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Clinical trial on type of calving pen and the risk of disease in Holstein calves during the first 90 d of life

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ABSTRACT

The objective of this study was to evaluate the efficacy of single cow calving pens that are cleaned between calvings vs. multiple cow calving pens for the prevention of calf diarrhea (scours), respiratory disease (pneumonia) and morbidity attributable to any cause.

Every other pregnant cow or heifer was moved to either the single cow calving pen (treatment) or the multiple cow calving pen (control) within 48–72 h prior to actual calving. The calves born in the single cow calving pens were assigned to the treatment group while the calves born in the multiple calving pens were assigned to the control group. Fecal materials, placental remains, and any other conspicuous dirt were removed from the single cow calving pens between each calving prior to the introduction of the next pregnant cow. The calves were then separated from their dams within 2 h of birth. Multiple cow calving pens were managed as usual at the producers' discretion. Upon birth, the calf managers monitored each enrolled calf for signs of diarrhea, pneumonia plus other morbidity up to 90 d of age. The effects of single cow calving pens (vs. multiple cow calving pens) that are cleaned between calvings on the risk of neonatal calf diseases were evaluated using multivariable logistic regression models. Risk of diarrhea (OR = 0.93, $P = 0.75$), pneumonia (OR = 1.23, $P = 0.64$), and morbidity due to any cause (OR = 0.93, $P = 0.74$) were not significantly different between calves born in single cow vs. multiple cow calving pens.

The current study found that, given the management situation evaluated, calves born in single cow calving pens were no different than calves born in multiple cow calving pens with respect to calf diseases risk. Long-term follow-up of the calves enrolled in the present study is ongoing to determine the efficacy of single cow calving pen use for the possible prevention of transmission of *Mycobacterium avium* subsp. *paratuberculosis* in Holstein calves.

1. Introduction

Economic losses attributable to calf diseases have been reported in the United States (US). A study undertaken in 43 California dairy herds found that calf diseases account for approximately 4% of the total cost of all diseases with diarrhea and pneumonia representing 86% of these costs (Sischo et al., 1990). Short-term losses due to increased calf mortality rates and the veterinary costs for treatment are well recognized (Sischo et al., 1990; Gardner et al., 1990).

To highlight the importance of the need to implement calf disease prevention and control programs, studies on long-term effects of morbidity on future health and performance have been undertaken. One finding of these studies is that heifers with a history of calf diseases are more likely to have a higher age at first calving compared to those that remained healthy as calves (Waltner-Toews et al., 1986a; Correa et al., 1988). Additionally, studies

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evaluating frequency (Waltner-Toews et al., 1986b; Curtis et al., 1988a; Wells et al., 1996; Sivula et al., 1996a) and risk factors (Curtis et al., 1993; Sivula et al., 1996b; Svensson et al., 2003, 2006) associated with the incidence of neonatal calf morbidity under field conditions have also been undertaken to identify specific farm and calf management risk factors, whose modification might promote raising healthier herd replacement heifers.

However, despite advances in knowledge of determinants of neonatal calf diseases, and concerted efforts towards prevention and control over the years, calf diarrhea and respiratory disease remain major causes of calf mortality on US dairy operations. In 2002, the National Animal Health Monitoring System (NAHMS) reported that approximately 8.7% of dairy heifers born alive died prior to weaning (NAHMS, 2002). Scours, diarrhea, and other digestive anomalies accounted for approximately 62.1% of all preweaned heifer mortality, while respiratory diseases were responsible for up to approximately 21.3% of all preweaned calf mortality (NAHMS, 2002).

The success of commercial dairies depends on a reliable supply of healthy replacement heifer calves with good genetic potential for milk production. Several management practices have been recommended to producers for reducing the frequency of calf morbidity and mortality on dairy farms. One area commonly emphasized is the calving pen. The management of calving pens influences the degree of early calf exposure to infectious environmental pathogens (Smith et al., 1989). The associations between calving site and incidence of calf morbidity have been previously identified in several studies. In one observational study of 25 New York herds, calves born in herds using calving pens were less likely to develop diarrhea than those born in herds using stanchions or loose housing areas for calving cows (Curtis et al., 1988b). Svensson et al. (2003) while studying the health of 3081 heifer calves from 122 dairy herds in southwest Sweden reported that calves born in herds using single cow calving pens were at lower risk of respiratory disease than those born in herds using cubicles or group calving pens. In Michigan herds with between 50 and 99 adult cows, the use of single cow calving pens and removal of bedding from these pens between calvings were associated with a lower incidence of diarrhea (Frank and Kaneene, 1993). Waltner-Toews et al. (1986c) reported that calves born in calving pens had a lower overall mortality rate compared to those born in unusual or unexpected sites on the farm. Consequently, single cow calving pens which are cleaned after each use have been recommended to farmers as a management tool with the potential for reducing early exposure of heifer calves to environmental pathogens (e.g. *Mycobacterium avium* subsp. *paratuberculosis* [MAP], *Escherichia coli, Salmonella* species) at the time of calving (Rossiter and Hansen, 2000). However, the effectiveness of this management tool with respect to calf disease prevention has not been evaluated using controlled field studies.

The aim of this study was to determine whether heifer calves born in single cow calving pens have a lower incidence of calf diseases than heifer calves born in multiple cow calving pens. The specific objectives were to evaluate the efficacy of individual or single cow calving pens that are cleaned between each calving vs. multiple cow calving pens for the possible prevention of (1) diarrhea or scours, (2) respiratory disease or pneumonia, and (3) overall morbidity attributable to any cause.

2. Materials and methods

This study was approved by the Institutional Animal Use and Care Committee at the University of Minnesota (Protocol Number 0406A1181).

2.1. Herd selection and description

The criteria for recruiting herds into the present study were (1) routine use of multiple cow calving pens as calving or maternity pens, (2) routine submission of production records to the Minnesota Dairy Records Processing Center, (3) willingness and interest to comply with the study protocols by the producer, and (4) proximity (within ≤160 km radius) to the College of Veterinary Medicine, University of Minnesota. A further criterion for herd inclusion was evidence for the endemic presence of Johne's disease (JD) in the herd. This evidence was initially ascertained based on a history of culling due to JD during the year prior to the start of the study and past serological test records for MAP, although herd screening testing to determine herd infection status was later undertaken. The requirement for JD infection was due to the fact that the long-term objective of this study was to evaluate the effect of single cow calving pens (vs. multiple cow calving pens) for prevention of transmission of MAP in Holstein calves. Prior to the start of this study, producers were asked to provide detailed information regarding their general calf management programs (e.g. nutrition, calfhood disease prevention measures and housing), average rolling herd milk production, herd size (number of lactating cows), and bulk tank somatic cell counts.

Three commercial dairy farms located in Eastern Minnesota satisfied the inclusion criteria and were conveniently selected to participate in this study. Table 1 shows a summary of characteristics of these herds. The size of participating herds ranged from 280 to 580 cows (median, 530 cows). Five percent of Minnesota dairy cattle herds fall within this range of herd-sizes (NASS, 2008). The rolling herd average milk production ranged between 9752 and 13,607 kg (median, 10,432 kg) while

Table 1
The general characteristics of three Minnesota dairy herds enrolled in a clinical trial for the effect of single cow calving pens (treatment) vs. multiple cow calving pens (control) on the risk of disease in Holstein calves in 2005.

	Herd 1	Herd 2	Herd 3
Herd size (*n*)	580	530	280
Number of calves enrolled (*n*)	229	157	63
Rolling herd average milk production (kg)	9,752	13,607	10,432
Average somatic cell count estimates (per mL)	230,000	180,000	230,000
Apparent MAP prevalence estimates (%)	16	10	3

bulk tank somatic cell counts ranged between 180,000 and 280,000 cells/mL (median, 230,000 cells/mL). The number of calves enrolled per herd ranged between 63 and 229 calves (median, 157 calves).

2.2. Calf management

Pregnant heifers and cows were vaccinated at approximately 3 months prior to the expected calving date to provide protection to the unborn calves against calf diarrhea using a multiple antigen vaccine (GUARDIAN®, Schering-Plough Animal Health Corp., Omaha, NE) which contained inactivated rotaviruses, coronaviruses, a bacterin-toxoid from *Clostridium perfringens* types C and D, and a free cell extract of *E. coli* in all study farms. Two of these herds (Herd 1 and Herd 3) raised their calves on the farm property while the third herd (Herd 2) transported calves at 1–3 d old to be raised offsite by a professional calf grower. Preweaning diets (up to 60 d of age) for all calves consisted of commercial milk replacer and free choice water and calf starter pellet. Calves were housed in calf hutches on the two farms (Herd 1 and Herd 3) that raised their calves on the farm property while barns with separated cubicles were used to house calves from the single farm that used the services of a professional heifer grower from birth until weaning (60 d of age). After weaning, all calves were transferred into loose houses with bedded pack.

2.3. Single and multiple cow calving pens

Prior to the study onset, the single cow calving pens were created within the existing multiple cow calving pens in each study herd by constructing a temporary barrier between the single cow calving pens and multiple cow calving pen area to prevent possible transfer of manure and other contaminants between the two calving locations. Each calving location was bedded with straw and adequate ventilation was provided by means of windows fitted with wire mesh and open entry and exit doors. The pens were shielded from cold winter drafts using polythene barriers fitted to the windows and by shutting the entry or exit doors to the calving pens. Water troughs in each calving location provided free source of fresh water for the cows.

2.4. Study design and treatment protocol

A clinical trial study design was used to evaluate the effect of single cow calving pens that are cleaned between each calving vs. multiple cow calving pens for the prevention of calf morbidity. The study unit was the calf and enrollment of calves in to the study was completed between January, 2005 and December, 2005. Each calf was monitored from birth up to 90 d of age for signs of diarrhea, respiratory disease and any other morbidity.

The intervention of interest was the improved hygiene within the single cow calving pens achieved through cleaning between calvings and presence of a single cow (and calf) at a time compared to the multiple cow calving pens which held several cows (and calves) at a given time. In the present study, every other pregnant cow or heifer was moved to either the single cow calving pens (treatment) or the multiple cow calving pens (control) within 48–72 h prior to actual calving. Every other calf born was assigned to the treatment group (single cow calving pens) while remaining calves were assigned to the control group (multiple cow calving pens). Upon birth, navels were disinfected for the majority of calves and all calves were bottle fed approximately 3.8 L of raw colostrum collected from their respective dams.

Feces, placental remains, and any other conspicuous dirty materials were removed from the single cow calving pens between each calving and prior to the introduction of the next about-to-calf cow or heifer. The calves were separated from their dams within 2 h of birth. The used bedding straw was completed replaced with fresh straw after 1 month of use in the single cow calving pens. The producers maintained their usual routine for cleaning the multiple cow calving pens. There was variation in frequency of cleaning the multiple cow calving pens across the 3 herds as follows: 1 herd (Herd 2) reported a once a month cleaning routine, a second herd (Herd 3) reported cleaning the multiple cow calving pens once every 6 months, while the third herd (Herd 1) confirmed cleaning their multiple cow calving pens once every year during the summer season.

2.5. Data collection

For each enrolled calf, the cow ID, calf ID, date of birth, sex of calves (although only heifer calves were included in the analysis), number of calves born (singleton or twins), whether or not the calving process needed to be assisted, calving pen (individual or multiple cow calving pen) in which each calf was born, and time to, method of feeding (all bottle fed), and volume of the first colostral meal fed to each calf plus time to separation of calves from the adult cows after birth were recorded.

2.5.1. Disease monitoring

Disease events were monitored by one manager per farm who recorded cases of diarrhea, pneumonia, and navel or joint illness, frequency of treatment, and whether the affected calf died or was successfully weaned at the end of the observation period (approximately 90 d of age). Each calf manager was blinded to the group (treatment vs. control) to which the calves were assigned at time of enrollment. Prior to the study onset, the calf mangers received standard training and definitions of each calf disease event of interest thus reducing the possibility of subjective assessments.

Disease monitoring involved checking each enrolled calf at the time of feeding for the following signs: depression, decreased appetite, abnormal fecal consistency, increased respiratory rate, cough, nasal and ocular discharges plus other abnormal appearances. When deemed necessary, the joints were evaluated for heat, swelling and abnormal gait, and navels were assessed for swellings or discharges. A calf displaying one or more of these signs was further examined by taking rectal temperature measurements to determine whether they were hyperthermic additional to complete assessment of fecal consistency and hydration status.

For the purpose of this study, a given disease event was recorded only once for each calf although some calves experienced multiple episodes of the same disease. Each calf was monitored from birth until 90 d of age for signs of disease at which point all calves were excluded from further observation. Some calves prematurely left their respective herds prior to attaining 90 d of age due to death. No treatment protocols were developed for this study and each herd relied on their regular herd veterinarian for advice on treatment decisions for the sick calves. Though no confirmatory calf disease diagnoses were made by a veterinarian, the author validated the calf manager recorded diagnoses using the veterinarians' treatment records upon visiting the farms. Farms were visited biweekly upon which calf enrollment sheets and records of calf morbidity were collected.

2.5.2. Disease outcome definitions

The following working definitions were used to enhance accuracy and uniformity of diagnosis across study farms: (1) diarrhea was reported in calves that voided abnormal feces with watery consistency, with or without dehydration or elevated body temperature ($\geq$40 °C), (2) pneumonia was reported in a calf that exhibited increased respiratory rate, nasal discharges, and cough with or without an elevated body temperature ($\geq$40 °C), and (3) joints and navel infections were reported in calves with associated signs of inflammation of joints or the umbilicus (heat, swelling, discharges and abnormal gait).

2.6. Statistical analysis

Data analysis was implemented using standard software (Stata® Corp., College Station, TX, USA) and values of $P \leq 0.05$ were considered statistically significant.

2.6.1. Descriptive analysis

The number of calves enrolled, calf disease incidence, mean number of treatment days for sick calves, proportion of assisted calving events, lactation number for the dams, birth season distribution, proportion of navel disinfections after birth, number of calves alive by 90 d of observation, mean time to feeding first colostrum, mean volume of colostrum fed, and mean time to physical separation of calves from their respective dams were summarized. Univariate associations between the preceding variables and the treatment (vs. control) variable were evaluated using *t*-tests for continuous variables and Pearson χ^2-test for categorical variables, respectively.

2.6.2. Multivariable analysis

The effect of the intervention (single cow calving pen [treatment] vs. multiple cow calving pens [control]) on number of calves diagnosed with diarrhea, pneumonia, and morbidity attributable to any cause was analyzed using three separate logistic regression models, respectively. Birth season (winter [November 01–April 30] vs. summer [May 01–October 31]), lactation number for the dams (1 vs. $\geq$2), and whether a calf was born via assisted calving due to apparent dystocia (yes/no) were screened for their possible association with each outcome variable using logistic regression models that included herd as fixed effect variable. The variables that were significantly associated ($P \leq 0.05$) with each outcome were selected for inclusion in the final models. A backward selection procedure was used to build the final models for each outcome, with a *P*-value for retention of a variable being $\leq$0.05. The intervention (single cow calving pen [treatment] vs. multiple cow calving pens [control]) variable was forced to remain in each model and herd was included in each model as a fixed effect variable. Two-way interactions for biologically plausible combinations of variables was tested and retained in the models if they satisfied a cut point of $P \leq 0.05$. Model fit to the data was tested using Hosmer–Lemeshow Goodness-of-fit test (GOF) (Hosmer and Lemeshow, 1989).

2.6.3. Power analysis

In this study, we tested the null hypothesis that calves born in single cow calving pens that are cleaned between every calving were at no greater risk of experiencing a calfhood morbidity event than calves born in multiple cow calving pens. The sample size needed to test this hypothesis was not determined *a priori* since the study was originally designed to compare the risk of MAP infection among calves born in clean single cow calving pens vs. multiple cow calving pens. Therefore a *post hoc* power analysis was conducted to determine if the sample of calves enrolled provided the current study with sufficient statistical power to enable the rejection of the null hypothesis or to mitigate a type II error problem. The parameters for this analysis were estimated from the current data and included the number of calves enrolled in the treatment and control groups plus the proportion of overall morbidity observed in the treatment and control groups, respectively.

3. Results

3.1. Descriptive summary

Between January and December, 2005, a total of 449 calves were enrolled in this study. Two hundred and thirty eight (53%) of these calves were born in single cow calving pens (treatment) while 211 (47%) calves were born in multiple cow calving pens (controls). The slight over enrollment in the treatment group was due to the fact that the single cow calving pen was occasionally used for cows that needed assistance at calving in 1 herd (Herd 2) at the onset of study implementation.

The incidence of calf diseases recorded up to 90 d of age and the distribution of other variables between groups is presented (Table 2). The mean $\pm$ SD treatment days for sick calves, mean $\pm$ SD time to feeding the first colostral meal (h), mean $\pm$ SD volume of colostrum fed to each calf after birth (L), mean $\pm$ SD time to physical separation of each calf from their respective dams (h), proportion of calves remaining alive within 90 d of observation, and proportion of calves

Table 2
The comparison of calf disease events, and the distribution of other variables in a clinical trial for the effect of single cow calving pens (treatment) vs. multiple cow calving pens (control) on the risk disease in Holstein heifer calves born on three Minnesota dairy farms in 2005.

Variable	Group		*P* value
	Treatment	Control	
Number of calves enrolled (*n*)	238	211	–
Calf disease incidence			
Diarrhea, *n* (%)	78 (33)	64 (30)	0.58
Pneumonia, *n* (%)	13 (5)	14 (7)	0.60
All cause morbidity, *n* (%)	91(38)	79 (37)	0.86
Mean $\pm$ SD number of days sick calves treated (d)	2.3 ± 0.66	2.3 ± 0.71	0.81
Assisted calving			
Yes, *n* (%)	92 (39)	37 (18)	<0.001
No, *n* (%)	146 (61)	174 (82)	–
Lactation			
1, *n* (%)	92 (39)	58 (27)	0.012
$\geq$2, *n* (%)	146 (61)	153 (73)	–
Birth season			
Winter, *n* (%)	102 (43)	81 (38)	0.34
Summer, *n* (%)	136 (57)	130 (62)	–
Navel disinfection			
Yes, *n* (%)	206 (87)	180 (85)	0.7
No, *n* (%)	32 (13)	31 (15)	–
Calf deaths within 90 d of study			
Yes, *n* (%)	4 (2)	1 (1)	–
No, *n* (%)	234 (98)	210 (99)	0.38[a]
Mean $\pm$ SD time-to-feeding first colostrum (h)	6 ± 0.02	6 ± 0.2	0.31
Mean $\pm$ SD volume of colostrum fed per calf (L)	4 ± 0.02	4 ± 0.0	0.35
Mean $\pm$ SD time-to-separation from dam (h)	1.72 ± 0.05	1.85 ± 0.1	0.09

[a] *P* value based on Fisher's exact test.

born in winter or summer were not significantly different between groups (treatment vs. control). However the proportion of calves born via assisted calving was significantly ($P < 0.001$) higher in the treatment vs. control group. This was expected since 1 herd (Herd 2) occasionally moved cows needing assistance at calving to the single cow calving pen during the early phase of the study.

The cumulative incidence of diarrhea was 32% (142/449), 33% (78/238) in the treatment vs. 30% (64/211) in the control group, respectively. Pneumonia was reported in 6% (27/449) of all calves, 5% (12/238) of these were in the treatment group vs. 7% (14/211) in the control group, respectively. When morbidity attributable to any cause was considered as the outcome of interest, the cumulative incidence was 39% (170/449), 38% (91/238) in the treatment vs. 37% (79/211) in control group, respectively.

3.2. Multivariable regression model analysis

The results from the logistic regression models detailing the effect of the intervention on risk of neonatal calf diarrhea, pneumonia, and overall morbidity is presented (Table 3). Except for the diarrhea outcome model (GOF test, $\chi^2 = 23.35$, $P < 0.0014$), the GOF analyses suggested that the pneumonia and overall morbidity outcome models fit the data reasonably well. The treatment showed a protective trend against diarrhea (OR = 0.93, $P = 0.75$) and overall morbidity (OR = 0.93, $P = 0.74$) but these effects were not significant (Table 3). However, the calves in the treatment group had 0.23-fold increase in the risk of pneumonia in comparison to controls though this apparent increase in risk of pneumonia was not significant (OR = 1.23, $P = 0.64$).

3.3. Power analysis

This analysis revealed that samples of these sizes (treatment [$n = 238$] vs. control [$n = 211$] provided the current study with a statistical power ≥ 0.83 to allow the rejection of the null hypothesis at $P \leq 0.05$ had the true biological difference in probability of a calf morbidity event in the population of calves born in single calving and multiple cow calving pens been $\geq$0.13.

4. Discussion

To the authors' knowledge, this is the first study performed under field conditions to evaluate the effect of using single cow calving pens (vs. multiple cow calving pens) for possible prevention of diseases in calves. The calves born in single cow calving pens in the present study were expected to experience less calf disease events. The rationale being the improved hygiene and lack of overcrowding of cows (and calves) within the single cow calving pens in comparison to the multiple cow calving pens, thus limiting degree of exposure to potential pathogens in the former group. There was however no significant difference in risk of calf diseases among calves born in single cow calving pens that are cleaned between uses and calves born in multiple cow calving pens. The

Table 3
Final logistic-regression models in a clinical trial of the effect of single cow calving pens (treatment) vs. multiple cow calving pens (control) on the risk of morbidity, diarrhea, and pneumonia in Holstein heifer calves born on three Minnesota dairy farms in 2005.

Effect of intervention	Level	Odds ratio	95% Confidence interval of odds ratio		P value
			Lower	Upper	
Diarrhea[a]					
Single cow calving pen	Yes	0.93	0.57	1.47	0.75
	No	Baseline	–	–	–
Birth season	Winter	0.59	0.37	0.94	0.03
	Summer	Baseline	–	–	–
Herd	1	Baseline	–	–	–
	2	45.5	16.1	128	<0.001
	3	17.6	5.7	54.4	<0.001
Pneumonia[b]					
Single cow calving pen	Yes	1.23	0.52	2.91	0.64
	No	Baseline	–	–	–
Lactation	1	0.29	0.09	0.91	0.03
	≥2	Baseline	–	–	–
Morbidity due to any cause[c]					
Single cow calving pen	Yes	0.93	0.63	1.40	0.74
	No	Baseline	–	–	–
Herd	1	Baseline	–	–	–
	2	4.18	2.61	6.67	<0.001
	3	1.82	0.94	3.51	0.07

[a] Lactation, calving difficulty, and two-way interactions were not significant and were consequently excluded from this model. The overall significance of herd variables based on likelihood ratio test (LR test) was $P < 0.0001$. Birth of a calf in winter was significantly protective against diarrhea after adjusting for the treatment and herd effects, respectively.

[b] Birth season, calving difficulty, and two-way interactions were not significant and were consequently excluded from this model. Note that records from two farms were excluded from this model because pneumonia outcomes were recorded in a single herd (Herd 1). In this herd (Herd 1) calves born to first lactation cows were significantly less likely to suffer an event of pneumonia after adjusting for the treatment and herd effects, respectively.

[c] Lactation, calving difficulty, season of birth, and two-way interactions were not significant and were consequently excluded from this model. The overall significance of herd based on LR test was $P < 0.0001$.

results of the present study must be interpreted cautiously because of lack of corroborative data since no studies had previously attempted to address similar questions using the study design employed in the present study.

Nonetheless the possible reasons for failure to detect a significant treatment effect in the present study could include the fact that the magnitude of the difference in incidence of calf morbidity between treatment and control groups in the present study was biologically negligible (difference $\leq$0.01) to be statistically significant ($P \leq 0.05$). The *post hoc* power analysis conducted in the present study although less appealing from a methodological viewpoint, revealed that samples of these size (treatment [n = 238] vs. control [n = 211] was adequate to enable the rejection of the null hypothesis at $P \leq 0.05$ with a statistical power $\geq$0.83 had the true population difference in probability of experiencing a calf morbidity event between the treatment and control groups been $\geq$0.13 and assuming the probability of experiencing a morbidity event among control calves was the same as that estimated in the current study.

It is possible that other herd and calf management practices in place on study farms may have overshadowed any effect of single cow calving pens for the prevention of calf diseases (e.g. housing from birth to weaning, sanitation, nutrition, colostrum management). In the present study, all herds housed calves in calf hutches until weaning. In addition all pregnant heifers and cows were vaccinated 3 months prior to actual calving to protect the yet to be born calves against calf diarrhea using a multiple antigen vaccine (GUARDIAN®, Schering-Plough Animal Health Corp., Omaha, NE). The effective control of calf diseases is a multifactorial function of several factors including the reduction of degree of exposure of newborn calves to putative risk factors (infectious agents inclusive), increasing levels of specific and nonspecific immunity resulting from provision of adequate colostrum, good nutrition, minimizing stressors and increasing specific resistance by vaccinating either the dams or newborn calves against specific pathogens (Radostits et al., 1994; Davis and Drackley, 1998). The calves in the present study were bottle fed approximately 3.8 L of raw colostrum collected from their respective dams within the first 6 h of birth. Since the farms in the present study already had many of these management practices in place to reduce exposure of calves to risk factors of disease and to enhance specific resistance to calf diseases (diarrhea or scours), it is not surprising that a possible effect of single cow calving pens if present was less obvious.

The complete removal of bedding straw from the single cow calving pens once every month as was the routine in the present study may have resulted in the slow accumulation of dirty materials inside these sites over time thereby compromising hygiene of the single cow calving pens. Consequently, calves born in single cow calving pens one week prior to removal of bedding straw may have had a similar risk of exposure to environmental pathogens as those born in the multiple cow calving pens with compromised hygiene conditions. It is also possible that calves born in the single cow calving pens one week prior to removal of bedding may have had greater risk of exposure to environmental pathogens than those born in the first week of the month following complete replacement of bedding material. These may have biased study findings towards the null although the authors were unable to verify the existence of this potentially serious source of bias. It should be noted that although in the authors' opinion, the ideal single cow calving pen management practice after each calving should involve complete removal of feces, placental remains, and bedding materials from the calving site, followed by disinfection of the floors with an appropriate disinfectant and placing fresh bedding material prior to introducing the next pregnant heifer or cow, this ideal program is less likely to be popular among many producers because of the potential costs (cost of bedding straw and labor for cleansing of the pens) associated with its implementation. The single cow calving pen cleaning protocol used in this study seem pragmatic and potentially adoptable by many producers despite the fact that once in a month removal of bedding has the potential of compromising hygiene of the single cow calving pens in the long run.

Finally it might be true that there really is little to no added protection provided by single cow calving pens against neonatal calf diseases in herds with management systems similar to those included in the present study.

Though a major strength of the present study was its design (i.e. being a clinical trial) that made it possible to potentially make casual inferences based on the current data, there were also a number of limitations. For example, while all calves in this study were bottle fed approximately 3.8 L of raw colostrum collected from their respective dams within the first 6 h of birth, the investigators did not collect blood samples from each enrolled calf to evaluate the passive transfer of serum IgG and total proteins between treatment and control groups of calves. It seems reasonable to hypothesize that calves born in single cow calving pens might experience lower failure of passive transfer rates (FPT) (FPT = a calf serum IgG level $<$10 g/L within 24 h of birth) in comparison to calves born in multiple cow calving pens, as the former group may have had less opportunity to potentially suckle alien cows that may have already been suckled, thus offering colostrum of inferior quality, although the converse might also be true. Another possible limitation of the current study is the fact that diagnosis of calf disease events by the calf mangers may have introduced misclassification of disease status bias, effectively driving the treatment effect estimates towards the null. The authors are however confident that this potential bias was limited because the calf managers were masked to the group (treatment vs. control) to which the calves were assigned at time of enrollment. Additionally calf mangers were trained about the calf diseases of interest prior to the study onset, thus reducing the possibility of subjective assessments of the outcomes. Though no confirmatory diagnoses by a herd veterinarian was required for the purpose of this study, the author validated the farmer recorded diagnoses using the veterinarians' treatment records at each biweekly farm visit. Other limitations included the fact that we were unable to evaluate the effect of treatment (single cows calving pens vs. multiple cow calving pens) on other outcomes such as number of treatments or sick calf days (surrogate for severity of disease), mortality, growth rates and possible still birth incidence.

In spite of the above limitations, findings of the present study suggests that dairy herds with adequate herd and calf management programs may not necessarily need single cow calving pens for the purpose of calf health improvement during the preweaning period. It is possible that the effect of single cow calving pens on calf health could be different in dairy herds using other calving location management systems. The findings reported here should not be construed to suggest an impetus for discouraging the use of single cow calving pens on commercial dairy operations since they may provide longer term benefits for the prevention of other diseases of economic importance to the dairy industry (e.g. MAP).

5. Conclusions

In the present study, we evaluated the possible efficacy of single cow calving pens for preventing neonatal calf diseases. Utilizing single cow calving pens that are cleaned between uses did not provide added protection to calves against calf diseases. Husbandry practices other than maternity pen management could have been relatively more important determinants of preweaning health than use of single cow calving pens. While it might be true that there really is little to no added protection provided by single cow calving pens against neonatal calf diseases, cautious interpretation of the current results is in order due to lack of corroborative data since no studies had previously attempted to address similar questions using the study design employed in the present study. These findings are inconclusive. Future studies that take into consideration the limitations outlined above are recommended. Additionally, the effect of treatment (single cows calving pens vs. multiple cow calving pens) on other outcomes including FPT rates, number of treatments or sick calf days, mortality, growth rates, and still birth risks should be explored in future studies. In spite of these findings, the authors do not recommend discontinuing the use of single cow calving pens in herds that are currently utilizing them since calves born in multiple cow calving pens might still be at greater risk of acquiring other infections (e.g. MAP). The long-term follow-up of the calves enrolled in the present study is ongoing to determine the efficacy of single cow calving pen use for prevention of MAP transmission.

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