Vaccination with type III secreted proteins leads to decreased shedding in calves after experimental infection with *Escherichia coli* O157

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Abstract

Escherichia coli O157:H7 remains a threat to humans via cattle-derived fecal contamination of food and water. Preharvest intervention strategies represent a means of reducing the pathogen burden before harvest. In this study, the efficacy of a commercially produced type III secreted protein (TTSP) vaccine was evaluated with the use of a commingled experimental calf infection model (30 placebo-treated animals and 30 vaccinates). The calves were vaccinated on days 0, 21, and 42 and infected with 10^9 colony-forming units (CFU) of *E. coli* O157 by oral–gastric intubation on day 56. Fecal shedding was monitored daily for 14 d. Serologic assessment revealed a robust immune response to vaccination; the serum titers of antibodies against EspA, Tir, and total TTSPs were significantly higher in the vaccinates than in the placebo-treated animals on days 21, 42, 56, and 70. Significantly less ($P = 0.011$) of the challenge organism was shed by the vaccinates than by the placebo-treated animals on days 3 to 10. Peak shedding occurred in both groups on days 3 to 6; during this period the vaccinates showed a mean log reduction of 1.4 ($P = 0.002$) and a mitigated fraction of 51%. The number of animals shedding was significantly lower among the vaccinates compared with the placebo group on days 3 to 6 ($P \leq 0.05$), with a mean prevented fraction of 21%. No differences in the duration of shedding were observed. Owing to the low challenge shedding in both groups on days 11 to 14 (mean CFU/g < 10; median = 0), no significant differences were observed. These data indicate that TTSP vaccination had protective effects through significant reductions in the number of animals shedding and the number of challenge organisms shed per animal and provides evidence that TTSP vaccination is an effective preharvest intervention strategy against *E. coli* O157.

Résumé

Escherichia coli O157:H7 demeure une menace pour l'humain via la contamination fécale des aliments et de l'eau par les bovins. Les stratégies d'intervention pré-récolte représente un moyen de réduire la charge des agents pathogènes avant la récolte. Dans la présente étude, l'efficacité d'un vaccin commercialement produit envers la protéine sécrétée de type III (TTSP) a été évaluée à l'aide d'un modèle d'infection expérimentale de veaux par mélange (30 animaux traités avec un placebo et 30 animaux vaccinés). Les veaux ont été vaccinés aux jours 0, 21 et 42 et infectés avec 10^9 unités formatrices de colonies (CFU) de E. coli *O157 par intubation oro-gastrique au jour 56. L'excrétion fécale a été surveillée quotidiennement durant 14 j. Une évaluation sérologique a révélé une réponse immunitaire robuste à la vaccination; les titres sériques des anticorps contre EspA, Tir et TTSPs total étaient significativement plus élevés chez les animaux vaccinés que chez les animaux témoins aux jours 21, 42, 56 et 70. Une quantité significativement plus faible (*P *= 0,011) du micro-organisme de défi était excrétée par les animaux vaccinés que par les animaux témoins aux jours 3 et 10. Le maximum d'excrétion a été noté dans les deux groupes aux jours 3 et 6; durant cette période, on observa chez les animaux vaccinés une réduction moyenne de 1,4 log (*P *= 0,002) et une fraction atténuée de 51 %. Le nombre d'animaux excréteurs était significativement inférieur parmi les vaccinés comparativement au groupe placebo aux jours 3 à 6 (*P *≤ 0,05); ainsi la fraction prévenue était de 21 %. Aucune différence dans la durée de l'excrétion n'a été observée. Étant donné la faible excrétion dans les deux groupes aux jours 11 à 14 (CFU/g < 10; médiane = 0), aucune différence significative n'a été observée. Ces résultats indiquent que la vaccination avec TTSP avait des effets protecteurs en réduisant significativement le nombre d'animaux excréteurs ainsi que le nombre de micro-organismes excrétés par les animaux, et fourni des évidences que la vaccination avec TTSP est une stratégie d'intervention pré-récolte contre* E. coli *O157.*

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Introduction

Over the past three decades, outbreaks of *Escherichia coli* O157:H7 infections have been associated with a variety of food products and water. Originally associated with hamburger in 1983 (1), *E. coli* O157:H7 remains a frequent contaminant of beef. The Food Safety Inspection Service of the US Department of Agriculture reported that 33 million pounds of beef were recalled in 2007 and more than 8 million pounds in 2008 (2,3). More recently, outbreaks of *E. coli* O157:H7 infection associated with produce, particularly leafy greens, have been increasingly reported (4).

The expanding spectrum of food products implicated in outbreaks of *E. coli* O157:H7 infection relates directly to the bovine reservoir (5). Fecal contamination of hides and carcasses contribute to beef contamination (6–8), and fecal run-off, composting, and poor on-farm hygiene practices play a role in produce contamination (9). Recent FoodNet data show a lack of reduction in *E. coli* O157:H7-related disease rates over the past 3 y. The lack of progress in reducing disease is thought to be associated with gaps in the food continuum that are not being effectively addressed (10).

On-farm intervention strategies offer methods to reduce the *E. coli* O157:H7 prevalence in cattle before harvest. Any tool that reduces bovine fecal *E. coli* O157:H7 levels represents an attractive means of minimizing contamination of beef-related and unrelated foodstuffs. Numerous strategies for reducing intestinal *E. coli* O157:H7 carriage have been investigated, with varying results. Dietary intervention strategies focus on altering hind-gut physiology (pH and volatile fatty acid concentrations) such that conditions are less favorable to *E. coli* O157:H7 colonization (11). Other strategies focus on increasing host resistance to *E. coli* O157:H7 infection or colonization; these include the use of probiotics, antibiotics, bacteriophages, sodium chlorate, feed additives, and vaccination (12,13). Recently, a risk assessment using quantitative criteria demonstrated that cattle vaccination is a cost-effective intervention strategy for reducing *E. coli* O157:H7 illness in humans (14).

In cattle, several vaccination strategies have been investigated. Type III secreted proteins (TTSPs) are recognized human vaccination targets (15) and have been shown to be involved in the colonization of cattle intestinal mucosa (16,17). Both humoral and rectal mucosal responses to TTSPs have been observed (17–22). Correspondingly, TTSP vaccines have reduced *E. coli* O157:H7 shedding in cattle (23–28). Also, vaccination strategies involving flagellin (27) and siderophore receptor and porin proteins (30,31) have been reported.

In this work, an experimental calf infection model was used to evaluate the use of a commercially licensed TTSP vaccine as a preharvest intervention tool for reducing the burden of *E. coli* O157 in cattle.

Materials and methods

Source and care of cattle

Seventy mixed British–Continental breed cattle were purchased from farms and auction marts in Saskatchewan. All steers and heifers showed no obvious signs of disease, were 5 to 8 mo old, and weighed 150 to 350 kg. Antimicrobials, other vaccines, and probiotics were not administered except in the treatment of foot rot in 2 animals.

The vaccinated and placebo-treated animals were commingled and housed in a single outdoor sloped pen (sandy-loam base) at the Vaccine and Infectious Disease Organization (VIDO) farm, University of Saskatchewan, Saskatoon, Saskatchewan, throughout the study. Within this pen, 3 subpens were used to house the cattle at a density of 340 ft^2 per animal, but the animals had access to the total pen area. The cattle were fed 6 to 7 lb of textured, barley-based finishing ration containing Rumensin (Elanco, Guelph, Ontario) and had free choice of cereal or alfalfa hay and access to water ad libitum via a trough. The housing conditions were in compliance with Agriculture and Agri-Food Canada's recommended code of practice for the care and handling of farm animals (32). Daily pen checks were performed throughout the study. Precautionary measures were taken by personnel to avoid contamination of the environment and the animals with the challenge organism. The facility received level 2 biosafety approval from the biosafety officer and the local animal care committee before study commencement. All the study cattle were strictly isolated from nonstudy cattle.

Study schedule

The study was begun in January and completed in May 2008. An overview of the study schedule can be seen in Table I. Before vaccination, the cattle were randomly assigned to the placebo group ($n = 30$) or the vaccine group ($n = 30$). The staff were blind to assignments. A commercially produced TTSP vaccine (Bioniche Life Sciences, Belleville, Ontario) was administered by subcutaneous shoulder injection with an 18-gauge × 1.6 cm needle. For placebo injection, saline was formulated with the same vaccine adjuvant. All vaccinations were delivered identically on days 0, 21, and 42. Blood samples were collected at each vaccination, on the day of challenge (day 56), and 14 d after challenge (day 70) for determination of serum titers of antibodies to EspA, Tir, and total TTSPs. Before challenge, the animals were screened for *E. coli* O157 by immunomagnetic separation (IMS) enrichment. After challenge, direct plating was used to quantify fecal shedding daily for 14 d. All experiments were conducted in accordance with the guidelines of the Canadian Council for Animal Care (33).

Production of vaccine, placebo, and challenge preparation

The *E. coli* O157 strain used for vaccine production was described previously (12). Culture was maintained as frozen stock at −70°C and propagated in Luria-Bertani (LB) agar and broth at 37°C. After fermentation, TTSPs were harvested and concentrated. The antigenic components were emulsified with the use of a commercial adjuvant. The placebo was formulated identically, except that physiologic saline replaced the antigenic components.

The challenge strain was a nalidixic acid derivative maintained and propagated as described above but with 15 μg/mL of nalidixic acid. Frozen stock was streaked onto LB agar with nalidixic acid (LB-N) and grown for 24 h at 37°C. One colony was inoculated into 5 mL of LB-N broth and incubated at 37°C for 6 h with 200 rpm shaking. Next, 30 μL of inoculumwas transferred into 30 mL of LB-N broth and incubated overnight with shaking. The challenge

Table I. Schedule for evaluation of a type III secreted protein (TTSP) vaccine by experimental infection of calves

Day of study	Day after challenge	Start of acclimatization	Randomization	Vaccination	Challenge	Blood collection	Feces collection	Direct plating	IMS detection
−7		x					x		x
0			x	x		x	x		x
21				x		x	x		x
42				x		x	x		x
56	0				x	x	x	x	x
57	1						x	x	
58	2						x	x	
59	3						x	x	
60	4						x	x	
61	5						x	x	
62	6						x	x	
63	7						x	x	
64	8						x	x	
65	9						x	x	
66	10						x	x	
67	11						x	x	
68	12						x	x	
69	13						x	x	
70	14					x	x	x	x
84	28						x		x
98	42						x		x
112	56						x		x

IMS — immunomagnetic separation.

inoculum, 3 L of LB-N broth, was warmed, inoculated with 30 mL of culture, and incubated at 37°C with shaking to an optical density at 600 nm (OD_{600nm}) of 0.34, at which point it was placed on ice and divided into aliquots of 50 mL. The target challenge dose was 5×10^9 colony-forming units (CFU) per animal. The challenge doses were administered by oral–gastric intubation and followed by 100 mL of saline.

Detection of *E. coli* O157 in feces

Quantification of *E. coli* O157 was by direct plating. Briefly, with a finger covered by a sterile glove, 4 to 7 g of feces from the rectum was placed in a sterile container. The feces were resuspended with 50 mL of tryptic soy broth (TSB) containing 40 mg/L of vancomycin and 0.05 mg/L of cefixime. The mixture was shaken vigorously and incubated for 30 min at ambient temperature. A 100-μL aliquot was serially diluted into 900 μL of 0.1 M phosphate-buffered saline (pH 7.2) to 10^{-3}, and 25 μL was plated from each dilution onto sorbitol–MacConkey (SMAC) agar with 0.05 mg/L of cefiximine, 2.5 mg/L of tellurite, and 15 mg/L of nalidixic acid. The plates were incubated at 37°C for 24 h and colony counts calculated on the basis of fecal weight. After enumeration, typical colonies (5 to 10) were randomly selected for confirmation of the O157 serogroup by means of the Oxoid Dryspot *E. coli* O157 O-Antigen Latex Agglutination Test Kit (Oxoid Canada, Nepean, Ontario).

Before and after challenge, IMS enrichment was used to detect O157. Briefly, 4 to 7 g of feces was suspended in 50 mL of TSB with 40 mg/L of vancomycin and 0.05 mg/L of cefiximine. The suspension was shaken vigorously and incubated at ambient temperature for 30 min. Samples were kept at 4°C overnight and then transferred to a 37°C incubator for 6 h. From the enriched culture, automated IMS was performed by means of the Dynal Dynabeads anti-*E. coli* O157 test kit (Invitrogen Canada, Burlington, Ontario). Aliquots were plated on SMAC agar as described above. Randomly selected typical colonies were O-antigen confirmed.

Serum analysis

For each animal, according to the schedule in Table I, 10 mL of blood was collected from the jugular vein into Vacutainer SST tubes (Becton Dickinson, Mississauga, Ontario) and centrifuged at $875 \times g$ at 4°C for 15 min. The serum was transferred to a deep-well serum block and frozen at −20°C. Titers of antibodies to EspA, Tir, and total TTSPs were determined by enzyme-linked immunosorbent assay (ELISA). Purified EspA and Tir were prepared by affinity chromatography, whereas total TTSPs were collected and concentrated with Millipore Centricon-Plus 70 filter devices (Fisher Scientific, Ottawa, Ontario). To determine the titers, 0.1 μg of the respective antigen was coated in carbonate buffer overnight at 4°C. The plates were washed 6 times with deionized water and blocked with 0.1 M Tris-base hydrochloride (pH 7.4), 0.14 M sodium chloride, and 0.05% Tween 20 (TBST). Samples were diluted appropriately in TBST plus 1% skim milk, incubated for 2 h at ambient temperature, and washed 6 times. Goat antibody against bovine IgG labeled with alkaline phosphate (KPL, Gaithersburg, Maryland, USA) was diluted 1:2500 in TBST plus 1% skim milk, added to each well, incubated for 1 h, and washed 6 times. The substrate pNPP (100 μL) (Sigma, St. Louis, Missouri, USA) was added to each well and the mixture incubated for

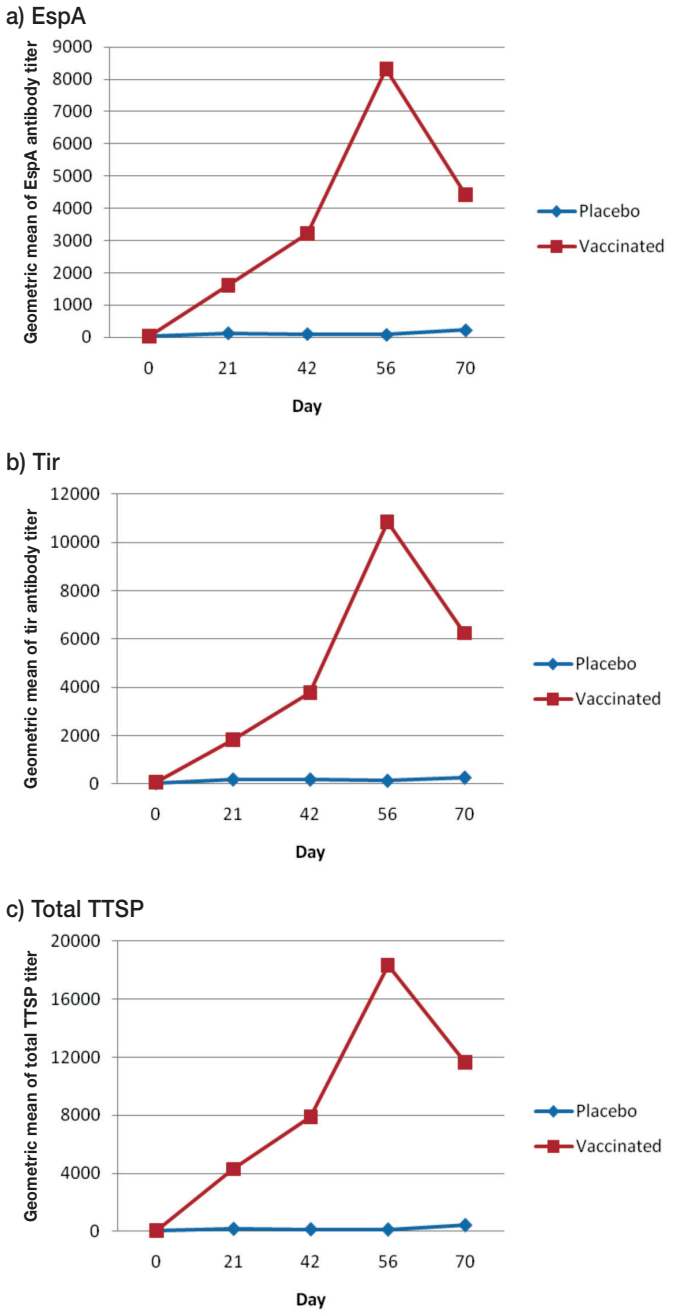

Figure 1. Serum titers of antibodies against EspA, Tir, and total type III secreted protein (TTSP) for 30 placebo-treated calves and 30 calves vaccinated with 3 doses of a TTSP vaccine, calculated from the geometric mean with the use of $\log_{10}$(titer + 1) values.

2 to 3 h. The reaction was terminated by the addition of 30 μL of 0.3 M ethylene diamine tetraacetic acid. Measurements at A_{405} with air as the reference (A_{490}) were taken for all samples and analyzed by the Mann–Whitney U-test.

Statistical analyses

The study was a 2-treatment, randomized design with repeated measures. Differences in fecal shedding were calculated as follows. Data were log-transformed (× + 1) and subjected to repeated-measures analysis of variance with treatment, day of measurement, and the treatment-by-day interaction as fixed effects and animal-within-treatment as a random effect, and repeated-measures analysis to model the covariance structure among the repeated measurements. Covariance structures were as follows: compound symmetry; first-order autoregressive; compound symmetry with unequal variances; first-order autoregressive with unequal variances; and unstructured. Akaike's information criterion was used to select the best covariance structure. The mitigated and prevented fractions were computed for individual days. The mitigated fraction describes a reduction in a specific outcome, whereas the prevented fraction focuses on the absence of a specific outcome (34). In this work, the prevented fraction would be defined as no detectable shedding of the challenge organism and the mitigated fraction as a reduction in shedding of the challenge organism. Differences in the duration of shedding were analyzed by means of the Wilcoxon rank–sum test, and the mitigated fraction was calculated with use of a 90% confidence interval.

Table II. Proportions of calves shedding *Escherichia coli* O157 after challenge

Day after challenge	Proportion with fecal shedding	
	Placebo-treated	Vaccinated
1	0.93	0.87
2	0.83	0.50
3	0.93	0.77
4	0.93	0.73
5	0.93	0.77
6	0.97	0.70
7	0.77	0.60
8	0.63	0.60
9	0.53	0.50
10	0.47	0.50
11	0.30	0.37
12	0.17	0.37
13	0.17	0.27
14	0.13	0.30

Results

Serologic response to vaccination

For all antigens, no significant differences between the placebo-treated and vaccinated groups were observed by ELISA at day 0, but the vaccinated group demonstrated significantly higher titers of antibodies to all antigens on days 21, 42, 56, and 70 compared with the placebo group (Figure 1). Maximum responses were observed at day 56.

Demonstration of successful infection

Before experimental infection (on day 56), all the animals were shown to be negative for *E. coli* O157. After infection, fecal shedding was monitored daily for 14 d. The target challenge dose per animal was 5.0×10^9 CFU; plate counts indicated that the actual infectious dose was 3.9×10^9 CFU. For success of the infection model, at least

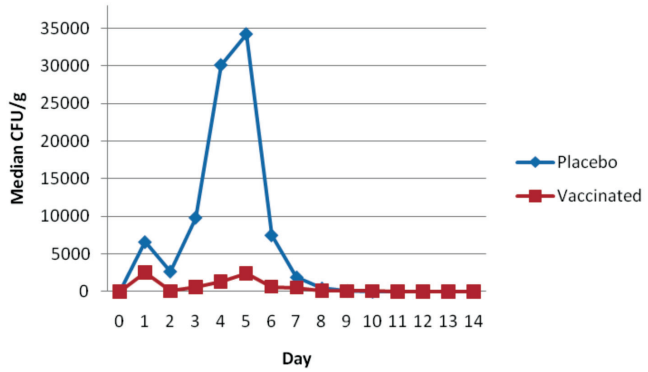

Figure 2. Fecal shedding [median number of colony-forming units per gram (CFU/g)] of the *Escherichia coli* O157 challenge strain in the 2 groups of calves for 14 d after challenge.

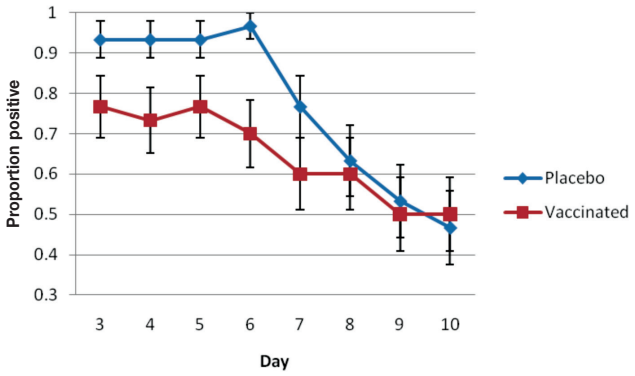

Figure 3. Proportion of placebo-treated or vaccinated animals shedding the challenge organism during the 14-d period after challenge.

Table III. Duration of *E. coli* O157 shedding over the 14 d after challenge

	No. of days positive for fecal shedding			
	Days 3 to 10		Days 3 to 14	
Measure	Placebo-treated	Vaccinated	Placebo-treated	Vaccinated
Mean	6.2	5.2	6.9	6.5
Median	6.5	5.0	7.0	6.0
Total	185	155	208	194

50% of the placebo-treated animals had to test positive for *E. coli* O157 on days 1 and 2 after infection. On day 1, 28 of 30 (93%) tested positive; on day 2, 25 of 30 (83%) tested positive (Table II). Thus, the model was considered successful.

Analysis of vaccine efficacy

Statistical analyses required to calculate vaccine efficacy after infection were affected by 2 factors: the intestinal transit time of the infection model and the low shedding of the challenge organism. As expected with an experimental oral–gastric challenge, high concentrations of *E. coli* O157 associated with intestinal transit were observed for 48 h after infection. Because it is impossible to distinguish cells colonized and subsequently shed from those transiently passing through the intestinal tract, the data for days 1 and 2 were excluded from the analyses. Further, low shedding in placebo-treated animals was observed from days 11 to 14 after challenge (Figure 2); the mean and median numbers of CFU/g were < 10 and 0, respectively, and the proportion shedding was 30% or less (Table II). Correspondingly, statistical analyses for days 11 to 14 revealed no significant difference between the treatment groups.

Quantity of *E. coli* O157:H7 shed from days 3 to 10 after challenge

Repeated-measures analysis was used to compare treatment means for individual days and to assess mitigated fractions. For both treatments, shedding peaked on days 3 to 6 and declined rapidly thereafter (Figure 2). Over all, vaccination significantly reduced the number of *E. coli* O157:H7 shed from days 3 to 10 after challenge. The mean $\log_{10}(\times + 1)$ number of CFU/g shed was significantly higher for the placebo-treated animals than for the vaccinates ($P = 0.011$). The mean values for days 3 to 6 were 3.7, 4.2, 4.2, and 3.8 for the placebo-treated animals and 2.5, 2.6, 2.9, and 2.5 for the vaccinates, correlating with mean log reductions in the vaccinates of 1.2, 1.6, 1.3, and 1.3 (mean 1.4; $P \leq 0.002$). As vaccination represents an intervention strategy that reduces *E. coli* O157:H7 carriage, the mitigated fraction, which has been proposed for severity-moderating interventions (that is, a reduction in shedding) (31), was calculated for days 3 to 6 for the vaccinates: the fractions were 51%, 59%, 50%, and 43% (mean 51%), respectively. Shedding decreased in both groups on day 7, and no significant differences were observed from days 7 to 10.

Number of animals shedding *E. coli* O157:H7

The proportion of vaccinates positive for *E. coli* O157:H7 was significantly less than the proportion of placebo-treated animals positive during peak shedding (0.742 versus 0.942; $P < 0.05$) (Table II, Figure 3). The prevented fraction, determined by the relative ratio of positive vaccinates to positive placebo-treated animals, was 18% to 28% on these days. No differences were observed for days 7 to 10.

Duration of *E. coli* O157 shedding

Although differences between the groups in the duration of shedding of the challenge organism were observed for days 3 to 10 (Table III), they were not significant. The differences for days 3 to 14 were even smaller.

Discussion

For a vaccine to be efficacious, it must induce a significant immune response such that antibodies to key antigens interfere with host–pathogen dynamics. Although calves do not normally mount a significant response to TTSPs, vaccination has been shown to stimulate the production of serum antibodies against EspA and Tir in cattle (23–25,36) and mice (37). In this work, vaccination led to significant priming of the cattle immune system: serum titers of antibodies to EspA, Tir, and total O157 TTSPs were significantly higher in vaccinates than in placebo-treated calves at all analysis points except day 0.

For traditional cattle vaccines, efficacy assessments are derived from measurable outcomes related to disease symptoms. For

vaccines intended to reduce *E. coli* O157 carriage in cattle, measurable outcomes are not based on disease symptoms. Although colonization in cattle is not commensal (17), cattle remain asymptomatic during *E. coli* O157 infection. Thus, monitoring *E. coli* O157 fecal shedding and sampling the terminal rectal mucosa are currently accepted standards for evaluating *E. coli* O157:H7 vaccine efficacy (23–28,30,31,37). Depending on the experimental design, quantification of shed *E. coli* O157 by direct plating, determination of *E. coli* O157 presence or absence with IMS, or both techniques are routinely employed. For this work, high levels of fecal shedding were expected over the 14-d period after challenge; thus, direct plating was used to quantify efficacy.

In a previous study using a similar animal model, high challenge shedding (10^3 to 10^4 CFU/g) on days 11 to 14 after challenge was observed (23). In the current study, significant reductions in shedding occurred much earlier. For days 7, 8, and 9, the median CFU/g decreased to 1922, 445, and 114, respectively, whereas the median count for days 10 to 14 was 0 CFU/g. The poor long-term colonization limited our ability to ascertain the biologic effect of vaccination at the end of the period after challenge. Recently, McNeilly et al (38) observed a similar decrease in shedding in a model of 9-wk-old calves. Although the challenge organism was shed at relatively high concentrations until day 10 (10^4 to 10^5 CFU/g), a rapid decrease was seen on days 12 to 14, when 10^2 to 10^3 CFU/g were shed.

Considering days 3 to 10 in this study, vaccination resulted in lower overall *E. coli* O157 shedding. Peak shedding in both groups was observed from days 3 to 6, with the maximal media shed occurring on day 5 (3.4×10^4 CFU/g in the placebo group and 2.4×10^3 CFU/g in the vaccinated group). With the mitigated fraction calculation, the effect of vaccination becomes significant. In vaccinated cattle, mitigated fractions of 51%, 59%, 50%, and 43% were observed for days 3 to 6, respectively. This represents a significant reduction in the voiding of *E. coli* O157 from the bovine host into the environment. Under field conditions, *E. coli* O157:H7 infection in cattle is linked closely to environmental conditions that increase the opportunity for oral exposure (24–26,39–41,42). Thus, under the conditions in this study of artificially high levels of exposure and commingling of the animals, the vaccinated animals shed significantly less during peak shedding. Moxley et al (28) reported that, under conditions of natural exposure, TTSP vaccination of cattle led to a 65% lower likelihood of *E. coli* O157:H7 shedding. Further, in a separate study, similar efficacy and significant reductions in hide contamination were attributed to TTSP vaccination (27). Consequently, both natural-exposure studies and artificial experimental challenge have demonstrated significant reductions in *E. coli* O157 shedding after vaccination with a TTSP vaccine.

In addition to reducing the quantity of challenge organisms shed, vaccination proved effective at reducing the total number of animals shedding. There were significantly fewer vaccinates shedding from days 3 to 6, resulting in prevented fractions of 18%, 21%, 18%, and 28%, respectively. Compared with placebo-treated animals, on day 6, for example, 28% fewer animals were positive for *E. coli* O157, which represents a significant reduction in herd-level shedding.

A proof-of-principle study in 2004 by Potter et al (23) using an experimental challenge model also showed significant protective effects of TTSP vaccination. The vaccine was laboratory-produced and -formulated. A second study was conducted to examine vaccine efficacy under conditions of natural exposure (35). Considering the size of this trial, it was necessary to scale up vaccine production through a third-party contract research organization. In addition to different antigen production and purification methods, the final formulation of the vaccine was altered in two significant ways: a different oil-based adjuvant (VSA3) was used, and formalin, known to denature antigens, was added as a preservative. It was not surprising that no significant protective effects were observed after administration of the vaccine. Much effort then went into developing a commercially viable production process and a formulation that excluded formalin and included an adjuvant similar to that used in the original study. The resulting commercial vaccine formulation was optimized and evaluated in a number of natural-exposure studies that demonstrated significant protective effects (24–28). Also, importantly, data from the current study confirm the efficacy of this vaccine formulation in an experimental challenge model that demonstrated protective effects similar to those of the original study by Potter et al (23).

In the current study, the impact of vaccination was 2-fold: compared with the placebo-treated animals, the vaccinated group demonstrated significantly less overall *E. coli* O157 shedding, and significantly fewer of the vaccinates shed *E. coli* O157. This has clear implications for reducing the preharvest *E. coli* O157 burden in cattle. First, decreases in shedding will significantly reduce the quantity of *E. coli* O157 cycled into the environment and minimize the burden at harvest. Further, the use of vaccination has been shown to reduce hide contamination (27), which is a significant source of carcass contamination (6–8). Thus, the effectiveness of abattoir-related intervention strategies will be maximized. Second, gross reductions in shedding reduce the opportunity for fecal-based contamination of produce commodities.

In conclusion, in this evaluation of a TTSP vaccine under conditions in which cattle were artificially exposed to *E. coli* O157 and subjected to ongoing environmental exposure through commingling of the treatment groups, vaccinates shed significantly less of the challenge organism than the placebo animals during peak shedding (mean mitigated fraction 51%), and significantly fewer vaccinated animals were positive for *E. coli* O157 (mean prevented fraction 21%) compared with the placebo-treated animals. Considering the recent suggestion of food safety gaps in our food continuum (10), the frequent implication of beef and produce food commodities in *E. coli* O157 outbreaks, and a quantitative risk assessment that identified cattle vaccination as an effective means of ameliorating *E. coli* O157:H7 illness in humans (14), adoption of *E. coli* O157 vaccination as an intervention strategy seems prudent. The results of this study were submitted to the Canadian Food Inspection Agency and contributed to the vaccine's subsequent commercial licensure. As such, this study provides regulators and scientists with a framework for evaluating the efficacy of future Shiga toxin-producing *E. coli* vaccines.

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