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Assessment of selenium supplementation by systemic injection at birth on pre-weaning calf health

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Abstract

The objective of this randomized clinical trial was to evaluate the effect of selenium and vitamin E supplementation by systemic injection on dairy calf health and growth during the pre-weaning period. The study was conducted at 39 dairy farms in Ontario, selected through a convenience sample of farms in close proximity to either Guelph or Kemptville. A total of 835 Holstein heifer calves were enrolled in this study. At birth, calves were randomly allocated to receive selenium and vitamin E supplementation by injection (3 mg sodium selenite and 136 IU dl-α-tocopherol acetate) or a placebo solution. At enrollment, producers recorded time of birth, calving ease, and colostrum feeding status. At weekly visits to the individual dairy farms, trained technicians collected measurements and samples from enrolled calves. Blood was collected from calves that were up to 8 d of age to assess the concentration of serum total protein and selenium. Between 8 and 15 d of age, fecal samples were collected to identify the presence of rotavirus and *Cryptosporidium parvum* (*C. parvum*) using a commercially available lateral immuno-chromatography antigen detection kit. Each enrolled calf was also weighed and assessed for health scores during the first, second, and seventh weeks of life. The average serum selenium concentration (SD) in treated calves was 0.08 µg/mL (0.02), versus 0.06 µg/mL (0.01) in control calves. The incidence of failure of passive transfer (FPT) among study calves was 21%, and did not differ between treatment groups. The mean average daily gain (ADG) for the study period was 1.43 lb (0.65 kg)/day and was not associated with selenium and vitamin E supplement injection. Of the 761 fecal samples tested, 272 (36%) and 118 (16%) tested positive for *C. parvum* and rotavirus antigen, respectively. Selenium and vitamin E treatment had a protective effect against rotavirus infection. However, there was no effect of experimental treatment on *C. parvum* infection status. Reduced odds of treatment for diarrhea was also seen in the selenium and vitamin E treatment group. This study suggests that selenium and vitamin E injection at birth could improve pre-weaning health by reducing rotavirus infection and diarrhea.

Key words: selenium, vitamin E, calf health

Résumé

L'objectif de cet essai clinique randomisé était d'évaluer l'effet de l'ajout de sélénium et de vitamine E par injection systémique sur la santé et la croissance de veaux laitiers avant sevrage. L'étude a été menée dans 39 fermes laitières de l'Ontario représentant un échantillon de commodité des fermes proches de Guelph ou de Kemptville. L'étude incluait un total de 835 génisses Holstein. À la naissance, les veaux ont été alloués au hasard dans deux groupes l'un recevant un ajout de sélénium et de vitamine E par injection (3 mg de sélénite de sodium et 136 UI d'acétate de dl-α tocophérol) et l'autre une solution placebo. Au recrutement, les producteurs notaient l'heure de la naissance, la facilité du vêlage et le statut de l'alimentation au colostrum. Lors de visites hebdomadaires aux différentes fermes laitières, des techniciens formés ont fait les mesures et recueilli les échantillons des veaux recrutés. Des échantillons de sang ont été prélevés chez les veaux jusqu'à huit jours suivant la naissance pour mesurer la concentration sérique des protéines totales et du sélénium. Entre les jours 8 et 15 suivant la naissance, des échantillons fécaux ont été recueillis pour déterminer la présence de rotavirus et de *Cryptosporodium parvum* (*C. parvum*) avec une trousse commerciale de détection d'antigène latéral par immunochromatographie. Chaque veau recruté était aussi pesé et recevait un score de santé aux semaines 1, 2 et 7 suivant la naissance. La concentration sérique moyenne de sélénium (EC) chez les veaux traités était de 0.08 µg/mL (0.02) et de 0.06 µg/mL (0.01) chez les veaux témoins. L'incidence de défaut de transfert passif chez les veaux recrutés était de 21% et n'était pas différente dans les deux groupes de traitement. La moyenne du gain moyen quotidien pendant la durée de l'étude était de 1.43 lb (0.65 kg)/jour et n'était pas associé à l'ajout de sélénium et de vitamine E par injection. Parmi les 761 échantillons fécaux recueillis, 272 (36%) se sont révélés positifs pour l'antigène de *C. parvum* et 118 (16%) pour l'antigène du rotavirus. Le traitement par l'ajout de sélénium et de vitamine E avait un effet protecteur contre l'infection par le rotavirus. Toutefois, il n'y avait pas d'effet du traitement expérimental sur le statut d'infection par *C. parvum*. Le traitement avec l'ajout de sélénium et de vitamine E a aussi réduit les chances de traitement pour la diarrhée. Cette étude suggère que l'injection de sélénium et de vitamine E à la naissance pourrait améliorer la santé avant sevrage en réduisant l'infection par le rotavirus et la diarrhée.

Introduction

Selenium content in North American soil is highly variable. Feeds grown east of the Mississippi River and west of the Rocky Mountains typically contain < 0.01 mg of Se/kg dry matter.[23] In Ontario, the majority of the dairy industry is located in regions with selenium-deficient soils. As a consequence, plants grown under these conditions contain low amounts of selenium. Although oral supplementation of dairy cattle with selenium has become a routine practice, treatment of newborn dairy calves is not widely implemented. In a study of calf management practices in Ontario, less than 30% of Ontario dairy herds routinely administer selenium and vitamin E to newborn calves.[35]

Selenium is an important element for the immune system and has been shown to impact animal health.[27,28] It activates phagocytosis by neutrophils, increases antibody production, and enhances lymphocyte proliferation.[30,31] Selenium is also well known for its role in the enzyme glutathione peroxidase, which is an important component of the cellular antioxidant system.[25]

Ruminants are more susceptible to selenium deficiency compared to other animal species.[11] Specifically, in dairy cattle, selenium deficiency has been associated with a wide range of conditions, such as increased rates of retained placenta and intramammary infection, as well as impaired reproductive performance.[8] Despite the importance and emphasis placed on selenium supplementation in mature dairy cattle, selenium concentration in calves has been given little attention. In calves, selenium concentration is associated with growth[7,26,42] and morbidity.[7,39,41] A recent study found that the injection of a multi-mineral preparation containing selenium at 3 and 30 days after birth reduced the incidence of diarrhea, and the combined incidence of pneumonia, otitis, or both.[34] It is suspected that Ontario dairy producers have primarily focused on supplementation of cows during their dry period, and have assumed that calves receive adequate quantities of selenium from dams in utero, and through consumption of colostrum and milk. However, Waalderbos showed that selenium concentrations in Ontario calves were well below 0.08 ug/mL selenium in serum.[38] Thus, selenium concentrations in Ontario dairy calves could be inadequate, potentially limiting calf health and growth.

The objective of this randomized clinical trial was to evaluate the effect of selenium and vitamin E supplementation, by systemic injection, on dairy calf health and growth during the pre-weaning period. The hypothesis of this experiment was that systemic treatment of newborn dairy calves with selenium and vitamin E would improve early life health and growth.

Materials and Methods

This randomized clinical trial was conducted in accordance with the University of Guelph Animal Care Committee requirements (Animal Use Protocol: #09R051).

Study Farms

Dairy farms were selected from a convenience sample of commercial Holstein herds that were within a 2-h radius of either the University of Guelph (Guelph, Ontario, Canada), or University of Guelph Kemptville Campus (Kemptville, Ontario, Canada). A total of 39 farms were enrolled in the study, including 20 farms surrounding Kemptville and 19 farms in the Guelph area. Inclusion as a study herd required that a detailed birth record on each calf was completed, and records of all occurrences of health problems and treatments administered up to weaning were kept.

Treatments, Randomization, and Blinding

Calves were randomly assigned to receive a subcutaneous injection from a numbered vial containing 1 of 2 treatments: 1 mL injection of either a placebo or an injectable selenium and vitamin E supplement[a] approved for use in dairy cattle that contains 3 mg Se (sodium selenite) and 136 IU vitamin E (dl-α-tocopherol acetate) per mL of solution. These concentrations are slightly higher than other comparable injectable products available in the United States. The placebo used in this study was created by adding a coloring agent to sterile water to create a visually similar solution to the Se-vitamin E solution.

Treatment allocation was randomized using a random number generator. Three separate randomized blocks of 10 were created with an equal number of treatments and controls. In total, 1200 vials were created numbering "0001" to "1200", with the randomization repeating every 30 vials. Treatment vials were distributed to farms in multiples of 10 to ensure that an equal number of treated and control calves were enrolled on each farm.

Throughout the trial, producers administrating the experimental solutions, assessors of the outcomes, and persons responsible for data analysis were blinded to the treatment allocation.

Calf Enrollment and Sampling

Holstein heifer calves born between May 1 and September 1, 2009 were enrolled in this study. At the time of discovery or delivery of a newborn calf prior to colostrum feeding, producers on each farm were instructed to administer the contents of a numbered treatment vial to study calves via subcutaneous injection. Following the injection of a newborn calf, producers completed a birth record documenting information about the birth event, including: date, time, calving ease, quantity, and source of colostrum fed to each calf, and the vial number assigned. Rather than an exact time, birth time was recorded by producers as being within a time of day interval.

On a regular weekly interval, trained technicians visited each farm to assess newly enrolled calves, as well as to collect measurements, samples, and recorded data from previously enrolled animals. Blood was collected from each calf up to 8 d of age that had not been sampled the previous week. Blood was collected by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle,[b] into a 10-mL sterile glass vacuum tube without anticoagulant.[c] Following blood collection on-farm, blood was transported on ice to the laboratory, where it was allowed to clot and was centrifuged at $970 \times g$ for 10 minutes at approximately 68°F (20°C). Serum was harvested and analyzed for serum total protein (STP) using a digital refractometer.[d] Calves with a STP < 5.2 g/dL were considered to have failure of passive transfer (FPT). Adequate passive transfer (APT) was defined as calves with a STP ≥ 5.2 g/dL. Recent research has established that STP can be reliably assessed in calves from 1 to 9 days of age.[44]

A second aliquot of serum was frozen and submitted to the University of Guelph Animal Health Laboratory (Guelph, ON, Canada) for selenium determination using inductively coupled plasma mass spectrometry.[16]

Each calf was weighed and assessed for diarrhea during the first week of life, the second week of life, and again at approximately 7 weeks of age. Calf weight was recorded using a heart girth weight tape for Holstein calves.[e] Based on weight measurements obtained at the first and final calf visit, average daily gain (ADG) was calculated for the study period. Fecal consistency was scored on a scale of 0 to 3, where fecal score 0 = normal consistency; 1 = semi-formed or pasty; 2 = loose feces; 3 = watery feces.[20] A fresh fecal sample was collected directly from each calf between 8 to 15 d of age, and frozen at -4°F (-20°C) for later processing in batches of approximately 100 samples. Each fecal sample was analyzed for the presence of *Cryptosporidium parvum* (*C. parvum*) and rotavirus antigen using a commercially available lateral immunochromatography antigen detection kit.[f]

During the course of the study, all treatment events on study calves up to weaning were recorded by producers. Treatment was defined as the administration of a product to an animal in response to a health event during the study period. Treatments included, but were not restricted to, antibiotics and other veterinary drugs, but excluded vaccinations. Administration of electrolytes and antibiotic tablets for neonatal calf diarrhea were considered treatments. All treatment events were recorded on treatment record sheets supplied to each farm at the start of the study. On each treatment record, date and duration of treatment, type of treatment, and reason for treatment were recorded. The number of treatment records and completeness of each individual record were somewhat variable by farm. Therefore, only the first treatment event was considered in treatment models.

Estimation of Sample Size

Sample size was calculated using ADG and *C. parvum* infection as outcomes of interest. It was assumed that ADG in pre-weaned Ontario dairy calves was 1.26 lb (0.57 kg)/day, with a variance of 0.15 lb (0.07 kg)/day and estimated that an injection of selenium and vitamin E would increase ADG to 1.28 lb (0.58 kg)/day. Using these assumptions and a two-sided hypothesis test ($\alpha = 0.05$, Power = 0.80), the <sampsi> command in Stata 10[g] generated sample size estimates of 393 animals per group. Sample size was also estimated using *C. parvum* infection as an outcome. It was assumed that mean within-herd prevalence of *C. parvum* infection was 40% in control calves and could be reduced to 30% in treated calves. Using a two-sided hypothesis test ($\alpha = 0.05$, Power = 0.80), it was determined that 376 calves per group would be required.

Statistical Analysis

Data were entered using Microsoft Office[h] Access 2007 and checked for entry errors using SAS 9.1.3[i] software. If abnormal or missing values were found, data were checked against the original hard copy records. Descriptive statistics were generated for all explanatory variables in the dataset.

All variables hypothesized to be related to the outcome of interest were screened for unconditional associations with the outcome variable in univariate analysis, while controlling for the random effects of farm. Variables that were significant at the $P \leq 0.2$ were included in a multivariable model. Collinearity was assessed by calculating Pearson correlation coefficients. If variables were highly correlated ($r > 0.75$), a separate model was constructed including each variable. The variable that produced the model with the lowest AIC was included in the final model. Linearity of continuous variables was assessed visually. Confounding was assessed by observing changes in coefficients of predictor variables in the model, following stepwise removal of non-significant variables in the multivariable model. Variables were retained in the model, if their removal from the model resulted in a change of more than 30% in a model coefficient. Main effects variables in the model were checked for interaction by inclusion of biologically appropriate two-way interaction terms, identified in the causal diagram. All hypothesized two-way interactions were tested in a multivariable model. Statistically significant interaction terms ($P \leq 0.05$) were considered to be evidence of interaction and were retained in the final model. Main effects variables were considered significant and included in the final model if $P \leq 0.05$. In all models, injection with selenium and vitamin E at birth was included as a variable in the final model, regardless of significance, since it was the predictor of interest.

For models created using the MIXED procedure in SAS, model fit was observed by plotting residuals and observing heteroscedasticity and normality visually. If these criteria were not met, all appropriate transformations of the outcome were assessed, and the transformation that most corrected heteroscedasticity and normality was selected.

Mixed logistic models were created to determine the impact of selenium and vitamin E injection on 1) success of passive transfer, 2) having profuse watery feces (fecal score = 3 vs fecal score < 3) at the time of sampling at 8 to 15 days of age, and 3) a calf having loose or watery feces (fecal score > 2 vs fecal score < 2) at the time of sampling. Fecal scores were recorded at the time of fecal collection from the calf (8 to 15 days of age) and were an accurate representation of fecal consistency at this sampling time.

Mixed linear models were constructed to evaluate 1) the impacts of predictor variables on serum selenium concentration, and 2) association between selenium and vitamin E treatment at birth and ADG. In both models, farm was included as a random effect. In the first model, to account for some of the variability in regional soil selenium concentrations, farm was nested within region (East vs West) and to correct for the normality of the residuals, serum selenium was log transformed.

Generalized linear mixed models were constructed to determine associations between selenium and vitamin E injection and 1) the probability of a positive test for *C. parvum* or rotavirus antigen, and 2) the probability of treatment for: any reason, neonatal calf diarrhea, and respiratory disease. In these models, farm of origin was included as a random effect.

Results

Animal Enrollment and Calving Environment

In total, 835 Holstein heifer calves were enrolled in this study between May 1 and September 1, 2009 with no difference being found with respect to number of calves enrolled into each treatment group. Animal enrollment by farm was highly variable, with a mean of 21 animals enrolled per farm. Calves were considered to be enrolled in the study at the time of injection by producer, regardless of whether or not they survived to the first sampling event (1 to 8 d of age).

A descriptive summary of continuous and categorical variables related to birth events and colostrum feeding, according to treatment group, are presented in Tables 1 and 2, respectively. There were no differences between the 2 treatment groups with respect to location of birth, calving time or calving difficulty. The majority of calves were born in the maternity pens, between 1000 to 1600 h and having an unobserved calving.

Colostrum Feeding Practices

There were no differences between the 2 treatment groups with respect to colostrum management practices (Tables 1 and 2). The mean (SD) colostrum volume fed in the first 24 h was 6.1 qt (5.8 L [1.6]), over an average of 1.9 feedings (0.8). Colostrum was most commonly delivered via esophageal feeder (46%) and by nipple bottle (42%), or a combination of the 2 methods (8%). Fewer than 4% of calves were fed using a bucket feeder, either alone or in combination with another method. Less than 1% of calves received colostrum by suckling the dam. The majority of calves on the study received colostrum either from their dams (64%) or pooled colostrum (32%). A small number of calves received colostrum replacement products (3%). Of the calves receiving colostrum either from their dam or pooled colostrum, most had fresh colostrum (68%) with a minority receiving either frozen (16%), pasteurized (16%) or a combination of multiple forms (1%).

Table 1. Descriptive summary of continuous intervening and outcome variables recorded according to treatment group (Mean)(SD)(Range).

Item	Dystosel*	Placebo**	*P*-value
Calves enrolled	426	409	
Age at visit 1 day (d)	4.4 (1.99) (0 – 8)	4.5 (2.01) (0 – 8)	0.70
Age at visit 2 d	11.4 (1.99) (8 – 15)	11.5 (2.02) (7 – 16)	0.54
Age at visit 3 d	50.9 (5.06) (36 – 63)	51.0 (5.22) (35 – 71)	0.79
Birth weight (lb)	94.8 (9.74) (64 – 135)	94.3 (8.85) (78 – 123)	0.38
Volume of colostrum fed (L)	5.8 (1.60) (1 – 12)	5.7 (1.48) (0 – 12)	0.92
Minimum time to colostrum (h)	1.7 (3.00) (0 – 17.7)	1.7 (3.10) (0 – 16.0)	0.96
Maximum time to colostrum (h)	6.5 (3.98) (0.5 – 23.7)	6.4 (3.98) (0.0 – 22.0)	0.92
Minimum time with dam (h)	1.7 (3.34) (0.0 – 17.8)	1.6 (3.27) (0.0 – 17.5)	0.68
Maximum time with dam (h)	6.1 (4.50) (0.0 – 23.8)	6.0 (4.37) (0.0 – 23.5)	0.75
Serum total protein (g/dL)	5.7 (0.76) (3.3 – 8.1)	5.8 (0.72) (4.1 – 8.7)	0.50
Serum selenium (ug/mL)	0.077 (0.021) (0.04 – 0.17)	0.057 (0.013) (0.028 – 0.12)	<0.001

*Dystosel, Zoetis Canada. Injectable supplement containing 3 mg sodium selenite and 136 IU dl-α-tocophenol/mL

**Sterile water with coloring added to mimic Dystosel

Passive Transfer of Immunity

The average STP was 5.7 for calves in the selenium and vitamin E treatment group, and 5.8 g/dL in the placebo

Table 2. Descriptive summary of categorical variables related to birth events and colostrum feeding according to treatment group

	Prevalence	
Item	**Dystosel* (%)**	**Placebo** (%)**
Birth location		
Free stall	11.0	12.7
Maternity pen	82.1	81.3
Tie stall	4.8	2.0
Other	2.1	4.0
Calving assistance		
Easy pull	29.4	24.1
Hard pull	4.5	4.2
Malpresentation	1.0	1.5
Observed but unassisted	14.0	15.1
Surgery	0.7	0.3
Unobserved	50.4	54.8
Number of feedings in first 24 h		
0	0.0	0.3
1	31.7	30.7
2	47.3	50.1
3+	21.0	17.9
Source of colostrum		
Dam	62.7	64.2
Pooled	32.3	32.6
Replacement	3.4	2.7
Combination	1.6	0.5
Type of colostrum		
Fresh	67.0	68.6
Frozen	17.4	14.9
Pasteurized	15.1	16.0
Combination	0.8	0.5

*Dystosel, Zoetis Canada. Injectable supplement containing 3 mg sodium selenite and 136 IU dl-α-tocophenol/mL
**Sterile water with coloring added to mimic Dystosel

Table 3. Descriptive summary of dichotomous outcomes according to treatment group.

	Prevalence	
Item	**Dystosel***	**Placebo****
Passive transfer		
>5.2 g/dL	78.1% (324/415)	80.1% (314/392)
<5.2 g/dL	21.9% (91/415)	19.9% (78/392)
Fecal score (8-15 days of age)		
3	14.5% (60/413)	17.5% (68/389)
<3	85.5% (353/413)	82.5% (321/389)
Rotavirus test result		
Negative	86.8% (334/385)	82.2% (309/376)
Positive	13.2% (51/385)	17.8% (67/376)
C. parvum test result		
Negative	66.0% (254/385)	62.5% (235/376)
Positive	34.0% (131/385)	37.5% (141/376)
Treatment during study (any reason)		
No treatment	79.1% (337/426)	76.5 (313/409)
Treatment	20.9% (89/426)	23.5% (96/409)
Treatment for diarrhea		
No treatment	89.2% (380/426)	85.6% (350/409)
Treatment	10.8% (46/426)	14.4% (59/409)

*Dystosel, Zoetis Canada. Injectable supplement containing 3 mg sodium selenite and 136 IU dl-α-tocophenol/mL
**Sterile water with coloring added to mimic Dystosel

Table 4. Final mixed logistic model for successful passive transfer (STP > 5.2 g/dL) after controlling for the random effect of farm (n=762).

Variable	**Coefficient (SE*)**	**OR (95% CI**)**	***P*-value**
Treatment group			
Placebo	0.12 (0.19)	1.12 (0.78 – 1.63)	0.54
Selenium and vitamin E	Ref†		
Age at sampling *For every 1 day increase in age at sampling*	-0.14 (0.05)	0.87 (0.83 – 0.91)	<0.01
Volume of colostrum *For every 1 L increase in volume of colostrum fed*	0.27 (0.08)	1.31 (1.21 – 1.42)	<0.01

*Standard error
**95% confidence interval
†Referent category

group (Table 1). The average STP was not different between treatment groups. Likewise, the incidence of FPT was not different between treatment groups. The incidence of FPT among calves receiving the selenium and vitamin E treatment was 22%, as compared to 20% incidence of FPT in calves receiving the placebo. A summary of the categorical data for passive transfer of immunoglobulins of the study calves by experimental treatment group is presented in Tables 2 and 3. Rates of FPT were highly variable by farm, ranging from 0% to 55% of calves on-farm. Selenium and vitamin E injection was not associated with passive transfer in the univariable model (P=0.47), nor in the final multivariable model (Table 4; P=0.54) after controlling for age of calf at sampling, and volume of colostrum fed in the first 24 h.

Description of Serum Selenium Concentration

Serum samples were submitted for analysis for 803 calves enrolled on the study. On average, the serum selenium concentration in selenium and vitamin E-treated calves was significantly higher than in control calves, according to a t-test (P<0.001). The average serum selenium concentration (SD) was 0.08 µg/mL (0.02) and 0.06 µg/mL (0.01) in selenium and vitamin E-treated calves and control calves, respectively (Table 1; Figure 1). There was considerable inter- and intra-farm variation in selenium concentrations. Selenium and

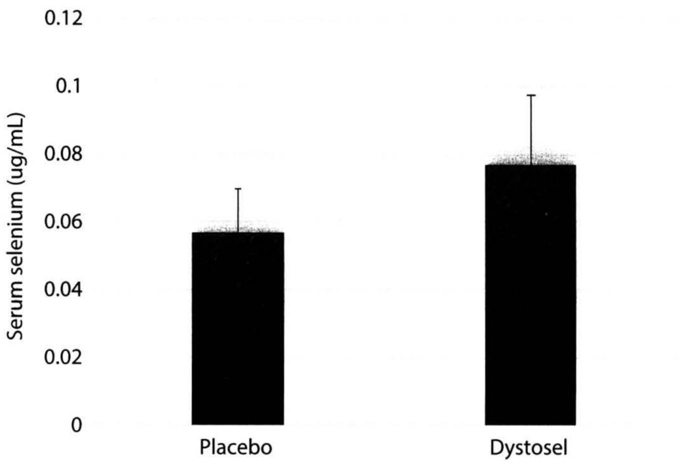

Figure 1. Descriptive summary of serum selenium levels in calves that were supplemented with selenium (Dystosel) and those that were not supplemented (Placebo).

vitamin E treatment (*P*<0.01) and age of calf at sampling (*P*<0.01) were associated with the log transformed serum selenium concentrations (Table 5).

Calf Growth during the Study Period

Calf weight was obtained at the time of enrollment, and at the end of the study period for all calves enrolled on the study. These weights were used to calculate ADG during the study period. The mean birth weight was 94.8 lb (43 kg) and was not different between treatment groups. The mean ADG for all study animals for the entire study period was 1.43 lb/day (SD 0.44; 0.65 kg/day [SD 0.2]).

Average daily gain during the pre-weaning period was not associated with selenium and vitamin E injection (*P*=0.86). Variables that were significant in the model included birth weight, success of passive transfer, and whether or not a calf was treated for illness during the pre-weaning period. Calves that experienced FPT on average gained 0.11 lb (0.05 kg)/day less than calves with APT (*P*=0.01). The final model for ADG is presented in Table 6.

Table 5. Final mixed linear model for log transformed serum selenium concentrations (µg/mL) in calves 1 to 8 d of age, after controlling for the random effects of farm and region (n=725).

Variable	Coefficient (SE*)	*P*
Treatment group		
Placebo	-0.29 (0.02)	<0.01
Selenium and vitamin E	Ref**	
Age at sampling: For every 1 day increase in age at sampling	-0.03 (0.004)	<0.01

*Standard Error
**Referent category

Table 6. Final mixed linear model for average daily gain (kg/day) during the entire study period, controlling for the random effects of farm (n=761).

Variable	Coefficient (SE*)	*P*
Treatment group:		
Placebo	0.01 (0.01)	0.36
Selenium and vitamin E	Ref.**	
Birth weight: For every 1 lb increase in birth weight	-0.002 (0.001)	0.01
Passive transfer		
< 5.2 g/dL	-0.05 (0.02)	0.01
> 5.2 g/dL	Ref[2]	
Treatment (any)		
Not treated	0.06 (0.02)	<0.01
Treated	Ref**	

*Standard Error
**Referent category

Lateral Immunochromatography Fecal Antigen Detection Test Results and Scouring

In total, fecal samples were collected from 761 Holstein heifer calves on 38 dairy farms. Total animals sampled from these farms ranged from 1 to 109 calves. A total of 131 (34%) and 141 (38%) calves in the treatment and placebo groups, respectively, tested positive for *C. parvum* antigen using the lateral immunochromatography antigen detection kit (Table 3). Within-herd prevalence of positive *C. parvum* tests ranged from 0% to 63%. Selenium and vitamin E treatment was not significant in the final model for *C. parvum* infection (OR = 1.15 (95% Confidence Interval (CI) = 0.84 – 1.58); *P*=0.33). Variables significant in the final model were fecal score of 3 and positive test for rotavirus antigen. Calves with a fecal score of 3 were more likely to test positive for *C. parvum* than calves with fecal scores less than 3 (OR = 2.49 (95% CI = 1.64 – 3.77); *P*<0.01). Rotavirus was found to have a protective effect against *C. parvum* infection. Calves testing negative for rotavirus were more likely to test positive for *C. parvum* infection than calves testing positive for rotavirus (OR = 2.33 (95% CI = 1.44 – 3.79); *P*<0.01). Fecal score was included in the univariable model in 3 separate levels (Fecal score = 3 vs < 3, Fecal score ≥ 2 vs < 2, Fecal score ≥ 1 vs 0). Because these variables were similar, and contained the same observations, only Fecal = 3 was included in the final model, since it produced the model with the smallest pseudo-AIC.

Fecal samples were also analyzed for presence of rotavirus antigen using the same lateral immunochromatography antigen detection kit used for *C. parvum.* In total, 51 (13%) and 67 (18%) of the selenium and vitamin E injection and placebo calves, respectively, had positive tests for rotavirus antigen (Table 3). Within-herd prevalence of rotavirus infection ranged from 0% to 66.7%. In total, 12 farms had no positive tests for rotavirus. The final model for rotavirus infection is presented in Table 7. Selenium and vitamin E treatment had a protective effect against rotavirus infection (*P*=0.05), after controlling for *C. parvum* infection and fecal score at time of sampling. Interestingly, calves testing negative for *C. parvum* infection were more likely to test positive for rotavirus compared to calves that tested positive for *C. parvum* (*P*<0.01). Fecal score was associated with antigen test results.

Morbidity and Mortality

Overall, the pre-weaning mortality rate in this study was 4% (30/835). A total of 12 calves on the study were lost to follow-up. When calves lost to follow-up were excluded from mortality analysis, there was no difference in calf mortality rates between the treatment groups (Chi-square *P*=0.22). The mortality rate in calves receiving the selenium and vitamin E injection was 3% compared to 5% in calves that received placebo injection.

A total of 89 calves (21%) in the selenium and vitamin E group received an individual treatment, whereas, 96 calves (24%) in the placebo group received an individual treatment. A model was constructed to determine the associations between predictor variables and the probability of a calf receiving any treatment during the pre-weaning period. Selenium treatment, passive transfer status, and presence of *C. parvum* infection were offered to the multivariable model. In the final model, calves that tested negative for *C. parvum* had reduced odds of being treated during the pre-weaning period, as compared to calves testing positive (OR = 0.67 (95% CI = 0.46 to 0.99); *P*=0.05). Selenium and vitamin E injection was not associated with pre-weaning treatment (OR = 1.11 (95% CI = 0.76 to 1.62); *P*=0.24). Failure of passive transfer status tended toward association with pre-weaning treatment (OR = 1.54 (95% CI = 0.96 to 2.48); *P*=0.07).

Table 7. Final mixed logistic model for shedding rotavirus after controlling for the random effect of farm (n=755).

Variable	Coefficient (SE*)	OR (95% CI**)	P
Treatment group:			
Placebo	0.41 (0.21)	1.50 (0.99 – 2.26)	0.05
Selenium and vitamin E	Ref†		
C. parvum			
Negative	0.83 (0.25)	2.29 (1.42 – 3.72)	<0.01
Positive	Ref†		
Fecal score: < 3	-0.62 (0.27)	0.54 (0.32 – 0.91)	0.02
3	Ref†		

*Standard Error
**95% Confidence Interval
†Referent category

Of the calves enrolled in the selenium and vitamin E group, 46 (11%) were treated for calf diarrhea in the study period, whereas, 59 (14%) in the placebo group were treated for diarrhea. A model was constructed to determine the associations between selenium and vitamin E injection and probability of being treated for diarrhea. Selenium treatment, passive transfer status, and presence of *C. parvum* infection were entered into the final model. In the final model, only selenium and vitamin E treatment was retained with the odds of being treated for diarrhea being greater in control calves (OR = 1.61 (95% CI = 1.03 to 2.49); *P*=0.04), compared to calves injected with selenium and vitamin E.

A total of 50 calves (6%) were treated for respiratory disease during the study period. A third model was constructed to determine the association between selenium and vitamin E injection at birth and treatment for respiratory disease during the pre-weaning period. Similar to the model for treatment for any reason, none of the predictor variables collected were associated with treatment for respiratory disease. In univariable analysis, selenium and vitamin E treatment was not associated with treatment for respiratory disease (*P*=0.81).

Associations between Fecal Score and Selenium Treatment

Of the calves that received selenium and vitamin E supplementation, 15% had a fecal score of 3 at sampling, whereas 18% had a fecal score of 3 in the placebo group. In the logistic model for predicting fecal score 3, variables that were significant in the final model (Table 8) included age at sampling (*P*=0.02), rotavirus test results (*P*=0.02), and *C.*

Table 8. Final mixed logistic model for probability of a calf having a fecal score of 3 at the time of fecal sampling, after controlling for the random effect of farm (n=802).

Variable	Coefficient SE*)	OR (95% CI**)	P
Treatment group:			
Placebo	0.23 (0.20)	1.50 (0.84 – 1.87)	0.26
Selenium and vitamin E	Ref†		
Age at sampling: For every 1 day increase in age at sampling	-0.12 (0.05)	0.89 (0.80 - 0.98)	0.02
C. parvum			
Negative	-0.59 (0.26)	0.55 (0.33 – 0.92)	0.02
Positive	Ref†		
Fecal score: < 3	-0.96 (0.21)	0.38 (0.25 – 0.57)	<0.01
3	Ref†		

*Standard Error
**95% Confidence Interval
†Referent category

parvum test results ($P<0.01$). Selenium treatment was not significant in this model ($P=0.26$). There was a strong trend for passive transfer to influence fecal score at the time of sampling, but it was not significant in the final model ($P=0.06$). Calves that tested positive for either rotavirus or *C. parvum* had increased odds of having a fecal score of 3 compared to calves testing negative (rotavirus OR = 1.8, *C. parvum* OR = 2.6). Similar to the fecal score 3 model, selenium treatment was not associated with calves having a fecal score of 2 or greater at the time of sampling ($P=0.20$). Predictors associated with fecal score of 2 or greater at the time of sampling were weight of the calf ($P=0.04$), rotavirus (OR = 2.2; $P<0.01$), and *C. parvum* (OR = 3.3; $P<0.01$).

Discussion

There has been a long-standing interest in selenium concentrations in dairy cattle. Until recently, the research focus in this area has been primarily on the effects of selenium supplementation on reproduction and mammary gland health in lactating dairy cattle, and on the chemical forms of selenium available for supplementation. Although numerous mature cow supplementation studies have included blood sampling of calves to determine associations between selenium status of the dam and calf, these studies did not characterize the impact of selenium concentration on neonatal calf health and growth. In this study, it was demonstrated that selenium and vitamin E supplementation at birth can have a positive impact on pre-weaning health, with reduced odds of the supplemented calf being treated for diarrhea and testing positive for rotavirus infection. Thus, improving selenium status whether through injection of selenium or through improving selenium status of dams could lead to improved preweaning health in calves.

There are some limitations to consider in this study. The amount of selenium being supplemented to the mature dry cow group and the selenium levels of the cows were not controlled for in the analysis. Bias could have been introduced as selenium concentration in beef cows has been shown to be correlated with selenium concentration in calves.[42] However, as the calves were randomly assigned within farms, these biases would have been distributed throughout both treatment groups, minimizing its effect. The effect of time since injection and age at sampling on selenium concentrations were completely confounded, and it could not be determined what impacted selenium status. Thus, additional research is required to determine whether selenium declines due to age, time from selenium supplementation, or both. An additional limitation was the inability to evaluate the effect of vitamin E supplementation. Although the amount of vitamin E that was supplemented was low, vitamin E could have also contributed to the effect that was found within this study.

In this study, selenium determination was performed on serum collected from calves aged 1 to 8 d. While some have suggested that whole blood selenium might be preferred to measure selenium concentration,[18] others have demonstrated that serum selenium is more accurate than whole blood in measuring the short-term changes in selenium status.[7] As we were interested in evaluating the effect of a selenium injection in a short period and due to ease of collection, a serum sample was used in this study. University of Guelph Animal Health Laboratory uses a reference interval of 0.08 to 0.15 µg/mL for serum selenium concentrations in all cattle, regardless of age. However, when this reference interval is applied to calves sampled on this study, very few calves fell within this range, with 95.2% of control calves and 59.8% of supplemented calves falling below the reference interval. Stowe and Herdt suggested for calves aged 1 to 9 d old, the expected serum selenium concentration was found to range between 0.05 to 0.07 µg/mL, with 28.2% of control calves and 4.6% of supplemented calves falling below the reference interval.[29] In fact, in supplemented calves, 40.2% had concentrations between 0.05 to 0.07 µg/mL, and 55.1% of calves had concentrations greater than 0.07 µg/mL. Given the inability for the young calves sampled in this study to meet a higher laboratory reference interval, even after treatment, it is reasonable to question whether or not this higher interval appropriately reflects selenium status relative to underlying requirements in neonatal calves.

Passive transfer in the current study was defined by a cut-point of 5.2 g/dL serum total solids. The observed rate of APT (79.1%) among study calves is similar to previous rates reported in North American dairy herds.[4,36] Kamada et al and Hall et al demonstrated an effect of selenium addition to colostrum on passive transfer of immunoglobulin.[10,14] However, in the current study, no association was found between injectable selenium/vitamin E treatment and passive transfer after controlled analysis. It is possible that the oral route of selenium supplementation is more effective for increasing immunoglobulin absorption.

Selenium treatment at birth had no effect on ADG over the study period. There is conflicting evidence on the effect of selenium supplementation on weight gain in calves with some studies demonstrating a positive effect[5,7,26,42] and others that have resulted in no effect.[15,17,22,33] Selenium is thought to influence growth through its effects on thyroid hormone metabolism. However, because of the known biological mechanism by which selenium influences thyroid hormone metabolism, the lack of an effect on ADG in the current study is likely due to basal selenium concentrations that are adequate for normal Type 1 iodothyronine 5'-deiodinase function.[2] Thus, supplementation likely had no effect on Type 1 iodothyronine 5'-deiodinase function.

C. parvum is a major pathogen involved in neonatal diarrhea in Ontario dairy calves. Trotz-Williams et al on dairy farms[37] and Spinato et al during necropsy,[28] detected *C. parvum* in 41% and 24% of calves tested, respectively, suggesting that the prevalence of *C. parvum* is likely quite high in Ontario dairy calves. In the current study, the prevalence of *C. parvum* in fecal samples was 36%. Despite the high prevalence of *C.*

parvum and its association with diarrhea in this study, there was no association detected between selenium and vitamin E treatment and *C. parvum* infection.

Rotavirus is also another major causative agent of neonatal calf diarrhea. The prevalence of rotavirus can also be high. For example, 18% of Dutch dairy calves,[3] 90% of dairy calves in California,[6] and 80% of scouring calves in Australia[13] had rotavirus detected in feces. In the current study, the prevalence of rotavirus was 16%, and the presence of rotavirus isolated in calf feces was associated with a fecal score of 3. Co-infection between rotavirus and *C. parvum* had a synergistic action on the incidence of diarrhea, which is similar to previous reports.[3] Calves receiving selenium and vitamin E at birth were less likely to have a positive fecal test for rotavirus. Selenium supplementation is known to increase killing efficiency of neutrophils by increasing glutathione peroxidase activity.[1,12,43] Thus, neutrophils in selenium and vitamin E-supplemented calves may have an increased ability to phagocytose and kill rotavirus upon exposure, preventing the virus from establishing infection and replicating in the small intestine.

Selenium and vitamin E treatment reduced the probability of a calf being treated for diarrhea during the study period. It is likely that selenium injection had a role in disease prevention through an increase in the supplemented calves' innate immune response.[32] Associations between selenium and vitamin E injection at birth and probability of treatment for diarrhea have also been previously reported.[7,40] In a very recent study of the associations between management practices and within-pen prevalence of calf diarrhea and respiratory disease on dairy farms using automated milk feeders, there was a lower within-pen prevalence of diarrhea when selenium and vitamin E were administered at birth.[21] In another observational project involving surveillance of neonatal calf disease in Ontario, an excessive incidence of muscular dystrophy and other signs of selenium deficiency have been recently reported.[9] However, in the current experiment, when all 185 treatment events were considered together, selenium and vitamin E injection at birth was not associated with the probability of a treatment event during the pre-weaning period. These results contrast several observational studies which reported associations between selenium and vitamin E injection at birth, and the probability of being treated during the pre-weaning period.[7,39,41]

Conclusions

In this study, a single injection of selenium and vitamin E at birth was found to increase serum selenium concentrations in calves sampled between 1 and 8 d of age. Serum selenium concentrations were highly variable, both between farms and within farm. Selenium and vitamin E injection at birth was found to reduce the number of calves treated for diarrhea over the pre-weaning period, and the number of calves that tested positive for rotavirus between 8 and 15 d of age. No association was found between selenium and vitamin E treatment and passive transfer, ADG, *C. parvum* infection, and the probability of treatment for illness during the pre-weaning period. In regions with selenium-deficient soil, injectable supplementation with selenium and vitamin E at birth has considerable potential as a cost-effective method to improve the health status of calves prior to weaning.

Endnotes

[a] Dystosel, Zoetis, CA
[b] BD Vacutainer Precision Glide, Becton Dickinson and Co., Franklin Lakes, NJ
[c] BD Vacutainer, Becton Dickinson and Co.
[d] KS – 0050, Kernco Instruments
[e] Nasco, Modesto, CA
[f] Bio K 248, Bio-X Diagnostics, Jemelle, Belgium
[g] Stata Corporation, College Station, TX
[h] Microsoft Corporation, Redmond, WA
[i] SAS Institute Inc., Cary, NC

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