ABSTRACT

In the western United States, it is common practice for dairy and beef x dairy calves to be sold and shipped within the first few days of life making transportation one of the first challenges a calf will experience. To date there is no published research that has examined the impacts of calf transport within the western United States. The objectives of this observational study were to describe health statuses of calves leaving the source dairy, characterize transportation conditions and calf behavior during transportation, and determine if age at transportation and transportation duration are associated with dehydration, blood glucose and lactate, and behavior. An observational study was performed on 2 source dairies in the Western United States. Initial enrollment consisted of dairy bull calves and beef x dairy calves (n = 126 total) across 16 transport cohorts which were sold and transported to separate calf-raising facilities approximately 80 km from the source dairy. Health exams and measurements were performed on all study participants 2–3 h before transport and ≤2 h after transport. Two researchers performed health exams using the Wisconsin calf health score which included clinical respiratory scores, lung ultrasound, and fecal, navel, and joint scores. Hydration status was assessed using skin tent duration. Blood samples were collected and immediately analyzed for blood glucose and lactate using glucose and lactate meters. Accelerometers were attached at the source dairy on ≤10 calves in each of the 16 transport groups to record movement and behavior during transport (n = 90 had accelerometers attached). Results showed that about half of calves (49%) were identified with at least one health abnormality before transport. To the authors knowledge, this is the first observational study that investigates the impacts of transportation on dairy bull and dairy x beef calf health and welfare in the Western United States. Our findings support the need for improved management and decision-making before transporting calves to reduce the negative impacts of transport.

(Key Words: Surplus, welfare, neonate)

INTRODUCTION

Nearly 90% of dairy bull calves born on US dairies are sold or raised at operations other than the dairy of origin, resulting in millions of calves being transported at a young age every year (USDA, 2016). It is increasingly common for “beef x dairy” calves (i.e., calves born to dairy cows bred with beef semen) to enter the supply chain; while data on beef x dairy calves are limited it is expected that over 5 million head of cattle with dairy influence will enter the beef supply chain annually in the US (Mckendree et al., 2020; Foraker et al., 2022; McCabe et al., 2022). In the US, preweaned dairy calves fall under the same transport regulations established for all food production animals which states that animals may not be confined in transport for more than 28 consecutive hours without unloading for food, water, and rest (49 U.S.C. § 80502). Transportation stressors include commingling, changes in environmental temperature, limited to no milk or water access, and a variety of handling techniques (Trunkfield and Broom, 1990; Stull and Reynolds, 2008). Furthermore, male dairy calves are reared in suboptimal conditions compared with heifer calves due to their perceived lower monetary value (Cave et al., 2005; Creutzinger et al., 2021) however, transportation practices for male dairy calves in the US, as well as early life management of beef x dairy calves are unknown.

Although transport research in calves is limited, data demonstrates that calves arrive to auction markets and veal operations in suboptimal condition (Pempek et al., 2017; Wilson et al., 2020a). For example, 14% of...
calves were identified with diarrhea, 0.5% with respiratory disease, 14% with depressed attitude, 27% with inflamed navels, and 35% with dehydration at arrival to veal facilities in the US (Pempek et al., 2017). In Canada, studies have found that 20% of calves had at least one health abnormality at arrival to auction (Wilson et al., 2020a) and that 17%, 9%, 3%, 8%, and 14% arrived at a calf grower with an abnormal behavioral attitude score, pyrexia, clinical bovine respiratory disease, abnormal fecal score, and abnormal navel score, respectively (Wilson et al., 2020b). Thus, this indicates that calves were sick or injured before transportation, calves became compromised during transportation, transportation worsened existing conditions, or a combination thereof. Understanding the point or points in the supply chain at which calf condition deteriorates is critical to intervene and ensure compromised calves are not shipped, and if illness or injury occurs during transit, they receive prompt veterinary care.

Another important element of calf transport to understand how calf age at the time of transport impacts welfare. While data on the age at which dairy bull or beef x dairy calves are transported is limited, national data shows that heifer calves are transported off-site at an average of 3 d of age (USDA, 2012). The immature physiological responses to stress and underdeveloped immune systems of young calves make them particularly vulnerable to stressors, such as transportation (Pardon et al., 2015; Hulbert and Moisá, 2016). For example, a Canadian study reported that calves who were transported at more than 7 d of age had reduced incidence of an abnormal respiratory score compared with calves 2 to 6 d old at the time of transport (Goetz et al., 2023b). Given that calves in the US are transported at a younger average age (USDA, 2012) than in these studies, it is necessary to understand the impact of transporting neonatal calves so that negative welfare consequences can be mitigated.

In the US, calves are transported varying distances, with approximately 25% of calf shipments traveling more than 161 km from the source dairy, and approximately one-third of these crossing state lines (USDA, 2012). Longer times or greater distances in transit have been associated with greater mortality during transport, lower body weight at arrival to slaughter, and physiological measures indicative stress in calves (Cave et al., 2005; Boulton et al., 2020; Rot et al., 2022). Greater transport durations also mean that milk and water are likely withheld for longer periods, and that exposure to other stressors, such as thermal stress, is prolonged. Numerous measurements relative to transporting calves have been assessed, including physiological, health, performance, and behavior, which provide insight into the potential immediate and long-term welfare consequences associated with calf transport (reviewed by Goetz et al., 2022). For example, blood glucose provides information about energy metabolism and was found to be lower in calves transported for 16 h versus 6 h (Goetz et al., 2023a). Blood lactate can be an indicator of stress during transportation, as levels are elevated in fatigued animals (Thomson et al., 2015). Understanding risk factors for morbidity and mortality post-transport could help the industry make more informed decisions before shipping calves off the source dairy. For example, calves with navel inflammation and smaller heart girths (a proxy for body weight) were more likely to develop diarrhea during the 2 weeks after transport compared with calves without navel inflammation and larger heart girths (Wilson et al., 2020b). Furthermore, calves with a depressed behavioral attitude were 2.5 times as likely to die in the 2 weeks post-transport compared with calves with normal attitude (Wilson et al., 2020b). Thus, a variety of calf characteristics can be impacted, both immediately and long-term, by transportation. Further understanding how transportation affects calf welfare can help reduce negative welfare outcomes, and better inform decision-making before transport.

To the authors’ knowledge, there are no studies that have examined transportation practices of calves in the western US. Given the young age at which calves in the US are transported, there is a need to better understand transportation practices in the western US for young calves. Thus, the objectives of this observational study focus on beef x dairy or male dairy calves in the western US and were to 1) describe health status of calves before transportation, 2) characterize transportation conditions and calf behavior during transportation, and 3) determine if age at transportation and transportation duration are associated with dehydration, blood glucose and lactate, and behavior. Given the preliminary and observational nature of this research, the research team did not have prespecified hypotheses.

**MATERIALS AND METHODS**

The study methods were approved by Colorado State University’s Institutional Animal Care and Use Committee (protocol #3157).

**Study overview**

The study took place on 2 source dairies in the Western United States from March through April in 2022. Source dairies, and their respective calf-raising facilities) were recruited based on previous productive
Cramer et al.: Effects of transport on calf welfare

Research relationships with the research team. Source dairy 1 had approximately 1,500 lactating cows and raised all replacement heifers on-site. Source dairy 1 had approximately 1,500 lactating Holstein cows and raised all replacement heifers on-site. Source dairy 2 had approximately 12,000 lactating Holstein cows and raised replacement heifers at a nearby facility owned by the same company. More details regarding the source dairies and pre-transport management are described in subsequent sections. Surplus calves from each source dairy were sold and transported to 2 separate calf-raising facilities within approximately 80 km of the source dairies. Research staff arrived at the source dairies within 2–3 h before calves being loaded onto trailers to collect pre-transportation measurements (health exams including assessing hydration status, blood samples, weights, attach activity loggers). Transporters coordinated with research staff regarding arrival time at the calf-raising facilities, thus post-transportation measurements (blood samples, assessing hydration status, and remove activity loggers) were collected ≤2 h after unloading at the calf-raising facilities. Measurements are described in more detail in subsequent sections. Transporters picked up calves from other source dairies before unloading enrolled calves at calf-raising facilities; the total number of source dairies represented in each trailer group was provided by drivers and recorded by the research team.

**Animal management before transportation on source dairies**

On both source dairies, calves were removed from the dam after being licked off and subsequently received approximately 2L of colostrum of a known quality (≥22% BRIX) via a bottle within 1–2 h after birth followed by a second 2L feeding approximately 4–6 h later. Both source dairies used an iodine-based solution to disinfect navels and sold all male Holsteins and all beef x dairy crosses. Calves on source dairy 1 were housed in outdoor pens measuring approximately 3m x 3m; calves were picked up 3 times per week and stayed in the pen until transported to the calf-raising facility, while receiving 2L of whole milk twice per day. Calves on source dairy 2 were housed in an indoor pen measuring approximately 6m x 6m and were transported to the calf-raising facility daily, but only after receiving 2 feedings of colostrum. The time of feeding milk or colostrum relative to transportation varied by source dairy and time of transport; however, all calves received either milk or colostrum within 6 h of being transported. A cohort was defined as calves that were transported together from the same source dairy on the same day.

**Calf handling during loading and unloading**

Research staff recorded the time at which the trailers departed the source dairy and arrived at the calf-raising facilities to determine transportation duration; this information was recorded and reported at the cohort level. The drivers loaded calves on and off the trailers and research staff did not assist with this process. Calves were loaded onto trailers by either gently walking them from their pen onto the trailer (source dairy 1) or picking calves up with one arm around the calf’s hind end and one arm under the calf’s chest (source dairy 2). Calves were unloaded either by gently walking them off the trailer and onto an electronic scale to obtain body weights at arrival according to existing facility procedures and then into calf hutches (calf-raising facility 1) or by picking calves up in the same manner as when loading and placing calves in their hutches (calf-raising facility 2).

**Trailer measurements**

Trailer measurements were collected and reported at the cohort level. Ambient temperature and humidity loggers (Elitech USB Temperature Data Logger; RC-51; San Jose, CA) were attached to the interior of the trailer at the center of the side panel, at 60 cm from the floor of the trailer. The logger was set to record the temperature and humidity every minute. Bedding depth was assessed at the center of the trailer by placing a tape measure perpendicular to the trailer floor such that the tape measure touched the bare surface of the trailer floor but did not disturb the bedding. Square meters per calf was determined by measuring the interior of the trailer and dividing it by the total number of calves in the trailer after loading at the source dairy (square meters per calf at loading) and the total number of calves in the trailer just before unloading at the calf ranch (square meters per calf at unloading).

**Health measurements and weight**

Health measurements were collected and analyzed at the calf level. Two researchers performed all health exams throughout the study and interobserver agreement was calculated before study commencement using 20 calves <1 week of age (kappa >0.9; R^2 = 0.93). Research staff performed health exams in the group pens where calves were housed before transportation. Health scores were recorded in an app (https://www.vetmed.wisc.edu/dms/fapm/apps/chs.htm) and included a clinical respiratory score (McGuirk and Peek, 2014), lung ultrasound exam (Ollivett and Buczinski, 2016), and fecal (McGuirk, 2008), navel (Fecteau et al., 1997),
Blood collection and analysis

Blood samples were collected and analyzed at the calf level. Research staff collected approximately 3–5 mL of jugular venous blood using a serum blood collection tube (BD Vacutainer®, Becton, Dickson, and Company; Franklin Lakes, NJ) and a 1-in x 20-gauge needle before and after transportation (Exelint International Co., 2500 Santa Fe Avenue, Redondo Beach, CA). Blood was immediately analyzed for glucose and lactate by applying a small drop of blood to strips inserted into meters. Blood glucose was analyzed using a glucose meter and test strips (ReliOn Premier, Walmart Inc. Bentonville, AR). Blood lactate was analyzed using a lactate meter and test strips (Lactate Plus; Nova Biomedical; Waltham, MA; Burfeind and Heuwieser, 2012). Blood samples were then stored on ice until research staff returned to laboratory facilities, at which time samples were centrifuged at 3,000 × g for 20 min at 20°C. Serum was then pipetted onto a refractometer to determine serum total protein values (Misco DD-3; Solon, OH). Serum total protein values were categorized into excellent (total protein ≥6.2 g/dL), good (total protein from 5.8 to 6.1 g/dL), fair (total protein from 5.1 to 5.7 g/dL), or poor (total protein <5.1 g/dL; Lombard et al., 2020).

Behavioral data

Behavioral data were collected and analyzed at the calf level. Accelerometers (IceQube; Ice Robotics; South Queensferry; United Kingdom) were attached to the lateral aspect of the right hind leg using several 10 cm x 10 cm pieces of gauze to provide cushion between the logger and the calf’s leg and secured with self-adhesive flexible bandages (Finney et al., 2018). Accelerometers were attached to calves before loading at the source dairy and removed after unloading at the calf ranch. Trailer departure and arrival times were recorded; only behavioral data from the time calves were in transit was used in analysis. Ten accelerometers were available for use; if more than 10 calves were transported on a given day, research staff randomly selected which calves would have an accelerometer attached to them. To randomly select calves, calf ID numbers were recorded on a data sheet in numerical order. Then, calf ID numbers were cross-referenced with a pre-determined randomized list numbered 1–30 (a maximum of 30 calves could be transported by either source dairy), with 10 of those randomly assigned to receive an accelerometer.

Statistical analysis

Data were stored and cleaned in Excel (Microsoft Corp., Redmond, WA) and analyzed in SAS v. 9.4 software (SAS Institute Inc., Cary, NC). A sample size calculation (PROC POWER) was performed using a difference of 1.61 and a SD of 2.7 mmol/L in blood glucose between calves that were transported 6 h compared with 18 h (Marcato et al., 2020); the estimated sample size was inflated by 25–30% to account for lack of independence between cohorts and potential loss to follow-up, thus our target sample size was approximately 120 calves total.
Raw calf body weights are reported as mean ± SD. The PROC FREQ procedure was used to calculate frequencies for health events and serum total proteins. Only 6 calves were 3 d old at the time of transport; thus, for analysis purposes, age categories of 2 d and 3 d were collapsed into one category (“2-3 d”).

**Multivariable analyses**

The predictors of interest were age (categorical: < 24 h, 1, and 2–3 d) and transportation duration (continuous). The effects of age and transportation duration were modeled separately. The following explanatory variables were assessed for their association with all outcomes in initial screening to determine which variables should be offered into the multivariable models: serum total protein category, breed, sex, diarrhea, navel inflammation, and joint inflammation. Univariate analyses (Chi-squared and 2 sample t-tests) were used to determine if an explanatory variable was associated with the outcome of interest and the predictor of interest. No explanatory variables were associated with both the predictor of interest and the outcome of interest at the P < 0.2 level, and therefore were not offered to the multivariable models (Dohoo et al., 2014). Final models included either age or transportation duration, and source dairy as a fixed effect and cohort as a random effect to account for the lack of independence between calves transported together in the same cohort. Continuous data were assessed for normality using PROC UNIVARIATE in SAS. Cook’s Distance was used to identify potential outliers (defined as Cook’s Distance > 4/n; Dohoo et al., 2014).

Separate logistic multivariable models with binary distributions (PROC GLIMMIX) were used to determine if the predictors of interest (age: categorical; transportation duration: continuous) were associated with dehydration status before transportation (yes/no) and dehydration status after transportation (yes/no). Only calves who were not dehydrated before transportation were included in the analysis for dehydration status after transportation.

Changes in blood glucose and lactate were calculated as: change = value after transportation – value before transportation. To determine if the predictors of interest (age: categorical; transportation duration: continuous) were associated with the change in blood glucose and the change in blood lactate, separate linear multivariable models (PROC MIXED) were constructed.

Total time spent lying and standing were calculated for each calf by summing durations for each behavior while calves were in transit; data from accelerometers that were recorded when calves were not on the trailer were excluded from analysis. To account for varying transportation duration, the proportion of time spent lying or standing was calculated by (total time in minutes / total transportation duration). The proportions of time spent lying and standing were modeled separately for each outcome using PROC MIXED. For linear mixed models, residuals were plotted to ensure model assumptions were met. Interactions between all fixed effects were assessed in every model, but none were significant (P > 0.8).

**RESULTS**

**Description of population**

A total of 126 calves were enrolled in the study. Forty-three percent of calves were male beef x dairy (n = 54/126), followed by 41% Holstein males (n = 52/126), and 16% female beef x dairy (n = 20/126). Calves were enrolled across 16 cohorts (median: 7 calves per cohort; min: 4; max: 12) and 2 source dairies (farm 1: n = 34/126 calves; farm 2: n = 92/126 calves). Sixty-four percent (n = 81/126), 19% (n = 24/126), and 13% (n = 17/126) of calves were < 24 h, 1 d, and 2–3 d old at the time of transportation, respectively. Age at transport did not differ between breeds (P = 0.21).

**Transportation conditions**

Calves were transported mean ± SD 249 ± 73 min, and transport duration did not differ between source dairies (P > 0.05; Figure 1). Ambient temperature and relative humidity in trailers during transportation are shown in Table 1. Trailers had 2.95 ± 1.5 cm of bedding depth and were bedded with straw (n = 5 cohorts), shavings or sawdust (n = 9), or a combination thereof (n = 2). The mean ± SD square meters per calf was 262.78 ± 146.07 at loading and 105.12 ± 68.17 at unloading. Milk or water were not provided in the trailers. Transportation drivers picked up additional calves from an average of 4 (min: 2; max: 4) sources dairies for transport to calf-raising facilities.

**Weight, health status, and serum total proteins**

Calves weighed an average of 54 ± 3.97 kg before transportation. Nearly half of all calves (49%, n = 62/126) were identified with at least one of the following health abnormalities before transportation: diarrhea, navel inflammation, joint inflammation, dehydration (Table 2). Ultrasonographic lung lesions were found in 5% (n = 7/126) of calves and 2% (n = 3/126) had a combination of at least 2 clinical abnormalities associated with the respiratory tract (nasal discharge, droopy ears, and fever) despite normal ultrasonographic ap-
pearance of their lungs. Thirty-eight calves were >24 h of age at the time of transport, and thus were of the acceptable age to test serum total proteins; however, the research team was unable to obtain blood samples from 3 of these calves due to dehydration and 1 sample was damaged and therefore unable to be analyzed for serum total protein. Thus, serum total protein was assessed for n = 34 calves. Of those calves, 82% (n = 28/34), 9% (3/34), 6% (2/34), and 3% (1/34) had serum total protein values in the excellent, good, fair, and poor categories, respectively (Lombard et al., 2020).

Dehydration

Thirty-seven percent (n = 47/126) of calves were dehydrated before transportation, whereas 56% (n = 71/126) of calves were dehydrated after transportation. Dehydration status pre and post transportation, by calf age is shown in Table 3. Age was associated with hydration status at loading whereby calves < 24 h of age were 5.29 times as likely to be dehydrated at loading compared with calves that were 1 d of age (95%CI: 1.53 to 18.34; P = 0.02). For calves that were not dehydrated before loading (n = 79), age (P = 0.35) was not associated with the odds of being dehydrated after transportation. Similarly, for calves that were not dehydrated before loading (n = 79), there was no association between transportation duration and the odds of being dehydrated after transportation (P = 0.60). Source dairy was not significant in any models for dehydration (P > 0.20).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (Q1, Q3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum temperature (°C)</td>
<td>17.9 (13.1, 24.3)</td>
</tr>
<tr>
<td>Minimum temperature (°C)</td>
<td>6.4 (3.3, 6.8)</td>
</tr>
<tr>
<td>Maximum relative humidity</td>
<td>50.9 (49.3, 68.4)</td>
</tr>
<tr>
<td>Minimum relative humidity</td>
<td>28.9 (18.8, 36.4)</td>
</tr>
<tr>
<td>Temperature change within a trip</td>
<td>13.6 (10.2, 17.8)</td>
</tr>
</tbody>
</table>

1Measured using a temperature and humidity logger; for each cohort of calves, the maximum and minimum temperature and relative humidity were recorded.

Table 2. Proportion and number of preweaned calves (age range: < 24 h to 2-3d; n = 126) with abnormal health conditions identified at the source dairy before transportation. Health categories are not mutually exclusive.

<table>
<thead>
<tr>
<th>Condition</th>
<th>%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Joint inflammation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Navel inflammation</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 1. Histogram of transport duration (minutes) for preweaned calves (n = 126) transported from the source dairy to a calf raising facility.

Table 1. Median (1st quartile, 2nd quartile) trailer temperature and relative humidity during transit.
Blood glucose and lactate

The research team was unable to obtain a blood sample from 3 calves because calves were dehydrated, thus analysis for blood glucose and lactate included n = 123 calves. Raw mean ± SD of blood glucose was 7.05 ± 1.51 and 6.95 ± 1.66 mmol/L pre- and post-transportation, respectively. Age was not associated with glucose pre-transport (P = 0.45) or change in glucose (P = 0.28). There was no association between transportation duration and the change in glucose (P = 0.97). Raw mean ± SD of blood lactate was 2.05 ± 5.3 and 2.34 ± 5.9 mmol/L pre- and post-transportation, respectively. Age was not associated with lactate pre-transport (P = 0.19) or change in lactate (P = 0.94). There was no association between transportation duration and the change in lactate (P = 0.62). Source dairy was not significant in any models for blood glucose or lactate (P > 0.30).

Behavior

Ninety calves were fitted with loggers on the source dairy. Thus, analysis for behavior included a total of 90 calves. Calves spent an average of 21.93% (±12.92%) of their time on the trailer standing and 78.07% (±12.73%) of their time lying. Age was not associated with the proportions of time spent lying (P = 0.63) or standing (P = 0.5195). Transport duration was also not associated with behavior (P > 0.60). Behavior did not differ between source dairies (P > 0.52).

DISCUSSION

To the authors’ knowledge, this is the first study to collect pre- and post-transportation health measurements, as well as to characterize behavior during transportation in beef x dairy and Holstein bull calves less than 4 d of age in the Western United States. Although the study only included 2 source dairies, the study provides foundational information about transportation practices in the US. While exact numbers of beef x dairy and dairy bull calves that are transported off of dairies are unknown, it is estimated that approxi-mately 5 million head of cattle with dairy influence (either pure dairy or beef on dairy) could enter the US beef supply annually (Foraker et al., 2022). Thus, there are potentially millions of these calves leaving the dairy and at risk for poor welfare before, during, and after transportation if not managed properly. With increasing attention to welfare concerns regarding transportation of young calves (Creutzinger et al., 2021; Roadknight et al., 2021), understanding the impacts of transportation in a variety of populations is critical to mitigate transportation stress. In addition to describing the health status of these calves leaving source dairies and characterizing transportation conditions and calf behavior during transportation, we also identified an association between age and dehydration status before transportation.

Condition of calves leaving the dairy

Ensuring calves are healthy before transportation is a critical component of calf welfare during and after transportation. Condition of adult cattle leaving the dairy has been a big focus in the dairy industry (i.e., “fitness for transport”; (Edwards-Callaway et al., 2019), but is recently becoming more prominent in calves. For example, the Calf Care Quality Assurance (CCQA) program, addresses calf fitness for transport in their Animal Care Manual (CCQA, 2021). The CCQA manual provides guidance on characteristics that would deem a calf unfit for transport, which include dehydration and active cases of disease (CCQA, 2021). In the present study, approximately half of all calves were identified with at least one abnormal health condition (i.e., navel inflammation, diarrhea, dehydration), which would deem them unfit for transportation. Similarly, Wilson et al. (2020) identified at least one health abnormality in 37% of bull calves (median age: 5 d) before transportation in Canada, with health abnormality prevalence ranging from 15 to 67% depending on the farm. Some welfare concerns associated with transporting young calves include food and water deprivation, dehydration, stress, muscle fatigue, discomfort, morbidity, and mortality (Creutzinger et al., 2021; Roadknight et al., 2021). When a calf is ill, not only are the
stressors associated with transportation compounded, but disease and dehydration can become exacerbated during transport (CCQA, 2021). Pre-transport health is also an important consideration for subsequent health (Wilson et al., 2020b). For example, navel inflammation and behavioral attitude identified before transport were associated with increased odds of diarrhea post-transport at a grower (Wilson et al., 2020b). Transporting calves who are in poor condition may also help explain the high morbidity observed in bob veal calves in the US; for example, one study reported that 68% of calves arrived at a slaughter facility dehydrated and 26% had inflamed navels (England et al., 2023). Our results as well as the results of others (Wilson et al., 2020b), indicate that there is ample opportunity to improve decision-making at source dairies to ensure calves are fit for transport.

Dehydration

Dehydration may occur when an animal is losing fluid (e.g., during diarrhea), has inadequate fluid intake (e.g., not drinking colostrum or milk), or a combination thereof. Dehydration is a welfare concern because moderate to severe dehydration may result in thirst, lethargy, or other unpleasant affective states (Smith, 2009; Kells et al., 2020; Roadknight et al., 2021). Notably, the present study found that calves <24 h of age had greater odds of being dehydrated before transport compared with calves aged 1 d. Given that diarrhea prevalence did not differ by calf age, this could be due, in part, to the normal weight and fluid loss that occurs when a neonate is adapting to extrauterine life (Morton and Brodsky, 2016). In the present study, we used skin tent duration to identify calves who were at least mildly dehydrated (Smith, 2009). We chose to use skin tent duration as a measure of dehydration because this test can be easily performed on-farm in commercial settings and could potentially be used in the industry to assess hydration status to determine if calves are fit for transport. Further, we chose a skin tent duration threshold of 2 s as the cutoff for dehydration, which is indicative of mild dehydration. Given that dehydration only worsens during transport, we wanted to have a low threshold before transportation.

Age and transport duration were not associated with the odds of being dehydrated after transport. However, this analysis was only performed using calves with normal hydration status pre-transport. With a larger sample size, we could have further investigated if transportation worsened dehydration in calves who were dehydrated before transport. Dehydration in calves after transportation is not uncommon. For example, England et al. (2022) found that 68% of bob veal calves were dehydrated on arrival to an abattoir. Not only is dehydration an important indicator of calf health and welfare, dehydration at arrival to veal facilities has been associated with at least a 0.2 kg/day reduction in ADG and greater hazard of mortality (Renaud et al., 2018a; b). Although calves in the present study were transported for a relatively short duration, longer transport times have been associated with increased odds of dehydration post-transport (Goetz et al., 2023b). More research is needed to understand the impact of transportation on hydration status in calves, especially calves ≤3 d of age given that is the average age at transport in the US (USDA, 2012). Additionally, accurate methods to assess dehydration that can easily be performed on-farm, especially in calves ≤3 d of age, are necessary to ensure that calves who are dehydrated are not transported and are treated with fluid therapy immediately.

Blood glucose and lactate

Blood glucose is an indicator of energy metabolism and is important for transport studies, as calves are typically off feed during transport, resulting in prolonged fasting (Creutzinger et al., 2021; Roadknight et al., 2021). We did not observe an association between transport duration on the change in blood glucose concentrations. Similarly, blood glucose concentrations were not different between calves transported 6 versus 12 h in an Australian study (Fisher et al., 2014). In contrast, calves transported for 16 h had significantly lower glucose concentrations post-transport compared with calves transported for 6 h (Goetz et al., 2023a). In the present study, age was also not associated with the change in blood glucose concentrations, which agrees with Goetz et al. (2023a). Different results between studies examining blood glucose are likely due to several factors including transport duration, calf age, energy requirements during transit, and time of transport relative to feeding. Hypoglycemia has been noted in 73% of bob veal calves arriving to slaughter in the US (England et al., 2023). No calves in the present study were identified as hypoglycemic, defined as 4.95 mmol/L (Renaud et al., 2022; England et al., 2023), likely due to the relatively short transport duration (and therefore shorter feed withdrawal times) and because all calves received either milk or colostrum within 6 h of being transported. Standard protocol on the source dairies in the present study was to provide adequate colostrum and milk before transportation, which is not always standard practice for dairy bull calves (Shivley et al., 2019; Renaud et al., 2020; Creutzinger et al., 2021) and this could therefore influence blood glucose levels. To our knowledge, no studies to date have measured
the effect of transportation on glucose in calves <24 h of age, thus, our study provides important insights of transporting neonatal calves. More work is needed to determine how long-distance transport, and therefore prolonged feed-withdrawal, affects blood glucose in calves <3 d of age. Future studies should focus on transport impacts in calves that might receive suboptimal colostrum management before leaving the dairy.

Blood lactate concentrations assess the metabolic state of livestock, in particular anaerobic states, and greater concentrations may indicate hypoperfusion, sepsis, exercise, and hypoxemia (Smith, 2019). Lactate is a useful measurement in transportation studies because increased concentrations can indicate physical exertion and may be a physiological indicator of poor welfare during transport (Thomson et al., 2015). In the present study, there was no association between transport duration or age and the change in lactate concentration, which agrees with previous studies in calves 5–10 d of age transported varying durations (Todd et al., 2000; Fisher et al., 2014). However, Bernardini et al. (2012) found that blood lactate was significantly greater immediately post-transport compared with pre-transport in 37 d old calves transported 19 h. Differences between studies could be due to calf age, as neonatal calves are still developing their stress and immune responses and therefore may respond differently physiologically compared with older animals (Hulbert and Moisá, 2016). Future studies should consider collecting a variety of physiological and behavioral measurements to better understand the neonatal calf’s stress response to transportation.

**Behavior**

Understanding calf behavior during transport may help inform decisions about trailer design and provide additional insight as to how transportation impacts welfare. Our results provide important information about the behavior of young calves during short distance transportation. Lying behavior during transport is especially important because when calves lay down, they are able to rest and the risk of slips and falls is reduced (Roadknight et al., 2021). Calves in the present study spent nearly 80% of their time lying during transport. Similarly, veal calves were found to spend the majority of their time lying during transit; however, calves in this study were an average of 18 d of age at the time of transport (Marcato et al., 2020). Previous studies of calves in various housing systems (i.e., not during transportation) have also reported that calves spend over 70% of their day lying down (Wilson et al., 1999; Chua et al., 2002; Panivivat et al., 2004; Camiloti et al., 2012). Although we did not observe an association between age and behavior in the present study, a previous study found that 3-d old calves lay down more compared with 5 or 10 d old calves during transportation (Jongman and Butler, 2014). Bajus et al. (2023), also found that transport duration and proportion of time spent lying in 2–19 d old calves were positively correlated. Calves in the present study were in transit for a shorter amount of time overall compared with Jongman and Butler (2014) and Bajus et al. (2023), which could explain why behavior and transport duration were not associated in the present study.

Given that calves spend most of their time lying, it is important that trailers have adequate dry and comfortable bedding. For example, previous studies have shown that calves avoid lying down on bare concrete, and that calves prefer dry surfaces with adequate bedding such as straw or sawdust (Le Neindre, 1993; Camiloti et al., 2012). Data in calves ≤ 3 d of age is limited, however data, including the present study, consistently demonstrate calves spend the majority of time lying. Thus, trailers should be comfortable and include ample, dry bedding to encourage natural lying behavior in calves.

The present study provides useful information about calf transport in the Western US, for which there is currently a paucity of data. The study results are most applicable to dairies transporting calves <3 d of age for shorter transport durations or distances. The authors are not aware of any data that describes transport durations for bull calves in the western US, however approximately 85% of dairies in the US shipped pre-weaned replacement heifers to a heifer-raising facility within 80 km (USDA, 2016). Thus, at least for pre-weaned replacement heifers, shorter duration transport is not uncommon. The present study had a small sample size within each age strata. Previous work has demonstrated that age at transport impacts a variety of factors including growth and health (Goetz et al., 2023b). While we did observe an association between age and dehydration before transportation, age was not associated with behavior, glucose, lactate, or dehydration post-transport. A larger sample size within each age category may allow for detectable differences in these outcomes.

**CONCLUSION**

This was the first study to collect pre- and post-transport health measurements and to quantify behavior during transport in calves ≤ 3 d of age in the United States. Almost half of the calves in the study had health abnormalities (e.g., dehydration, diarrhea, and joint and navel inflammation) before transportation. Calves <24 h of age were more likely to be dehydrated before transportation compared with 1 d old calves and
nearly 60% of calves were dehydrated post-transport. Calves spent most of the time during transit laying down. These findings support the need for: 1) improved decision-making at the dairy so that sick or injured calves are not transported, 2) management interventions that can help reduce the impact of transportation (e.g., providing milk or electrolytes before, during, and immediately after transportation), and 3) special attention to the trailer environment to ensure the space has plenty of dry and comfortable bedding, as well as space to accommodate natural calf behavior. Finally, there is a need to identify accurate ways to assess calf fitness for transport that are practical on farm.

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Conflict of interest and author roles The authors have no conflicts of interest to declare. The first author (Cramer) conceived the study design, conducted the study, drafted the manuscript. Authors Edwards-Callaway, and Roman-Muñiz contributed to study design, data interpretation, and drafting of the manuscript. Machuca assisted with data collection and drafting the manuscript.

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Cramer et al.: Effects of transport on calf welfare


