

Short Communication: Association between neonatal calf diarrhea and lying behaviors

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ABSTRACT

The objective of this study was to determine the association of neonatal calf diarrhea (NCD) with step activity and lying behaviors in pre-weaned dairy calves. Calves were housed in individual hutches for the first 6 days of life, and then moved into a group pen. On the day of birth, calves ($n = 30$) were fitted with an accelerometer, and step activity and lying behaviors were recorded. Calves were assigned a fecal score (FS) twice daily using a 0 to 3 scale, and were diagnosed with NCD ($n = 10$) when the score was a 3. To ensure the only association noted was due to NCD, calves that had any other health complications were excluded from analyses ($n = 1$). Calves with NCD were pair matched by age, breed, and birthdate to a healthy calf. Day 0 was designated as the date of NCD diagnosis. Calves with NCD spent less time lying ($P < 0.05$) and displayed more lying bouts ($P < 0.05$) of a shorter duration ($P < 0.01$) than healthy calves. Specifically, calves with NCD displayed more lying bouts on days -7 ($P < 0.05$), -6 ($P < 0.01$), -5 ($P < 0.01$), -4 ($P < 0.01$), and -3 ($P < 0.05$). Similarly, lying bout duration was shorter for calves with NCD on days -6 ($P < 0.05$), -5 ($P < 0.05$), -4 ($P < 0.01$), and -3 ($P < 0.01$). Additional research is needed to examine if these tools can be used to identify diseased calves prospectively.

Introduction

Group-housing of pre-weaned calves has gained popularity with approximately 20% of dairy operations across the United States utilizing this type of facility (Urie et al., 2018a). Studies have demonstrated benefits to social housing, such as improvements in socialization (Duve, Weary, Halekoh, & Jensen, 2012), cognitive performance (Gaillard, Meagher, von Keyserlingk, & Weary, 2014), and greater concentrate intake before weaning (Babu, Pandey, & Sahoo, 2004; Miller-Cushon & DeVries, 2016). The benefits of social housing continue into the weaning period, as noted by an improvement in average daily gain (Miller-Cushon & DeVries, 2016) and body weight (Chua, Coenen, van Delen, & Weary, 2002) when compared to individually-housed calves. Another advantage to group-housing calves with an automatic calf feeder is the reduction in labor required to feed larger volumes of milk (Kung et al., 1997). While there are numerous benefits to socially housing pre-weaned calves, identification of diseased calves in group-housing can be challenging when compared to individually-housed calves (Svensson & Jensen, 2007; Svensson, Lundborg, Emanuelson, & Olsson, 2003). In fact, some studies have shown that mortality rates were greater in group-housed

calves (Gulliksen, Lie, Løken, & Østerås, 2009; Waltner-Toews, Martin, & Meek, 1986), and this appears to become more evident in calves housed in large groups when compared to calves reared in small groups or individually-housed (Losinger & Heinrichs, 1997). Although, it should be noted these associations with social housing and mortality are not always found (Costa, von Keyserlingk, & Weary, 2016).

Several methods of disease detection in calves currently exist including health examinations (McGuirk, 2008), non-invasive infrared thermography (Schaefer et al., 2012), and laboratory procedures such as polymerase chain reaction (Asano, Souza, Silva, Richtzenhain, & Brandão, 2009). The recent advent of precision dairy technologies opens a new avenue for the detection of disease (Rutten, Velthuis, Steeneveld, & Hogeveen, 2013). For pre-weaned calves, technologies such as an automatic calf feeder have identified behavioral changes associated with disease (Borderas, Rushen, von Keyserlingk, & de Passillé, 2009; Knauer, Godden, Dietrich, & James, 2017; Svensson & Jensen, 2007). For example, sick calves on a high milk allowance (*ad libitum* or 12 L/d) consumed less milk, visited the feeder less often, and had a longer visit duration when compared to healthy calves (Borderas et al., 2009). Restrictively-fed diseased calves had shorter visits (Borderas et al., 2009) and fewer unrewarded visits (Svensson &

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Table 1 Definitions of the scoring system used to determine disease status adapted from the Calf Health Scorer app (University of Wisconsin, School of Veterinary Medicine).

	0	1	2	3
Fecal scoring	Normal	Pasty, semi-formed	Loose, but remains on top of the bedding	Watery, sifts through the bedding
Fecal consistency	Normal	Mild or small amount of discharge	Moderate amount of discharge on both eyes	Heavy discharge
Respiratory scoring	Normal serous discharge	Ear flick or head shake	One ear with a slight droop	Both ears droop and/or severe head tilt
Eye discharge	Normal	Unilateral slightly cloudy discharge	Bilateral cloudy discharge	Heavy bilateral purulent discharge
Nasal discharge	Normal, clear discharge	Single induced cough	Induced multiple coughs or occasional spontaneous cough	Repeated spontaneous coughs
Coughing	No coughing	38.3 to 38.8	38.9 to 39.4	Greater than 39.4
Rectal temperature (°C)	37.8 to 38.2			
Other scoring				
Navel scoring	Normal	Slightly enlarged, but not warm or painful	Slightly enlarged, with some pain or moisture	Enlarged, painful, and warm, with a foul odor
Joint scoring	Normal	Slight swelling, no pain or warmth	Swelling with pain, some lameness	Swelling with severe pain, heat, and lameness

Jensen, 2007) than healthy calves. Furthermore, calves with NCD had a slower drinking speed and consumed less milk starting a few days prior to treatment and persisted for at least a week after treatment when compared to healthy calves (Knauer et al., 2017). However, when applying analytical techniques to identify diseased calves using feeding behaviors, these associations lack sufficient accuracy (Knauer, Godden, Dietrich, Hawkins, & James, 2018). Therefore, identification of other behavioral alterations associated with diseased calves may be valuable for on-farm disease detection.

In previous work, we focused on the use of an accelerometer to measure step activity and lying behaviors in pre-weaned calves (Swartz, McGilliard, & Petersson-Wolfe, 2016). We found reductions in lying bouts and step activity in calves with respiratory disease when compared to healthy controls (Swartz, Findlay, & Petersson-Wolfe, 2017). However, with digestive disorders occurring in 56% of ill calves (Urie et al., 2018b), identification of alterations in lying behaviors and step activity may be useful in identifying calves with neonatal calf diarrhea (NCD). Therefore, the objective of this study was to examine the association of NCD with step activity and lying behaviors using an accelerometer. We hypothesized that calves with NCD would take fewer steps, have fewer lying bouts, and spend more time lying than healthy calves.

Material and methods

This study was conducted from March 2016 through August 2016 at the Virginia Tech dairy in Blacksburg, VA, USA in accordance with guidelines set by the Institutional Animal Care and Use Committee (16-002). The results presented in this paper characterize a different subset of data from a previous study that reported behavioral changes associated with respiratory disease, and all experimental procedures were previously described (Swartz et al., 2017).

Female Holstein and Jersey calves (n = 30; 19 Holstein and 11 Jersey) were enrolled at birth, housed outside in individual hutches (4.1 by 1.2 m; Hampel Corp., Germantown, WI), ear tagged, and fed 4 L of colostrum after birth. Calves remained in individual hutches for the first 6 days of life. While in hutches, calves were bottle-fed 2 L of 40.6°C water mixed with 300 grams of milk replacer (13% Total Solids; Cow's Match®, Warm Front® PB, Land O'Lakes Animal Milk Products Co., Shoreview, MN) twice daily.

When the calf was 7 days of age, calves were moved into group-housing with an automatic calf feeder (FA Förster-Technik GmbH, Engen, Germany) at approximately 0800 h just prior to the first feeding. The pen had a 6.3 by 6.0 m sawdust bedded pack, and a 4.3 by 6.0 m concrete alley which was maintained by the farm staff. The number of calves in the group was dynamic, as calves entered group-housing 7 days after birth, and left after weaning. The automatic calf feeder gradually increased milk replacer allowances (13% Total Solids; Cow's Match®, Warm Front® PB, Land O'Lakes Animal Milk Products Co., Shoreview, MN) from 6 L to a maximum of 12 L of milk per day; maximum milk allowance was reached when calves were 16 days of age. Weaning began at 46 days of age until 55 days of age when calves were weaned and removed from the pen. In group-housing, calves were allowed *ad libitum* access to both water (WaterMatic 150, Ritchie Industries Inc., Conrad, IA) and calf starter in a metal feed bunk (Intensity 22% Textured Calf Starter Medicated, Southern States, Richmond, VA). Grain refusals were discarded daily, and new grain was provided to ensure *ad libitum* access. No other feedstuff was provided.

Previously validated accelerometers (AfiTag II, AfiMilk LTD., Kibbutz Afikim, Israel) on dairy calves were attached to the calves' right rear legs at birth to collect step activity and lying behaviors (Swartz et al., 2016). These variables were collected wirelessly every 15-min and transmitted to a computer (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel). Data were then summarized into daily steps (no./d), lying time (h/d), and lying bouts (no./d) using Excel spreadsheets (Microsoft Corp., Redmond, WA). Lying bout duration was calculated

by dividing daily lying times by the number of lying bouts.

All calves were health scored twice daily (0800 and 1600 h) using the Calf Health Scorer App (University of Wisconsin, School of Veterinary Medicine). This app closely follows the criteria of the calf health scoring chart previously developed by McGuirk (2008). A description of this scoring system is provided in Table 1. Using digital rectal stimulation, a fecal score (FS) was assigned using a 0 to 3 scale, with a 0 or 1 representing normal, a 2 indicating loose manure that remains on top of the bedding, and a 3 representing a calf with NCD with watery fecal consistency that sifts through the bedding. Calves were also scored on eye discharge, ear disposition, nasal discharge, coughing, and temperature for respiratory scoring, with scores of 0 representing normal, and 3 being severely abnormal. Respiratory disease was indicated when the sum of these five variables were ≥ 5 , provided that at least two of the five variables were ≥ 2 (McGuirk, 2008). Respiratory, joint, and navel scores were performed to ensure that calves diagnosed with NCD were only affected by NCD.

For each diseased calf, a healthy calf of the same breed, similar date of birth (average difference in date of birth; mean \pm SD, 17.8 \pm 17.4 days), and same age (age at NCD diagnosis; range, 9 to 15; mean \pm SD, 11 \pm 2 days) was identified as the matched pair. Day 0 was assigned as the day when NCD was first diagnosed with a fecal score = 3. Healthy controls were defined as calves with fecal score ≤ 2 , joint, and navel scores of a 1 or 0, and a total respiratory score of ≤ 4 . A calf with NCD was defined as a calf with a fecal score = 3, with total respiratory score of ≤ 4 , joint, and navel scores of a 1 or 0. Calves were only included in analyses if the calf was free of other diseases, resulting in a single calf being removed due to respiratory disease. Our final sample size was 10 calves with NCD and 10 healthy control calves.

The residuals were analyzed for outliers and normality (PROC UNIVARIATE, SAS v. 9.4). All data were deemed to be normally distributed. A repeated-measures mixed model (PROC GLIMMIX, SAS) was used with health status, day (repeated measure), and the interaction of health status and day as fixed components, and the matched pair of calves and the interaction of pair and health status as random effects. The autoregressive covariance structure was used in all models, and the Kenward-Roger procedure was used for degrees of freedom approximation. The effect of health status was compared within each day using the SLICE option in SAS. Significance was declared when $P < 0.05$.

Results

The results of the statistical analyses are provided in Table 2. No effect of NCD was identified on the number of steps (Figure 1.). Daily lying time (h/d), lying bouts (no./d), and lying bout duration (min/bout) for calves with NCD are shown in Figure 2. For lying time, the main effect of health status was significant ($F_{1,56} = 6.68$; $P < 0.05$), as calves with NCD spent less time lying per day than healthy calves (17.8 \pm 0.2 vs 18.2 \pm 0.2 h/d, respectively). Specifically, when investigating the interaction term of health status and day, calves with NCD spent less time lying on days -3 ($F_{1,224} = 4.16$; $P < 0.05$) than

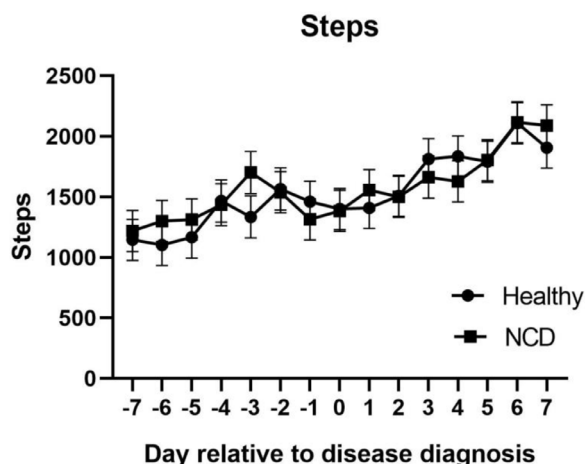


Figure 1. Daily steps (LSM \pm SE) of either healthy or calves with NCD. Day 0 represents the day of disease diagnosis.

healthy calves.

As for lying bouts, the main effect of health status was significant ($F_{1,22} = 6.02$; $P < 0.05$), as calves with NCD displayed more lying bouts than healthy calves (21 \pm 1 vs 18 \pm 1 bouts, respectively). When investigating the interaction of health status and day, calves with NCD displayed more lying bouts on days -7 ($F_{1,81} = 5.39$; $P < 0.05$), -6 ($F_{1,81} = 11.22$; $P < 0.01$), -5 ($F_{1,81} = 11.22$; $P < 0.01$), -4 ($F_{1,81} = 10.05$; $P < 0.01$), and -3 ($F_{1,88} = 6.22$; $P < 0.05$) than healthy calves. Lying bout duration followed a similar pattern, as the main effect of health status was also significant ($F_{1,30} = 8.34$; $P < 0.01$). Calves with NCD spent less time lying per bout than healthy calves (55 \pm 3 vs 66 \pm 3 min/bout, respectively). When investigating the interaction of health status and day, calves with NCD spent less time lying per bout on days -6 ($F_{1,114} = 5.71$; $P < 0.05$), -5 ($F_{1,114} = 5.53$; $P < 0.05$), -4 ($F_{1,114} = 7.24$; $P < 0.01$), and -3 ($F_{1,125} = 6.93$; $P < 0.01$) than healthy calves.

Discussion

Our study is the first to demonstrate alterations in lying behaviors from calves with NCD. A previous study reported that calves with NCD did not differ in lying bouts or lying time relative to healthy calves (Sutherland, Lowe, Huddart, Waas, & Stewart, 2018); however, in agreement with the present study, Studds and coworkers (2018) found that diseased veal calves spent less lying than healthy calves. This study poses a number of questions. Why would NCD alter lying behaviors a week in advance of disease diagnosis? Are calves exhibiting these behaviors more likely to succumb to NCD? Or is the disease already impacting calf behavior well in advance of clinical signs? We speculate that the behavioral response may be due to early subclinical stages of disease. An increase in lying bouts and a decrease in lying time from a severe gastrointestinal tract disorder could be an indicator of

Table 2

Summary of behavioral outcomes for either healthy or calves with NCD. Probability level and F-values provided for the fixed effects of health status, day, and the interaction of health status and day.

	Healthy	NCD	SE	Health status F-value	P-value	Day F-value	P-value	Health status * day F-value	P-value
Steps ¹ (steps/d)	1,535	1,572	111	0.20	NS	4.60	< 0.001	0.78	NS
Lying time ¹ (min/d)	1,093	1,070	10	6.68	< 0.05	4.21	< 0.001	0.68	NS
Lying bouts ¹ (no./d)	18	21	1	6.02	< 0.05	1.94	< 0.05	1.05	NS
Lying bout duration ¹ (min/bout)	66	55	3	29.72	< 0.01	1.54	NS	0.66	NS

¹ Numerator degrees of freedom are 1, 14, and 14 for health status, day, and the interaction for every model. Denominator degrees of freedom for steps 37, 223, and 223, lying time 56, 225, and 225, lying bouts 22, 226, and 226, and lying bout duration 30, 226, and 226 for health status, day, and health status * day, respectively. Den df approximated using the Kenward-Roger method.

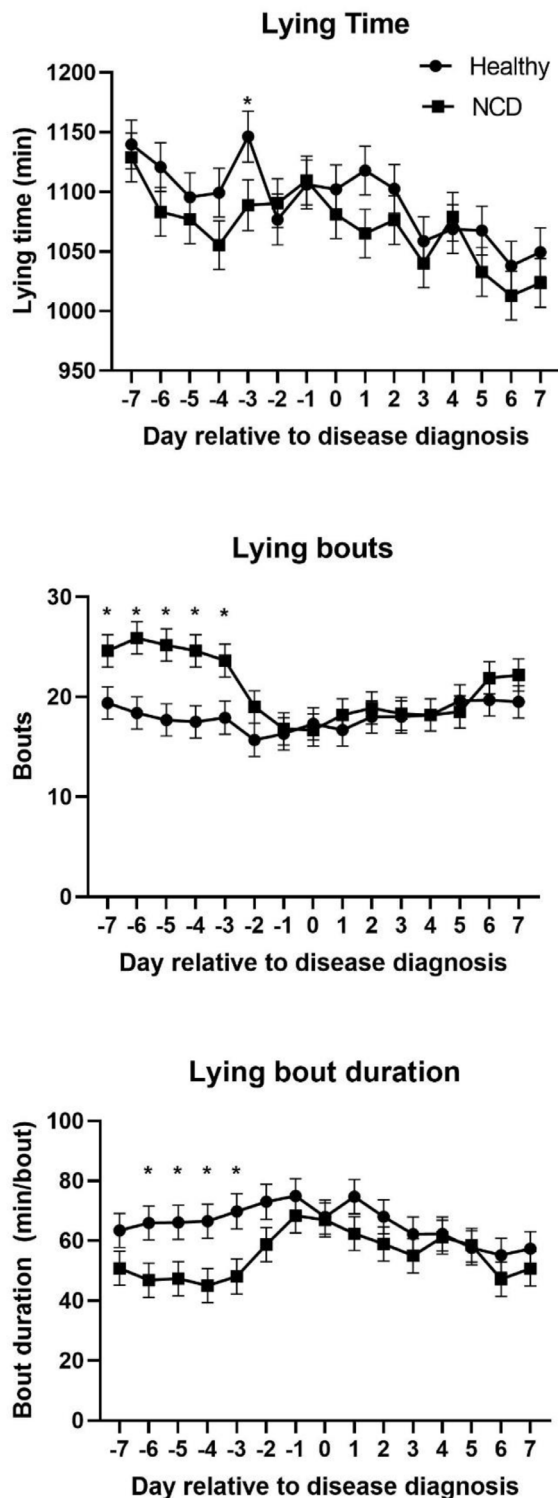


Figure 2. Daily lying time (A; LSM \pm SE, min), lying bouts (B), and lying bout duration (C; min/bout) for either healthy or calves with NCD. Day 0 represents that day of disease diagnosis. Differences between healthy and calves with NCD: * $P < 0.05$.

discomfort. Past studies have found that an increase in position changes, such as lying bouts, are generally suggestive of restlessness (Proudfoot, Huzzey, & von Keyserlingk, 2009), and discomfort from disbudding in calves reduced lying time (Sutherland et al., 2018). Indeed, the use of anesthetics during disbudding increased lying time when compared to calves disbudded with no pain management

(Sutherland et al., 2018). However, the incubation periods for most NCD pathogens range from 12 h to 5 days (reviewed by McGuirk, 2008). Consequently, changes in behavior would be expected to occur during the incubation period and thereafter. Indeed, past research has demonstrated that NCD reduced drinking speed starting 3 days prior to treatment, potentially occurring during the incubation periods (Knauer et al., 2017). However, the present study found alterations in lying behaviors even further in advance of the incubation periods. In fact, an increase in the number of lying bouts in calves with NCD was identified 7 days prior to disease diagnosis when compared to healthy calves. Similar studies in periparturient dairy cows have demonstrated behavioral deviations occurring weeks before disease diagnosis, suggesting that these changes may predispose cattle to disease (Huzzey, Veira, Weary, & von Keyserlingk, 2007; Itle, Huzzey, Weary, & von Keyserlingk, 2015). Taken together, it seems probable that calves exhibiting a greater frequency of lying bouts early in life are more likely to succumb to NCD. Moreover, in the present study, calves with NCD spent less time lying than healthy calves, consistent with a previous study in veal calves (Studds et al., 2018). This is particularly troublesome, as lying is a high priority behavior that typically increases as part of an adaptive response to overcome disease (Cyples et al., 2012; Hart, 1988). As suggested with lying bouts, this decrease in lying time may also have predisposed the calf to NCD. Considering these findings, improving our understanding of how lying behaviors relate to disease susceptibility could be useful in identifying high-risk calves for NCD.

Conclusions

Calves with NCD spent less time lying when compared to healthy calves. Furthermore, calves with NCD expressed more lying bouts of a shorter duration days in advance of disease diagnosis. These data suggest that the use of lying behaviors could be useful in identifying calves at risk for NCD. However, additional research is needed to examine the ability of these technologies to identify sick calves prospectively.

Ethical statement

This study was conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee (16-002) at the Virginia Polytechnic Institute and State University.

Declarations of Competing Interest

None of the authors have any personal or financial relations that would inappropriately influence the content of this study.

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