The influence of 3 different navel dips on calf health, growth performance, and umbilical infection assessed by clinical and ultrasonographic examination

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ABSTRACT

The objectives were to investigate the effect of 3 navel dips on (1) umbilical infection, (2) health events (pneumonia, diarrhea, and arthritis), and (3) average daily gain (ADG) in newborn dairy calves. A secondary aim was to compare the agreement of standardized ultrasonographic examination with clinical examination for the diagnosis of umbilical infection. In a randomized block design, newborn calves were assigned by birth order to 3 treatment groups: Navel Guard (NG; SCG-Solutions Inc., McDonough, GA), 7% iodine tincture (SI), and 2% chlorhexidine gluconate (CH). Treatment consisted of a single dip administration of the umbilicus immediately after removal of the newborn from the calving pen with 1 of the 3 navel dips. Weekly clinical examinations were carried out during the first 4 wk of life with special attention being paid to the umbilicus, joints, respiratory tract, and fecal consistency, and included ultrasonographic evaluation of the umbilical structures. Body weight was assessed by using a girth tape at first and last evaluation. Multivariable logistic regression demonstrated no statistical differences in umbilical infection or health events. Multivariable linear regression analysis showed statistical differences in ADG (least squares means $\pm$ standard errors) between groups, with 494 ± 29 , 571 ± 29 , and 516 ± 29 g/d in groups NG, SI, and CH, respectively. Overall mortality during the study period was 9.5% ($n = 40$). Postmortem examination identified diarrhea (80%) as the main disorder with 19, 4, and 9 calves in groups NG, SI, and CH, respectively. Kappa values yielded fair [0.30 (95% CI: -0.03 – 0.63)], good [0.61 (95% CI 0.46 – 0.75)], moderate [0.53 (95% CI 0.31 – 0.74)], and moderate [0.49 (95% CI 0.19 – 0.79)] agreement for detection of omphalitis between clinical and ultrasonographic evaluation in wk 1, 2, 3, and 4, respectively. Agreement was very good for detection of omphalophlebitis, with kappa

values (95% CI) of 0.91 (0.80–1.00), 0.87 (0.75–0.98), and 0.90 (0.76–1.00) in wk 2, 3, and 4, respectively, when omphalophlebitis was diagnosed. We detected no difference in the effectiveness of the 3 treatments in the prevention of umbilical infection, pneumonia, diarrhea, or arthritis.

Key words: calf, omphalitis, ultrasonography, umbilicus

INTRODUCTION

Umbilical infection is one of the most common diseases in newborn calves, occurring in 1.3% (Svensson et al., 2003) to 29.9% of newborn calves (Hathaway et al., 1993) and it is detrimental to the general condition and health of the calf (Mee, 2008). Besides the local infection and inflammation, bacteria can spread by hematogenous dissemination into joints, lungs, kidneys, and other organs, causing severe complications, decreased growth rate (Virtala et al., 1996a), and increased mortality (Donovan et al., 1998b).

Preventive measures for umbilical infection encompass maternity pen hygiene, decreased residency time of the newborn calf in the maternity pen, adequate colostrum management, and antiseptic umbilical cord care (Mee, 2008). For decades, 7% iodine tincture has been recommended as an antiseptic for umbilical cord care (Hadley, 1954) and is still considered the gold standard (Grover and Godden, 2011; Robinson et al., 2015). However, severe restrictions regarding the use of 7% iodine solutions were implemented in the United States by the Drug Enforcement Agency in 2007 (Department of Justice, 2007), limiting its availability. Consequently, the demand for alternative antiseptics for umbilical cord care by the cattle industry has increased (Robinson et al., 2015). Chlorhexidine is considered an alternative antiseptic agent with a wide spectrum and long duration of antimicrobial activity and high efficacy in the presence of organic matter (Mee, 2008; Robinson et al., 2015). Chlorhexidine had superior effects on calf survival compared with iodine for umbilical cord care in one epidemiological study (Waltner-Toews et al.,

Received June 23, 2016.

Accepted September 3, 2016.

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1986b), and thus has been recommended over iodine tincture by some researchers (Waltner-Toews et al., 1986a; Mee, 2008). Navel Guard (SCG-Solutions Inc., McDonough, GA) is a commercially available antiseptic solution for navel dipping that was recently evaluated for the prevention of umbilical infection by Grover and Godden (2011), who found a numerical but not statistically significant decrease in the incidence of umbilical infection compared with 7% iodine tincture ($n = 16$, 10.9% compared with $n = 24$, 16.4%).

Group housing and ad libitum feeding of preweaned calves is increasingly practiced as a method of calf rearing. Because little is known about the incidence of umbilical infection in this setting, and recommendations for preventive measures such as choice of navel dipping solution are lacking, the primary objective of our study was to investigate the influence of the 3 described navel dips on the incidence of umbilical infections in a group-housing setting. Although several authors have studied the effect of different navel dips in individual housing systems (Grover and Godden, 2011; Robinson et al., 2015), this is the first randomized controlled trial to investigate the effect of different navel dips in group-housed calves.

Umbilical cord care has been shown to reduce the risk of arthritis (Bennett and Jasper, 1978) and respiratory disease (Perez et al., 1990) in previous studies. Moreover, umbilical infection was shown to reduce ADG (Virtala et al., 1996a), whereas little information is available about the effect of umbilical cord care on the incidence of newborn calf diarrhea. Hence, our secondary objective was to investigate the influence of umbilical cord care on the incidence of pneumonia, diarrhea, and arthritis and on ADG in newborn calves.

Several observational studies on calves at slaughter indicate that most umbilical infections affect the intraabdominal umbilical structures (umbilical vein, umbilical arteries, urachus) as well as the extraabdominal umbilicus (Curtis, 1970; Hathaway et al., 1993; Biss et al., 1994). Despite this knowledge, routine examination of the intraabdominal structures has not been included in field studies (Virtala et al., 1996a; Grover and Godden, 2011; Robinson et al., 2015). Whereas ultrasonographic examination has gained importance as a diagnostic aid in the assessment of umbilical disorders in hospital settings (Lischer and Steiner, 1994; Staller et al., 1995; Steiner and Lejeune, 2009), no technique applicable under field conditions has been described. Therefore, the third objective of our study was to describe a standardized procedure for ultrasonographic evaluation of the umbilical structures using an ultrasound device that is commonly used in the field, and to compare the agreement of ultrasonographic ex-

amination with clinical examination for the diagnosis of umbilical infection.

MATERIALS AND METHODS

All procedures were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2014-0093). This randomized controlled trial was conducted between December 2014 and June 2015 on a commercial dairy farm milking 1,500 cows, located near Ithaca in New York State.

Animals and Treatments

All female newborn calves born to heifers and cows, including female twins, were eligible for enrollment. Calves ($n = 423$) were separated from their dams immediately after birth and moved into a segregated pen with straw bedding. Colostrum was harvested 3 times a day and stored in a refrigerator until use. Before administration, it was warmed in a water bath (35°C) for approximately 30 min. All calves received 3.8 L of raw, fresh colostrum via an esophageal feeding tube within the first 3 h of life. Newborn calves were moved twice daily to the adjacent calf barn and housed in groups of approximately 15 animals in pens with straw bedding on a deep gravel base. After administration of colostrum, all calves were fed acidified whole milk ad libitum offered in a custom-made feeding system with 5 nipples per group. Hospital milk was harvested 3 times daily and stored in a mobile stainless steel tank and refrigerated at 5°C until pasteurization at 72°C for 15 s (Terminator, Goodnature Products, Buffalo, NY). Subsequently, the pasteurized milk was cooled by using an integrated plate cooling system to 21°C, stored in a mobile polyethylene tank (PolyDome Portable Milk Master, Litchfield, MN) until distribution to the feeding system, and acidified with an organic acid until a pH of 4.0 to 4.5 was reached. Water and calf starter were available ad libitum.

Calves were assigned to 1 of 3 treatment groups following a randomized block design, blocking on birth order in groups of 3 animals. Allocation within block was performed following the sequence of a random number generator (Urbaniak and Plous, 2014). Treatment was administered by the herd personnel as a single immersion of the umbilicus in 60 mL of Navel Guard (group **NG**; SCG-Solutions Inc.); 7% iodine tincture (group **SI**; VetOne, Boise, ID); or 2% chlorhexidine solution (group **CH**; VetOne) immediately after removal of the newborn calf from the calving pen. The respective navel dips were prefilled into single-use plastic containers (VWR International, Radnor, PA) in the laboratory

to avoid contamination when handling the solutions at the farm. To ensure complete exposure of the entire umbilical cord, the container was agitated while pressing the edge of the container gently to the adjacent skin around the base of the navel. The individuals who administered the treatments were not responsible for assessing outcomes.

Calving ease on a scale from 1 to 5 (1 = no assistance; 2 = moderate assistance, provided by farm staff; 3 = moderate assistance, but veterinarian called as a precaution; 4 = difficult calving, with extraction done by skilled farm staff; 5 = very difficult calving, with maximum veterinary assistance) was recorded by the herd personnel attending each calving. Birth date was recorded and stratified into 2 seasons (winter = calves born between December 1 and March 20, spring = calves born between March 21 and June 30) for subsequent analysis.

A sample of each batch of navel dip was taken before distribution into the individual containers for bacterial culture to detect possible contamination and for quality control. Samples were spread on blood agar (trypticase soy agar 5% sheep blood + 0.1% esculin agar; bioMérieux Inc., Durham, NC), incubated at 37°C for 48 h, and evaluated for growth of bacterial colonies.

Ultrasonography and Clinical Examination

All investigations were carried out by one trained veterinarian who visited the farm at least twice a week. Calves were first examined between 2 and 5 d of age and weekly thereafter for the 4-wk period. Complete clinical examinations of each calf were done once per week during the 4-wk study period, with special attention being paid to the umbilicus, joints, respiratory tract, and consistency of feces. Rectal temperature was measured with a digital thermometer (VetOne).

Clinical evaluation of the umbilicus included inspection of the umbilical cord (amniotic remnant) for assessment of wetness; diameter of the umbilical stalk; presence or absence of a fistula; and presence, quality, and degree of discharge; as well as pain response. For the remainder of this report, the umbilical stalk will refer to the extraabdominal part of the umbilical structures surrounded by subcutaneous tissue and skin, with a dorsal margin at the internal umbilical ring at the abdominal wall and a ventral margin at the external umbilical ring at the insertion of the umbilical cord. For assessment of pain response, the umbilical stalk was placed between the fingers of one hand. A firm squeeze was applied and the calf was observed for any pain response (e.g., flinch or kicking). For comparison of pain reaction, a similar squeeze of the same intensity

was applied to a skin fold at a defined position, 10 cm to the right of the umbilicus, before and immediately after squeezing the umbilical stalk. The diameter of the umbilical stalk was palpated and compared with a selection of wooden dowels with diameters from 0.5 to 4.5 cm in 0.5-cm increments. In addition, the presence and size of a hernial ring was assessed. Intraabdominal umbilical structures were evaluated by placing both hands on the abdominal wall of the standing animal to palpate the umbilical vein and caudally localized intraabdominal umbilical structures (urachus and umbilical arteries) by applying pressure to the body wall and tracing the anatomical structure along the entire length if possible.

A standardized scoring chart was used for the assessment of the respiratory tract as described by Poulsen and McGuirk (2009). Fecal consistency was evaluated as 1 of 4 categories (firm, pasty, soupy, watery). All accessible joints (stifle, tarsus, fetlocks, elbow, pastern, and carpal joints) were palpated and evaluated for effusion, heat, and pain response. In addition, presence of lameness on a level concrete surface was documented. Body weight was estimated at the time of the first and last evaluation with a heart girth tape (The Coburn Company Inc., Whitewater, WI).

The extra- and intraabdominal umbilical structures were subjected to weekly ultrasonographic examinations with a portable ultrasound device (IbexPro, E. I. Medical Imaging, Loveland, CO) and a 6.5- to 10-MHz linear array transducer (L8.0; E. I. Medical Imaging). For preparation of ultrasonographic examination, the area around the umbilicus between the xiphoid and the pelvis was clipped and ultrasound gel (Aurora Pharmaceutical, Northfield, MN) was used as a coupling medium. The extraabdominal umbilicus was evaluated at 2 defined positions, at the distal end (**P1**) and the base (**P2**) of the umbilicus measuring the long and cross-sectional diameter, respectively. The umbilical vein was assessed at 2 defined cross sections, immediately cranial to the inner umbilical ring (**P3**) and halfway between the umbilical ring and the liver (**P4**). The urachus was assessed adjacent to its junction with the bladder apex (**P5**), and the umbilical arteries were measured either at P5 or more caudally, on the lateral side of the urinary bladder (**P6**) if one or both arteries could not be visualized at P5 (Figure 1). For each position, horizontal and vertical cross-sectional diameters were recorded. The final diameters of the umbilical structures were assessed as follows:

$$\text{Diameter} = [(\text{cross/horizontal diameter} + \text{long/vertical diameter})/2].$$

Case Definitions

An umbilical hernia was diagnosed when a hernial ring of any size was detected, or a protrusion of abdominal organs through the abdominal wall was identified by ultrasound or palpation. The case definition for an umbilical infection was met when one or more of the following criteria assessed by clinical or ultrasonographic examination were present at least once: positive pain response, presence of a fistula with suppurative discharge, diameter of the umbilical stalk larger than 25 mm, diameter of the umbilical stalk increased compared with a prior evaluation (≥ 10 mm), urachus of calves older than 2 d with a diameter of more than 10 mm (urachitis), diameter of an umbilical artery exceeding 15 mm (omphaloarteriitis), diameter of the umbilical vein larger than 25 mm and hyperechoic lumen (omphalophlebitis) or firm in consistency. These cut-off values were based on previously published research (Lischer and Steiner, 1993; Grover and Godden, 2011). The case definition for pneumonia was a score of 5 or greater on the respiratory health score (Poulsen and McGuirk, 2009). The case definition of diarrhea was presence of soupy or watery fecal consistency, and definition of arthritis was inflammation of one or more joints. For all health events (umbilical infection, umbilical hernia, pneumonia, diarrhea, and arthritis), only the first event of each disease was considered for subsequent analysis.

Blood samples (10 mL) were collected from the jugular vein by venipuncture with a 20-gauge needle (1.2 $\times$ 40 mm) into evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) at the time of the first evaluation. Blood samples were transported refrigerated to the laboratory, and centrifuged within 6 h at $1,800 \times g$ for 10 min at 25°C. Serum was separated and analysis of Brix % was performed with a standard digital refractometer (PA201X, Misco, Cleveland, OH). Field necropsy of all calves that died or had to be euthanized during the study period was performed by the investigator.

Protocols for Treatment of Disease

Treatment of umbilical infection and arthritis as diagnosed by the investigator consisted of 2 doses of an antimicrobial drug (florfenicol, 20 mg/kg of BW i.m.; Nuflor, Merck & Co., Kenilworth, NJ) 48 h apart, and a single application of an oral nonsteroidal anti-inflammatory drug (meloxicam, 1 mg/kg of BW; Carlsbad Technology Inc., Carlsbad, CA) at the time of diagnosis. All health events and treatments were recorded on paper and entered into a database (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). Calves

diagnosed with pneumonia by the investigator as described above were treated with a single administration of an antimicrobial drug (tulathromycin, 2.5 mg/kg of BW s.c.; Draxxin, Zoetis, Florham Park, NJ). In addition, pneumonia was recorded by the farm personnel daily and treated accordingly. Treatment of diarrhea was administered by herd personnel and consisted of 1.9 L of an oral electrolyte solution (Electrolyte Complete, Land O'Lakes, Shoreview, MN) once or twice per day, depending on the degree of dehydration after the first treatment. Oral electrolytes were first offered in a nipple bottle and only fed with an esophageal feeding tube in case the calf refused to drink. Treatments were repeated daily until fecal consistency was either pasty or normal.

Analytical Approach

Sample Size. The sample size calculation was based on the primary outcome of interest (treatment effect on incidence of umbilical infection). Prior observations on this farm using SI as a navel dip showed an incidence of 30% and we wanted to be able to identify a meaningful difference of 10% in incidence of umbilical infection. We applied a power of 0.80 and a significance level of 0.05, resulting in a sample size of 141 calves per group.

Figure 1. Positions of ultrasonographic examination of extraabdominal and intraabdominal umbilical structures: distal end of umbilical stalk (P1), base of umbilical stalk (P2), umbilical vein immediately cranial to the inner umbilical ring (P3), umbilical vein halfway between the umbilical ring and the liver (P4), and urachus adjacent to its junction with the urinary bladder apex (P5). The umbilical arteries were assessed either at P5, or further caudally (lateral side of the urinary bladder; P6) in the case of completed physiological retraction to this position.

Baseline Characteristics. Chi-squared tests were generated using PROC FREQ of SAS (version 9.3, SAS Institute Inc., Cary, NC) for differences in twin births and dam parity. Differences in calving scores were assessed with Fisher's exact test using PROC FREQ in SAS. One-way ANOVA were carried out with PROC ANOVA for differences in age at evaluation, BW at the first evaluation, and Brix % between treatment groups.

Incidence of Umbilical Infection and Umbilical Hernia. Differences in cumulative incidence of umbilical infection (omphalitis, omphalophlebitis, urachitis, or omphaloarteriitis) and umbilical hernia between treatment groups were assessed with chi-squared test using PROC FREQ in SAS. To determine the association between the 2 dependent variables of interest, umbilical infection and umbilical hernia, and the independent variables group, season, dam parity, calving ease, twin births, Brix %, BW at first evaluation, arthritis, pneumonia, diarrhea, and umbilical infection or umbilical hernia, respectively, a multivariable logistic regression model was fitted for each of the 2 outcomes using PROC GLIMMIX. After an initial screen of all variables, those with a P -value of ≤ 0.20 in univariable analysis were entered into the model. Backward stepwise selection was performed until each independent variable had a P -value of ≤ 0.05 . Treatment group was forced into the model. Two-way interactions between remaining variables were investigated and retained in the model if $P \leq 0.05$. Enrollment block was included as a random effect.

Disease Incidence and ADG. Chi-squared tests were generated using PROC FREQ of SAS for differences in incidence of pneumonia, diarrhea, and survival. Fisher's exact test using PROC FREQ of SAS was applied for differences in incidence of arthritis. One-way ANOVA was carried out with PROC ANOVA for differences in ADG between treatment groups. To study the association between health events (pneumonia, diarrhea, and arthritis) and the independent variables treatment group, season, dam parity, calving ease, twin birth, Brix %, and BW at first evaluation, a multivariable logistic regression model was used with the PROC GLIMMIX procedure in SAS. All variables with a P -value of ≤ 0.20 from univariable analysis were included in the initial model. Backward stepwise selection was carried out until each independent variable in the model had a P -value ≤ 0.05 . Treatment group was forced into the model. Two-way interactions between remaining variables were investigated and retained in the model if $P \leq 0.05$. Enrollment block was included as a random effect.

To determine the association between ADG and the variables treatment group, season, dam parity, calving

ease, twin birth, Brix %, BW at first evaluation, arthritis, pneumonia, diarrhea, and umbilical infection, a multivariable linear regression model was fitted with PROC MIXED in SAS. The initial model included variables with a P -value of ≤ 0.20 in univariable analysis. Backward stepwise selection was performed until each independent variable in the model had a P -value of ≤ 0.10 . Two-way interactions between remaining variables were investigated and retained in the model if P -value ≤ 0.05 . Enrollment block was included as a random effect. Tukey's post hoc test was used to compare the effect of treatment group. Enrollment block was included as a random effect. For the final model, the assumption of homoscedasticity and normality of residuals was assessed.

Ultrasonography and Clinical Examination. For comparison of diameters of the umbilical arteries and the urachus in wk 1 and differences in decrease of the diameter of the umbilical arteries from wk 1 to wk 2, one-way ANOVA was carried out with PROC ANOVA. Repeated-measures ANOVA was performed for repeated ultrasonographic measurements at positions P1, P2, P3, and P4 using PROC MIXED in SAS with the fixed effects of treatment and time, as well as the time $\times$ treatment interaction; enrollment block was included as a random effect. The degree of agreement between palpation and ultrasonographic examination as a diagnostic tool for umbilical infection (omphalitis and omphalophlebitis) was determined with kappa (κ) statistics using JMP (version 12.0, SAS Institute Inc.), evaluating each week separately. The results were interpreted according to Landis and Koch (1977), who define a κ value of < 0.21 as indicating poor agreement, of 0.21–0.40 as fair, of 0.41–0.60 as moderate, of 0.61–0.80 as good, and of 0.80–1.00 as very good agreement.

RESULTS

Quality control of navel dips yielded no evidence of contamination because no bacterial growth was detected in any of the samples taken for bacterial culture.

Description of Study Population

A total of 423 calves were enrolled in the study. One calf of each group was replaced the day after enrollment: 1 animal in group NG was euthanized due to an esophageal injury, and 1 animal of group SI and 1 animal of group CH died within the first day after birth. Descriptive statistics are presented in Table 1. We detected no group differences for BW at first evaluation, Brix %, calving ease, twin calves, parity of dam, or age at the 4 evaluations ($P \geq 0.38$).

Table 1. Descriptive statistics by treatment group (results presented as mean value $\pm$ SD unless otherwise noted)

Measurement	Treatment ¹			Overall	<i>P</i> -value
	NG	SI	CH		
Number of calves	141	143	139	423	—
BW at first evaluation (kg)	42 $\pm$ 5	41 $\pm$ 5	42 $\pm$ 5	42 $\pm$ 5	0.77
Brix %	9.2 $\pm$ 0.8	9.2 $\pm$ 0.8	9.2 $\pm$ 0.8	9.2 $\pm$ 0.8	0.82
Calving ease					
Score 1 [no. (%)]	124 (87.9)	124 (86.7)	122 (87.8)	370 (87.5)	0.99
Score 2 [no. (%)]	16 (11.4)	18 (12.6)	16 (11.5)	50 (11.8)	
Score 3 [no. (%)]	1 (0.7)	1 (0.7)	1 (0.7)	3 (0.7)	
Twins [no. (%)]	13 (9.2)	13 (9.1)	11 (7.9)	37 (8.8)	0.91
Dam parity					
Primiparous [no. (%)]	45 (31.9)	55 (38.5)	50 (36.0)	150 (35.5)	0.51
Multiparous [no. (%)]	96 (68.1)	88 (61.5)	89 (64.0)	273 (64.5)	
Age at evaluation (d)					
Wk 1	2 $\pm$ 1	2 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1	0.52
Wk 2	9 $\pm$ 1	9 $\pm$ 2	9 $\pm$ 1	9 $\pm$ 1	0.75
Wk 3	16 $\pm$ 2	16 $\pm$ 2	16 $\pm$ 1	16 $\pm$ 2	0.38
Wk 4	23 $\pm$ 2	23 $\pm$ 2	23 $\pm$ 2	23 $\pm$ 2	0.39

¹Treatments were single dip administration of the umbilicus with 1 of 3 products: NG = Navel Guard (SCG-Solutions Inc., McDonough, GA); SI = 7% iodine tincture; and CH = 2% chlorhexidine solution within the first 2 hours of life.

Incidence of Umbilical Infection and Umbilical Hernia

Cumulative 4-wk incidence of umbilical infection between treatment groups NG, SI, and CH did not differ [41/141 (29.1%), 40/143 (28.0%), and 34/139 (24.5%); $P = 0.66$]. Omphalophlebitis was diagnosed in 21/141 (14.9%), 14/143 (9.8%), and 16/139 (11.5%) calves in groups NG, SI, and CH, respectively ($P = 0.41$). Omphaloarteriitis and urachitis was documented in 1 calf in groups NG and CH, respectively. In 29 of 115 (25.2%) calves, the umbilical infection involved only the intraabdominal part of the umbilical vein, and

therefore could only be detected by deep abdominal palpation or ultrasonographic examination of the intraabdominal umbilical structures.

The logistic regression model for the absence or presence of umbilical infection contained the following independent variables: dam parity ($P = 0.05$), BW at first evaluation ($P = 0.007$), and absence of the umbilical cord at the time of first examination ($P = 0.04$); and none of the tested interactions remained in the model (Table 2). Controlling for the effect of group ($P = 0.70$), calves of primiparous dams had decreased odds of umbilical infection compared with calves of

Table 2. Logistic regression model showing factors associated with umbilical infection in 423 calves; enrollment block was included as a random effect

Parameter ¹	Regression coefficient β (SE)	<i>P</i> -value	aOR ² (95% CI)
Group ³		0.70	
NG	0.24 (0.29)		1.27 (0.72–2.23)
SI	0.18 (0.29)		1.20 (0.68–2.13)
CH	Referent		—
Dam parity		0.05	
Primiparous	−0.58 (0.29)		0.56 (0.32–0.99)
Multiparous	Referent		—
Umbilical cord at first evaluation		0.04	
Present	−1.12 (0.55)		0.33 (0.11–0.96)
Absent	Referent		—
Birth BW (kg)	0.07 (0.03)	0.007	1.08 (1.02–1.13)

¹Intercept omitted for clarity.

²Adjusted odds ratio.

³Treatments were single dip administration of the umbilicus with 1 of 3 products: NG = Navel Guard (SCG-Solutions Inc., McDonough, GA); SI = 7% iodine tincture; and CH = 2% chlorhexidine solution within the first 2 hours of life.

multiparous dams [odds ratio (**OR**) = 0.56, 95% CI: 0.32–0.99]. A 1-kg increase in birth BW increased the odds of umbilical infection marginally (**OR** = 1.08, 95% CI: 1.02–1.13). Calves with presence of the umbilical cord at the time of first examination had decreased odds of umbilical infection compared with those that did not have an umbilical cord at this time (**OR** = 0.33, 95% CI: 0.11–0.96).

Incidence of umbilical hernia over the study period did not differ among groups [24/141 (17.0%), 27/143 (18.9%), and 26/139 (18.7%) in the NG, SI, and CH groups, respectively; $P = 0.90$]. None of the offered independent variables remained in the logistic regression model for the presence or absence of umbilical hernia. Compared with calves in group CH, the odds for developing an umbilical hernia were 0.89 (95% CI: 0.48–1.65) and 1.01 (95% CI: 0.55–1.84) in groups NG and SI, respectively.

Disease Incidence and ADG

Treatment was not associated with the incidence of pneumonia [28/141 (19.9%), 31/143 (21.7%), and 29/139 (20.9%) in treatment groups NG, SI, and CH, respectively; $P = 0.93$]. The final multivariable logistic regression model for pneumonia included the following fixed effects: BW at first evaluation ($P = 0.005$), Brix % ($P = 0.001$), and season ($P = 0.002$), whereas none of the tested interactions remained in the model. Compared with group CH, the odds of pneumonia in groups

NG and SI were 1.00 (95% CI: 0.53–1.90) and 1.21 (95% CI: 0.64–2.28). Controlling for the effect of group ($P = 0.79$), a 1-kg increase in birth BW marginally increased the odds of pneumonia (**OR** = 1.08, 95% CI: 1.02–1.15). A 1-unit increase in Brix % decreased the odds of pneumonia (**OR** = 0.54, 95% CI: 0.37–0.78). Odds of pneumonia were higher for calves born in winter than in those born in spring (**OR** = 3.82, 95% CI: 1.63–8.97).

Diarrhea was diagnosed in 103/141 (73.1%), 109/143 (76.2%), and 102/139 (73.4%) calves in treatment groups NG, SI, and CH, respectively ($P = 0.80$). Compared with calves in group CH, the **OR** of developing diarrhea were 0.96 (95% CI: 0.56–1.66) and 1.14 (95% CI: 0.66–1.99) in groups NG and SI, respectively. A 1-kg increase in birth BW marginally decreased the odds of diarrhea (**OR** = 0.94, 95% CI: 0.90–0.99). Odds of diarrhea were higher in winter compared with calves born in spring (**OR** = 1.86, 95% CI: 1.09–3.17).

Cumulative incidences of arthritis were not different between groups: 3/141 (2.1%), 3/143 (2.1%), and 4/139 (2.9%) in the NG, SI and CH groups, respectively ($P = 0.85$).

Brix % was the only independent variable remaining in the final logistic regression model for the absence or presence of arthritis ($P = 0.002$). Compared with calves in group CH, the **OR** of arthritis in calves of group NG and SI were 0.87 (95% CI: 0.18–4.17) and 0.88 (95% CI: 0.18–4.23), respectively. A 1-unit increase in Brix % decreased the odds of arthritis (**OR** = 0.24, 95% CI: 0.10–0.58).

Table 3. Multivariable linear regression model showing the effect of treatment group, pneumonia, diarrhea, season, and Brix % on ADG (g/d); enrollment block was included as a random effect

Parameter ¹	Regression coefficient β (SE)	<i>P</i> -value	LSM estimate (SE)
Group ²		0.05	
NG	−22 (32) ^a		494 (29)
SI	55 (31) ^b		571 (29)
CH	Referent ^{ab}		516 (29)
Pneumonia		<0.0001	
Absent	235 (36)		644 (20)
Present	Referent		409 (35)
Diarrhea		0.006	
Absent	87 (31)		570 (31)
Present	Referent		483 (23)
Season		0.002	
Winter	−115 (37)		469 (22)
Spring	Referent		584 (34)
Brix %	32 (19)	0.07	—

^{a,b}Groups with different superscript letters differ at a level of $P < 0.05$ in Tukey's post hoc test.

¹Intercept omitted for clarity.

²Treatments were single dip administration of the umbilicus with 1 of 3 products: NG = Navel Guard (SCG-Solutions Inc., McDonough, GA); SI = 7% iodine tincture; and CH = 2% chlorhexidine solution within the first 2 hours of life.

The final multivariable linear regression model for ADG included the following fixed effects: pneumonia ($P < 0.0001$), Brix % ($P = 0.07$), diarrhea ($P = 0.006$), season ($P = 0.002$), and group ($P = 0.05$; Table 3).

A total of 40 calves (9.5%) died or had to be euthanized during the 4-wk study period (21, 8, and 11 in groups NG, SI, CH, respectively; $P = 0.02$). Postmortem examination was performed by the investigator in 38 calves. Postmortem diagnoses in the NG, SI, and CH groups, respectively were distributed as follows: diarrhea in 19/21 (13.5%), 4/8 (2.8%), and 9/11 (6.5%), pneumonia in 1 calf of each group, and peritonitis, aortic thrombosis, and mesenteric volvulus in 1 calf in groups NG, SI, and CH, respectively.

Ultrasonography and Clinical Examination

Supplementary Tables S1 and S2 (<https://doi.org/10.3168/jds.2016-11654>) describe the umbilical stalk diameter at position P1 and P2 at each of the 4 weekly evaluations by group in all calves, as well as stratified by groups of calves with an umbilical hernia only, calves with omphalitis only, calves with omphalitis and hernia, and calves with normal involution of the umbilical stalk. The umbilical stalk diameter increased in size in calves with umbilical hernia and calves diagnosed with umbilical hernia and omphalitis, whereas it decreased in size in all other groups. We detected an effect of time in all calves ($P \leq 0.005$) except for those diagnosed with both umbilical hernia and omphalitis ($P \geq 0.70$). There was no effect of treatment group ($P \geq 0.13$), and interactions between group and time were not identified ($P \geq 0.28$).

Umbilical vein diameter as measured at position P3 and P4 at each of the 4 weekly evaluations in all calves, as well as stratified by calves diagnosed with omphalophlebitis and those with normal involution of

the umbilical vein, are demonstrated in Supplementary Tables S3 and S4 (<https://doi.org/10.3168/jds.2016-11654>), respectively.

The umbilical arteries were imaged most clearly in the first week of life (right artery $n = 256$, left artery $n = 231$). The mean diameter ($\pm$ SE) of the right and left artery did not differ in groups NG, SI, and CH [right artery: 9.8 ± 0.2 ($n = 81$), 9.6 ± 0.2 ($n = 90$), and 9.6 ± 0.2 mm ($n = 85$), respectively, $P = 0.72$; left artery: 9.6 ± 0.2 ($n = 74$), 9.5 ± 0.2 ($n = 80$), and 9.3 ± 0.2 ($n = 77$) mm, respectively, $P = 0.61$]. At the second evaluation, the umbilical arteries could only be identified in 80 out of 256 (right artery) and 68 out of 231 (left artery) calves, respectively, and the mean difference in diameter ($\pm$ SE) from the first evaluation was not different among groups NG, SI, and CH, respectively [right artery: 1.0 (± 0.3), 1.0 (± 0.3), and 0.8 (± 0.3) mm, respectively, $P = 0.81$; left artery: 1.2 (± 0.4), 0.8 (± 0.4), and 0.9 (± 0.4) mm, respectively, $P = 0.72$].

The urachus could be imaged in 130 calves in the first week when the mean diameter ($\pm$ SE) of the urachus in groups NG, SI, and CH was 7.0 ± 0.2 ($n = 39$), 6.7 ± 0.2 ($n = 48$), and 6.9 ± 0.2 ($n = 48$) mm, respectively ($P = 0.60$). After wk 1, the urachus could only be identified and clearly differentiated from the surrounding tissue in 2 calves with urachitis.

Agreement between palpation and ultrasound as a diagnostic tool for detection of umbilical infection (omphalitis and omphalophlebitis) is depicted in Table 4. For detection of omphalitis the agreement of both diagnostic techniques for wk 1, 2, 3 and 4 was determined to be fair, good, moderate, and moderate, with κ values (95% CI) of 0.30 (-0.03 – 0.63), 0.61 (0.46 – 0.75), 0.53 (0.31 – 0.74), and 0.49 (0.19 – 0.79), respectively. Omphalophlebitis was not diagnosed in wk 1 and for the diagnosis of omphalophlebitis in wk 2, 3, and 4 the agreement between palpation and ultrasound was

Table 4. Measurement of agreement (κ value) between clinical and ultrasonographic examination for detection of umbilical infection (omphalitis and omphalophlebitis) in 423 dairy calves; each case was only considered once in the first week of positive diagnosis

Infection	Number of observations				κ value	95%CI	
	Positive on ultrasound and clinical exam	Positive on ultrasound	Positive on clinical exam	Negative on ultrasound or clinical exam		Lower	Upper
Omphalitis							
Wk 1	2	1	8	412	0.30	−0.03	0.63
Wk 2	20	7	16	370	0.61	0.46	0.75
Wk 3	9	7	8	354	0.53	0.31	0.74
Wk 4	4	6	2	370	0.49	0.19	0.79
Omphalophlebitis							
Wk 1	0	0	0	423	—	—	—
Wk 2	15	0	3	395	0.91	0.80	1.00
Wk 3	17	4	1	356	0.87	0.75	0.98
Wk 4	9	1	1	371	0.90	0.76	1.00

determined to be very good with κ values (95% CI) of 0.91 (0.80–1.00), 0.87 (0.75–0.98), and 0.90 (0.76–1.00), respectively.

DISCUSSION

Incidence of Umbilical Infection and Umbilical Hernia

The primary objective in the present study was to investigate the effect of 3 navel dips on umbilical infection. We demonstrated no association between the choice of navel dip and occurrence of umbilical infection or umbilical hernia. This suggests that both Navel Guard and 2% chlorhexidine gluconate are viable alternatives to 7% iodine tincture. These findings concur with those of Grover and Godden (2011), who demonstrated a numerical but not statistically significant reduction in the incidence of umbilical infection in calves receiving Navel Guard compared with calves in which the navel was dipped with 7% iodine tincture or solutions with lower concentrations of iodine (0.5 to 2%). Robinson et al. (2015) compared the efficacy of 4 antiseptics (7% iodine tincture, 0.1% chlorine solution, 4% chlorhexidine gluconate, and 10% trisodium citrate) on umbilical cord healing in the first 24 h of life in dairy calves, and concluded that all 4 navel dips were equally effective on healing rate of umbilical cords. Those authors measured the diameter of the umbilical cord (as an indicator of umbilical cord healing) with a digital caliper immediately and 24 ± 1 h after birth, whereas the surface temperature (measured using infrared technology) of the umbilical stump at the same time points was used as an indicator of umbilical infection. Because none of the experimental calves in the study by Robinson et al. (2015) was diagnosed with umbilical infection, it is difficult to compare those results to results in our study.

Umbilical cord care was considered the dairy farm's standard of care. Therefore, a limitation of our work is that assessments were not comparable to an untreated control and hence no interpretation can be made as to whether cord care was equal to or worse than no treatment for the prevention of umbilical infection. Grover and Godden (2011) demonstrated, in a controlled study with a total of 111 calves (58 calves in treatment group, 53 calves in control group), that umbilical cord care significantly reduced the risk of developing umbilical infection in newborn calves when applied shortly after birth.

Umbilical infections are considered to have a low incidence and are perceived as a low-priority disease by many producers and veterinarians (Grover and Godden, 2011). This is also reflected by the National Animal Health Monitoring System (NAHMS; USDA, 2010)

report, which indicated that only 31.2% of operations in the United States detect umbilical infections, with an average of only 1.6% of preweaned heifers being diagnosed and treated in these operations. In the present study, the overall cumulative incidence was 27.0% of all calves enrolled. Although this is significantly higher than previously reported by numerous investigators who documented the incidence of umbilical infections in dairy calves to be between 1 and 14% (Virtala et al., 1996b; Svensson et al., 2003; Grover and Godden, 2011), several factors could explain this discrepancy. One reason is likely due to the case definition for umbilical infection in the present study, which was much more inclusive than that used in previous studies (Virtala et al., 1996b; Grover and Godden, 2011) and likely increased case detection at any of the 4 evaluations. The current study is, to the best of our knowledge, the first field study to include evaluation of the intraabdominal umbilical structures. Because no infection of the external part of the umbilicus was detected in approximately 50% of calves diagnosed with omphalophlebitis, these calves would not have been diagnosed as having an infection in any of the previous studies. This may have resulted in a higher number of false negatives and could explain the lower reported incidence of umbilical infection (Virtala et al., 1996b; Grover and Godden, 2011). However, by increasing the sensitivity of our evaluation, we may also have increased the number of false-positive diagnoses over other methods previously used.

To date, little is known about the incidence of umbilical infections in group-housing systems. It is possible that group housing results in additional potential risk factors for umbilical infection, such as cross-sucking of the navel during the first days of life.

Although treatment group did not have a significant effect on the odds of developing an umbilical infection, several risk factors increasing the odds of umbilical infection could be identified. Calves born to multiparous dams had a higher risk of developing an umbilical infection. A possible explanation could be differences in postpartum behavior between primiparous and multiparous cows. Particularly, differences may occur in licking behavior, which is a potential source of contamination and even trauma of the umbilical cord of the neonate. Multiparous dams stand sooner after parturition than primiparous dams (Houwing et al., 1990). In addition, primiparous cows lick their calves for a shorter period (Edwards and Broom, 1982; Le Neindre and D'Hour, 1989) and more often fail to lick their calves compared with multiparous dams (Edwards, 1983). Because cows begin licking their calf as soon as 1 min after birth (von Keyserlingk and Weary, 2007), this natural behavior has to be taken into account as a possible risk factor for umbilical infection, even on farms with good calving

supervision. Although a walk through the close-up pens and maternity pen was performed hourly during the day and every other hour during night shifts and calves were removed promptly after birth, we did not record the time each calf spent with their dam after birth and this possible explanation remains to be tested.

In over 50% of the cases where the umbilical cord was short (ruptured at the aspect of the external umbilical ring), calves developed an umbilical infection. Although it appears reasonable to conclude that the absence of the natural barrier (umbilical cord) leaving an open wound at the umbilicus during the first days of life increases the odds of developing umbilical infection in newborn dairy calves, no recommendation on how to manage affected calves is available. Protocols on how to decrease the proportion of calves with a ruptured cord, documentation of affected calves and consequently an umbilical examination within the first days of life might improve umbilical health in newborn dairy calves.

The effect of birth BW on survival has been demonstrated in recent studies carried out in New York State, leading to a consensus that a birth BW of 38 to 41 kg in North American Holsteins is optimal for calf survivability (Henderson et al., 2011), whereas both lighter and heavier calves are more likely to die before weaning (Teixeira et al., 2013). Unlike these observations, the risk of developing an umbilical infection increased linearly with BW in the present study.

Disease Incidence and ADG

Our data indicated no difference in the incidence of pneumonia, diarrhea, or arthritis between the 3 treatment groups. Although Bennett and Jasper (1978) reported a significant decrease in the incidence of arthritis by implementation of 7% iodine tincture for umbilical cord care after having used a commercial teat dip, a causal inference should not be made from that report. Unlike in a controlled trial, the observed associations might have been influenced by other unidentified confounding factors as the 2 treatments were compared sequentially rather than contemporarily (Bennett and Jasper, 1978). The decrease in the relative risk for respiratory disease in dairy calves receiving umbilical cord care might have been confounded by other unobserved factors, as discussed by Perez et al. (1990).

Our choice of enrollment in blocks of 3 rather than blocks according to housing group might have reduced the likelihood of detecting an effect of the respective navel dips on infectious disease incidence. In this regard, it might have been more beneficial to house calves by treatment group. However, this could have resulted

in consecutive problems, such as different group size, or exclusion of whole groups in case of erroneously assigned calves. Although the incidence of diarrhea was diagnosed by weekly assessment of fecal consistency, it is inevitable that some events with shorter duration of disease remained undetected. Moreover, our results reemphasize the importance of passive transfer of immunity for the prevention of calfhood disease, as demonstrated by others (Godden, 2008; Windeyer et al., 2014).

Average daily gain differed among treatment groups, with the calves receiving 7% iodine tincture having the highest ADG (543 g/d). Although Virtala et al. (1996a) reported a reduction in ADG of 96 g/d in calves with umbilical infection, we failed to associate the detected difference in our study with differences in the incidence of umbilical infection between treatment groups. We observed an effect of pneumonia on ADG, as has been reported in previous studies (Virtala et al., 1996a; Teixeira et al., 2013). Controlling for the effect of pneumonia and treatment groups, the ADG of calves with one or more events of diarrhea was reduced by 94 g/d. Donovan et al. (1998a) found a decrease in ADG of 13.4 g/d per diarrhea treatment day in dairy calves from birth to 6 mo of age. In contrast, Teixeira et al. (2013) and Virtala et al. (1996a) found no effect of diarrhea on ADG in calves followed for 2 and 3 mo, respectively. Because our evaluations did not extend beyond 4 wk, a possible explanation might be the potential of the calf to compensate BW loss from diarrhea over time, as discussed by Virtala et al. (1996a). In respect to its limitation regarding accuracy and precision, differences in ADG in the range of 50 g/d, as assessed with a heart girth tape in the present study, should be interpreted with caution.

Season had a remarkable effect on the incidence of pneumonia and diarrhea, as well as on ADG. These results concur with observations made by Windeyer et al. (2014) and indicate additional opportunities to improve calf health such as adequate nutrition and proper ventilation in calf barns.

We were unable to explain the differences in survival, particularly due to diarrhea, between treatment groups in the absence of differences in diarrhea incidence between treatment groups. However, it is likely that survival in calves with diarrhea was influenced by one or more unmeasured confounders, which we believe are unlikely to be causal factors of any association between umbilical cord care and diarrhea disease outcome.

Another limitation of our study might have been the fact that although the clinician was masked to treatment, the 3 navel dips differed in color, which might have resulted in unsuccessful masking to treatment.

Ultrasonography and Clinical Examination

Several previous studies have described umbilical involution in a small number of clinically healthy calves using ultrasonography in hospital settings (Lischer and Steiner, 1993; Watson et al., 1994). Although the ultrasonographic measurements reported in those studies were carried out at different time points than the measurements reported here, the gradual decrease of umbilical diameter as well as the percentage size decrease in calves with normal involution of the umbilicus from the first to the last measurement were almost identical in the present and the 2 previous studies. Although Watson et al. (1994) reported difficulties in identifying the umbilical vein in calves at the third and fourth measurement, results of our study and those of Lischer and Steiner (1993) indicate more difficulties in identifying the umbilical arteries in calves older than 1 wk of age. A consensus exists concerning the difficulty in identifying the urachus, which appeared to be challenging in the present and previous studies (Lischer and Steiner, 1993; Watson et al., 1994).

The continuous increase in size of the umbilical stalk in calves with umbilical hernia and calves with omphalitis and umbilical hernia is also noteworthy. Some of the smaller umbilical hernias might not have been detected at an earlier examination, yet our results concur with the observation made by Virtala et al. (1996b), confirming that not all umbilical hernias are present at birth. In addition, our data suggest that there is a gradual increase of size of umbilical hernias within the first 4 wk of life. As the mean duration of umbilical hernia is reported to be 6.7 wk (95% CI: 5.8–7.6; Virtala et al., 1996b), we were not able to draw any conclusion from our data concerning the clinical course beyond the observation period of 4 wk.

A further aim of this study was to compare the agreement of ultrasonographic examination and clinical examination for detection of umbilical infection. Although agreement for detection of omphalophlebitis was very good, indicating that both techniques work equally well for detection of infection of the umbilical vein, agreement for detection of omphalitis was relatively weak. Although an acute infection could be easily detected by palpation via pain response or swelling, it appeared difficult to objectively observe any alterations by ultrasonography. In acute cases of omphalitis, the increase in size often appeared to be restricted to the subcutaneous tissue, which was reflected by anechoic structures and thereby difficult to distinguish from normal subcutaneous tissue surrounding the umbilical stalk. This resulted in a relatively low detection rate of omphalitis by ultrasonographic examination, especially in acute cases. In respect to these results, clinical ex-

amination for detection of umbilical infection proved a valid and even superior diagnostic technique during the first 2 wk of life. Farm technicians can be easily trained in the technique. Implementing such a protocol on dairy farms might substantially improve health in dairy calves by enabling early treatment of calves with umbilical infection. In contrast, ultrasonographic examination proved valuable for visualization of omphalitis in wk 4 and diagnosing infection of the umbilical vein. Ultrasonographic examination could therefore be implemented together with other herd health events such as dehorning and thoracic ultrasonography (Olivett and Buczinski, 2016). Bovine practitioners in the field should be encouraged to use both techniques and consider the information derived from physical examination and ultrasonography together.

As outlined by several authors (Mee, 2008; Grover and Godden, 2011), umbilical cord care in dairy calves is an area that requires further research. Several effective alternatives to 7% iodine tincture have been identified in recent studies, whereas different dipping techniques (dip or spray, single or repeated applications, use of navel clamps or ligation) have not been elucidated. Moreover, the case definition for umbilical infection that we used in this study should be further validated by comparison with a gold standard (bacterial culture and pathological examination). Finally, data on the influence of umbilical infection—with and without involvement of intraabdominal structures—on survivability, reproductive performance, and milk production will help in developing treatment protocols for umbilical disease in dairy calves.

CONCLUSIONS

All 3 navel dips were equally effective in the prevention of umbilical infections in dairy calves in a group-housed setting. Treatment had no effect on the incidence of pneumonia, diarrhea, or arthritis, whereas ADG was marginally higher in calves of the group dipped with 7% iodine tincture. Ultrasonography and clinical examination are interchangeable techniques for detection of omphalophlebitis. Clinical examination can be considered a sensitive technique to detect omphalitis during the first 2 wk of life, whereas ultrasonography yielded a higher detection rate in calves at 3 and 4 wk of age.

ACKNOWLEDGMENTS

This project was supported by the Resident Research Grants Program from Cornell University, College of Veterinary Medicine (Ithaca, NY). The authors thank

the staff at the dairy farm and acknowledge the collaboration of the farm owner.

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