantilly, VA
[c]Sheep anti-bovine IgG H&L HRP, The Binding Site, San Diego, CA
[d]ABTS, Kirkegaard & Perry, Gaithersburg, MD
[e]BioTek ELx405, BioTek Instruments, Winooski, VT
[f]9 mL vacuum serum tubes, Greiner Bio-One NA, Monroe, NC
[g]Temperature compensated refractometer, Reichert, Depew, NY
[h]Epitopix LLC, Willmar, MN
[i]Whirl-pak™ filter bag, NASCO, Fort Atkinson, WI
[j]Dynabeads® anti-E. coli O157, Invitrogen, Carlsbad, CA
[k]PickPen® 8-M, Bio-Nobile Oy, Turku, Finland
[l]CT-SMAC, Becton Dickinson, Franklin Lakes, NJ
[m]CHROMagar™ O157 plate, Chromagar, Paris, France
[n]RIMTM *E. coli* O157:H7 test kit, Remel, Lenexa, KS
[o]Emulsigen®, MVP Laboratories, Ralston, NE
[p]Excel®, Microsoft, Redmond, WA
[q]STATA® version 10, College Station, TX

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