



## Association between transfer of passive immunity, health, and performance of female dairy calves from birth to weaning

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### ABSTRACT

The objective of this observational study was to compare calf health, average daily weight gain, and calf mortality considering the proposed categories of transfer of passive immunity (TPI) by the consensus report of Lombard et al. (2020). The consensus report defines 4 categories of passive immunity (excellent, good, fair, and poor) of calves obtained after colostrum ingestion. The association between the 4 TPI categories was analyzed on calf health (i.e., hazards for morbidity and mortality), and average daily weight gain (ADG) of female Holstein Friesian calves during the first 90 d of age. A further aim of this study was to examine the effects of calving-related factors, such as dystocia or winter season, on TPI status. We hypothesized that calves with excellent TPI have greater ADG, lower risks for infectious diseases such as neonatal diarrhea, pneumonia, and omphalitis, and lower mortality rates. This observational study was conducted from December 2017 to March 2021. Blood was collected from 3,434 female Holstein Friesian dairy calves from 1 commercial dairy farm. All female calves aged 2 to 7 d were assessed for TPI status by determination of total solids (TS) in serum via Brix refractometry by the farm personnel once a week. Passive immunity was categorized according to Lombard et al. (2020) with excellent ( $\geq 9.4\%$  Brix), good (8.9–9.3% Brix), fair (8.1–8.8% Brix), or poor TPI ( $< 8.1\%$  Brix). For the analysis of ADG and calving ease 492 or 35 calves had to be excluded due to missing data. The distribution of calves according to TPI categories was as follows: 4.8% poor ( $n = 166$ ), 29.5% fair ( $n = 1,012$ ), 28.3% good ( $n = 971$ ), and 37.4% excellent ( $n = 1,285$ ). From the calving-related factors, parity of the dam, calving ease, birth month, calving assistance by different farm personnel, and day of life for TPI assessment were significantly associated with TS concentration. Out of 3,434 calves, 216 (6.3%)

had diarrhea, and 31 (0.9%) and 957 (27.9%) suffered from omphalitis and pneumonia during the first 90 d of life, respectively. Overall, the morbidity during the preweaning period was 32.6% ( $n = 1,118$ ), and the mortality was 3.1% ( $n = 107$ ). The ADG was  $0.90 \pm 0.15$  kg with a range of 0.32 to 1.52 kg. The Cox regression model showed that calves suffering from poor TPI tended toward a greater hazard risk (HR) for diarrhea (HR = 1.57, 95% CI: 0.92–2.69) compared with calves with excellent TPI. Calves suffering from TPI had a greater HR for pneumonia (HR = 2.00, CI: 1.53–2.61), overall morbidity (HR = 1.99, CI: 1.56–2.55), and mortality (HR = 2.47, CI: 1.25–4.86) in contrast to excellent TPI. Furthermore, calves with good and fair TPI had significantly greater HR for pneumonia (good TPI: HR = 1.35, CI: 1.15–1.59; fair TPI: HR = 1.41, CI: 1.20–1.65) and overall morbidity (good TPI: HR = 1.26, CI: 1.09–1.47; fair TPI: HR = 1.32, CI: 1.14–1.53) compared with the excellent TPI category. Average daily weight gain during the first 60 d of life was associated with TPI categories. Calves with excellent and good TPI status had ADG of  $0.90 \pm 0.01$  kg/d and  $0.92 \pm 0.01$  kg/d (mean  $\pm$  SE), respectively. The ADG of calves with fair TPI status was  $0.89 \pm 0.01$  kg/d, and calves suffering from poor TPI had  $0.86 \pm 0.01$  kg/d. Average daily weight gain differed in calves with poor TPI compared with the other categories. Fair and excellent TPI differed additionally from good TPI. We found no statistical difference between the TPI categories fair and excellent. In conclusion, poor TPI was associated with higher morbidity and mortality during the first 90 d of life. Furthermore, calves with fair, good or excellent TPI had greater ADG.

**Key words:** categories of passive immunity, neonatal calves, average daily weight gain, hazard for disease

### INTRODUCTION

Sound evidence suggests that adequate supply of colostrum to the neonatal calf after birth is crucial for its passive immunity and essential for calf health (Cutance et al., 2018; Lora et al., 2018; Godden et al., 2019). During gestation, the bovine cotyledonary syn-

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epitheliochorial placenta impedes the transfer of maternal immunoglobulins (Ig) from the dam to the fetus, and the calves are born immunonaive (Barrington and Parish, 2001; Furukawa et al., 2014; Fischer-Tlustos et al., 2021). Therefore, the intestinal absorption of maternal Ig, especially IgG, via colostrum is essential for successful passive transfer in neonatal dairy calves (Weaver et al., 2000; Godden et al., 2009; Godden et al., 2019). Because the calf is dependent on the maternal Ig, the timely delivery of high-quality colostrum in a sufficient quantity is necessary for a high intestinal absorption rate during the first 24 h after birth. This process is known as transfer of passive immunity (TPI) and it is necessary to protect the calf against pathogens and common diseases (Weaver et al., 2000; Godden et al., 2009; Godden et al., 2019). Furthermore, an appropriate colostrum volume for first feeding (~10% of bodyweight, 4 L in Holstein Friesian calves) positively affects the calf's metabolism (i.e., reduced mortality rates, higher ADG; Blum and Hammon, 2000; Fischer-Tlustos et al., 2021) and may influence its performance as an adult dairy cow (i.e., reduced age at first calving; Faber et al., 2005). This is related to other components of colostrum. Important non-immunoglobulin immune factors, termed milk-borne factors (e.g., insulin-like growth factor I, epidermal growth factor), are provided by colostrum feeding, as well as energy, fluid intake, and heat support for the thermoregulation (Blum and Hammon, 2000; Bagnell et al., 2009; Silva and Machado Bittar, 2019; Fischer-Tlustos et al., 2021).

Methods for TPI assessment can be performed in calves aged 24 h to 7 d by direct measurement of IgG or indirect measurement of total protein or TS in calf serum or plasma. During the last decades the standard for categorizing dairy calves with successful passive immunity or failed transfer of passive immunity (FTPI) was defined as serum IgG concentrations above or below 10 mg/mL in 24- to 48-h-old calves, respectively (Weaver et al., 2000; McGuirk and Collins, 2004; Godden, 2008). This threshold for serum IgG helped to reduce calf mortality in heifer calves (USDA, 1996, 2018). Interestingly, calf morbidity has still not declined in the United States (USDA, 1994, 2018; Lombard et al., 2020). According to Lombard et al. (2020), new recommendations for TPI assessment could improve this pending problem. These recommendations were made based on conference calls with various academic and calf health professionals to discuss different publications and the data from the NAHMS study (Dairy 2014, calf component; Urie et al., 2018a,b). Groups of calf experts were established to evaluate the data and identify whether changes to passive immunity standards were necessary to reduce morbidity and potentially mortality. New TPI suggestions were made based on 4 serum

IgG categories: excellent ( $\geq 25.0$  g/L;  $\geq 9.4\%$  Brix), good (18.0–24.9 g/L; 8.9–9.3% Brix), fair (10.0–17.9 g/L; 8.1–8.8% Brix), and poor ( $< 10$  g/L;  $< 8.1\%$  Brix; Lombard et al., 2020). Regarding the herd level,  $> 40\%$  of the calves should achieve the excellent category, and 30%, 20%, and  $< 10\%$  of calves should meet the good, fair, and poor TPI categories, respectively. This new classification has the potential to contribute a reduction of morbidity in dairy calves; thus the risks of pre-weaning morbidity and mortality and among these TPI categories need to be compared.

The objective of this observational study was to compare calf health (i.e., hazards for morbidity and mortality) and ADG between the proposed recommendations for TPI assessment by the consensus report of Lombard et al. (2020) of female Holstein Friesian calves during the first 90 d of age. In addition, we aimed to evaluate the effects of calving-related factors (e.g., dystocia, winter season, farm employee providing calving assistance and neonatal care) on TPI status. We hypothesized that calves with excellent TPI have greater ADG, lower risks for infectious diseases such as neonatal diarrhea, pneumonia and omphalitis, and lower mortality rates during the first 90 d of age. Furthermore, we hypothesized that calving-related factors, such as dystocia or the winter season, negatively affect the TPI status.

## MATERIALS AND METHODS

### Dairy Farm and Animal Enrollment

This observational study was conducted from December 2017 to March 2021 on a commercial dairy farm in Mecklenburg-Vorpommern, Germany. Because all the required material was obtained during routine farm management practices to monitor poor TPI, calf health, and heifer development, an exemption from protocol review of the Institutional Animal Care and Use Committee of the Freie Universität Berlin was granted. The farm owned approximately 2,500 Holstein cows with an average annual milk yield of 11,000 kg/cow. Heifers and cows were housed in a naturally ventilated transition management facility consisting of 4 freestall pens, providing cubicles with sand bedding and ad libitum access to feed and water from drying off to the first 20 DIM (36 heifer cubicles and 144 cow cubicles). All cows were vaccinated during the dry period, and prepartum heifers were vaccinated before the first calving against *Escherichia coli*, bovine rota- and coronavirus (Rotavec Corona, MSD Animal Health, Intervet Deutschland GmbH, Unterschleißheim, Germany) to improve colostrum quality. The vaccination was carried out approximately 6 wk before calving.

Prepartum cows and heifers were monitored every 30 min to detect signs of imminent parturition (Lange et al., 2017). The dams were moved into an individual maternity pen (3.5 m<sup>2</sup>), when the amniotic sac or calf feet were visible outside the vulva. A manual vaginal examination was conducted in every animal transferred to the maternity pen to assess dilatation of the vulva and cervix, as well as position, posture, presentation, and vitality of the calf. Calving assistance was provided to reduce calf losses, if the cow had not delivered the calf 1 h after moving the cow to the maternity pen (Schuenemann et al., 2011). Intensity of calving assistance was recorded on a 4-point scale (1 = no assistance; 2 = assistance by 1 person; 3 = assistance by at least 2 persons; 4 = cesarean section). Postpartum cows were milked 3 times daily (0600 h, 1400 h, and 2200 h). A detailed description of the calving management and the first milking after calving has been described previously (Sutter et al., 2019).

Immediately after birth, calves were separated from their dams and were weighed with an electronic scale (WA200 mobile platform scale, Meier-Brakenberg GmbH & Co. KG, Extertal, Germany) before placing them into a hutch (1.5 × 1 m) bedded with fresh straw for the first 24 h following birth. All newborn calves had their navels dipped using a 10% tincture of iodine solution (Braunol, B. Braun Melsungen AG, Melsungen, Germany). Standard operating procedures were in place for colostrum feeding and Brix refractometry. The first meal consisted of 4 L of pasteurized (Perfect Udder, Dairy Tech Inc., Greeley, CO), pooled, high-quality colostrum (containing ≥22% Brix; Biemann et al., 2010) and was fed approximately 30 min after calving using an esophageal tube feeder (Dairymac Drencher, Dairytop, Beilen, Netherlands). At 6 to 12 h after the first meal, the female calves received another 2 L of colostrum. All female calves aged 2 to 7 d were assessed for passive immunity by determination of TS in serum by the farm personnel. On average, 30 animals were tested per week. Calves with clinical signs of dehydration were excluded from the blood testing. Blood withdrawal was performed by jugular vein puncture once a week. Blood samples were centrifuged at 4,500 × g for 6 min at approximately 20°C on the farm 1 to 2 h after collection and were directly assessed via a digital Brix refractometer (Misco PA201, Misco, Solon, OH). Calibration of the Brix refractometer was performed once a week with distilled water by the farm personnel.

On d 2 of life the female calves were moved to the calf barn and placed into individual pens bedded with straw. Water and starter grain (20% CP; 6.7 MJ NE<sub>1</sub>/kg) were provided ad libitum from d 1 and 7 of life, respectively. The feeding protocol consisted of feeding twice daily pasteurized whole milk using individual teat

buckets. Milk replacer (Pro Elite 60, Dairytop, Beilen, the Netherlands; 24% CP, 18.5% crude fat, and 60% skim milk powder) was added to increase milk solids to reach 170 g/L. The quantity fed was 3.5 L twice daily in the first week of life, 2 × 4 L from d 7 to d 10, and 2 × 4.5 L from d 10 until 60 d of age. Afterward the milk volume was reduced stepwise during 24 d until 1 L once daily was fed. Weaning finally ended at 90 d of age. In the third week of life, female calves were reallocated into 35-m<sup>2</sup> group housing pens (10 calves/pen) until weaning. Each group was fed via a calf group feeder equipped with 10 teats (Milk Bar 10, Milk Bar, Oldenburg, Germany). During group housing the calves had access to TMR consisting of a mixture of concentrate, chopped straw, soy meal, molasses, and chalk.

Weighing was performed directly after birth and at d 60 with an electronic scale (WA200 mobile platform scale, Meier-Brakenberg GmbH & Co. KG, Extertal, Germany).

Calves were considered clinically healthy when visible signs of disease were not observed. Appearance of disease was monitored once daily by the farm personnel using the calf health scoring chart created by the University of Wisconsin. The chart applies validated methods for fecal scoring (McGuirk, 2008), respiratory screening (McGuirk and Peek, 2014), and detection of omphalitis. Farm personnel were trained to assess clinical signs and apply treatments according to veterinarian's instructions. Neonatal diarrhea was defined as visibly loose feces of decreased consistency (Renaud et al., 2020). Pneumonia was defined as visibly detectable tachypnea with nasal discharge (serous, mucous, or purulent) and presence of cough (McGuirk, 2005). Omphalitis was present when a combination of several signs was observed: clinical signs (redness, swelling, purulent discharge, painful response to palpation) in addition to fever (>39.5°C) or lack of appetite (Fordyce et al., 2018). Only cases of disease associated with treatment were included in the statistical analysis. Cases of diarrhea were treated once daily for 3 d with 1.25 mg/kg i.m. danofloxacin (Advocid, Zoetis GmbH, Berlin, Germany). Cases of pneumonia were treated with a single administration of 2.5 mg/kg i.m. tulathromycin (Draxxin, Zoetis GmbH, Berlin, Germany), and cases of omphalitis were treated once daily for 3 d with 15–20 mg i.m. procaine benzylpenicillin (Procain Penicillin G, AniMedica International GmbH, Frankfurt, Germany).

### Data Collection and Statistical Analyses

All prepartum cows and heifers having singleton calves and without cesarean section were included in the study. Relevant data such as date and time of parturition, sex of the calf, twin births, calving assistance

and cesarean section, birth weight and following weight recordings, time of first feeding, identification number, serum TS concentration (in % Brix) reflecting the TPI status, and treatment records during the preweaning period were obtained from the on-farm computer software (DairyCOMP 305; Valley Ag Software, Tulare, CA). The data were transferred to Microsoft Excel (Office 2013, Microsoft Deutschland Ltd., Munich, Germany). Passive immunity was categorized according to Lombard et al. (2020) with excellent ( $\geq 9.4\%$  Brix), good (8.9–9.3% Brix), fair (8.1–8.8% Brix), or poor TPI ( $< 8.1\%$  Brix). Farm personnel were blinded to the TPI category, though the TS result in % Brix was not blinded, as being part of the standard operating procedure of the farm.

Statistical analyses were performed using SPSS for Windows (version 22.0, IBM Corp., Ehningen, Germany). Average daily weight gain was calculated from the difference between weight at 60 d and birth weight divided by the age in days.

Cox proportional hazard models were used to observe the time to event outcomes—that is, occurrence of neonatal diarrhea, omphalitis, pneumonia, overall morbidity (occurrence of diarrhea, omphalitis, and pneumonia in total) as well as mortality—during the first 90 d of life. Cox regression models were performed to create a hazard model for the TPI categories referring to the disease prevalence (diarrhea, omphalitis, pneumonia, and overall morbidity) and mortality during the first 90 d of life, respectively. The hazard model specified the time until an event (disease or mortality) occurred and produced a hazard or survival function considering the different TPI categories. For the morbidity models, calves that died before 90 d of age were censored. Proportional hazard or survival assumptions were illustrated by plotting the  $-\ln [-\ln (\text{disease or mortality})]$  curves for calves during the preweaning period against the  $\ln$  (hazard time or survival time).

To evaluate the association between calving management (parity, calving ease, employee, colostrum harvesting time, colostrum feeding time, day of TPI assessment) and environmental factors (birth month, calving time) as calving-related factors and the TS concentration in % Brix, a generalized linear mixed model was constructed using the GENLINUX procedure of SPSS. Furthermore, 6 generalized linear mixed models were constructed to evaluate the association between TS concentration in % Brix as well as TPI category and ADG (continuous parameter), disease prevalence (diarrhea, omphalitis, and pneumonia), overall morbidity, and mortality during the preweaning period. The outcome variable was either ADG (continuous parameter), diarrhea, omphalitis or pneumonia prevalence, overall morbidity, or mortality (categorical parameters). Calf

was the experimental unit. Each parameter considered for the mixed model was separately analyzed in a univariate model, including the parameter as a fixed factor (i.e., categorical parameter) or covariate (i.e., continuous parameter; Dohoo et al., 2009). Only parameters resulting in univariate models with  $P \leq 0.20$  were included in the final mixed model. Selection of the model that best fit the data was performed by testing each effect separately in a multivariate model and finding the model with the lowest value for the Akaike information criterion, using a backward elimination procedure that removed all variables with  $P > 0.10$  from the model. A significant statistical difference was defined for variables between the levels of a classification when  $P < 0.05$ ; a statistical tendency was specified as differences between  $P \geq 0.05$  and  $P \leq 0.10$ .

The initial model for TS concentration in % Brix contained the following variables as fixed effects: parity (1, 2, and  $\geq 3$ ); calving ease (score 1 to 3); employee responsible for calving, milking, and neonatal care (1 to 8); birth month (1 to 12 mo); calving time during the day (1 to 24 h); colostrum harvesting time after calving (hours, continuous); time of colostrum feeding after calving (minutes, continuous); calf birth weight (continuous); and day of life for TPI assessment (2 to 7 d).

The initial models for ADG during the preweaning period contained the same variables as for % Brix, including TS concentration (continuous) and TPI categories (poor, fair, good, excellent).

The initial models for disease prevalence (diarrhea, omphalitis, or pneumonia), overall morbidity and mortality during the preweaning period contained the same variables as for % Brix, including ADG (kg/d; continuous), TS concentration (% Brix; continuous) and TPI categories (poor, fair, good, excellent). We tested all biologically plausible 2-way interactions. In the case of multiple comparisons, the  $P$ -value was adjusted using a Bonferroni correction.

To evaluate significant differences between ADG, disease prevalence (diarrhea, omphalitis, and pneumonia), overall morbidity and mortality, and the TPI categories (poor, fair, good, excellent) during the preweaning period, a one-way ANOVA was performed using SPSS. The dependent variable was either ADG (kg/d; continuous), disease event (diarrhea, omphalitis, and pneumonia), and overall morbidity and mortality (categorical parameters), and the independent variable was TPI categories (poor, fair, good, excellent).

## RESULTS

From a total of 3,951 records, only 3,434 female dairy calves were included in this observational study from

December 2017 to March 2021. A total of 517 animals were removed from the data set and excluded from the statistical analysis, because TPI data were missing in 340 cases, 5 animals received a cesarean section as calving assistance, and 172 calves were twins. Furthermore, to evaluate the association between calving assistance as well as ADG and TPI, additional 35 (1%) and 492 (14.3%) animals had to be excluded from the statistical analysis because calving was not observed or ADG data were missing, respectively.

The passive immunity of the calves classified in TPI categories was represented as follows: 4.8% poor ( $n = 166$ ), 29.5% fair ( $n = 1,012$ ), 28.3% good ( $n = 971$ ), and 37.4% excellent TPI ( $n = 1,285$ ). First colostrum was fed at  $48.3 \pm 43.4$  min (mean  $\pm$  SD) with a range of 8 to 431 min after calving. Calving assistance was provided in 1,210 dairy cows (score 2,  $n = 628$ ; score 3,  $n = 582$ ). The average birth weight was  $40.2 \pm 4.7$  kg. Second weighing took place at an average age of  $62.3 \pm 9.7$  d, and the average weight was  $96.4 \pm 13.5$  kg. The ADG was  $0.90 \pm 0.15$  kg with a range of 0.32 to 1.52 kg.

Out of 3,434 calves, 216 (6.3%) calves had diarrhea, and 31 (0.9%) and 957 (27.9%) calves suffered from omphalitis and pneumonia, respectively, during the first 90 d of life. Overall, the morbidity during the preweaning period was 32.6% ( $n = 1,118$ ), with 1,032 calves suffering from 1 disease (30.1%) and 86 calves having multiple diseases (2.5%). The mortality was 3.1% ( $n = 107$ ). Descriptive statistics for the TPI categories, parity, calving ease, and ADG, and disease and survival prevalence are summarized in Table 1. Disease prevalence and mortality stratified by TPI category are illustrated in Figure 1A–D.

According to the Cox regression model, the TPI category of the dairy calves affected morbidity and mortality. Compared with calves with excellent TPI, calves suffering from poor TPI had a greater hazard risk (HR) for pneumonia (HR = 2.00;  $P < 0.01$ ; 95% CI: 1.53–2.61), overall morbidity (HR = 1.99;  $P < 0.01$ ; CI: 1.56–2.55), or mortality (HR = 2.47;  $P < 0.01$ ; CI: 1.25–4.86). We found a tendency toward a greater HR for diarrhea (HR = 1.57;  $P = 0.10$ ; CI: 0.92–2.69) for calves suffering from poor TPI compared with calves with excellent TPI. Furthermore, calves with good and fair TPI had significantly greater HR for pneumonia (good TPI: HR = 1.35,  $P < 0.01$ , CI: 1.15–1.59; fair TPI: HR = 1.41,  $P < 0.01$ ; CI: 1.20–1.65) and overall morbidity (good TPI: HR = 1.26,  $P < 0.01$ , CI: 1.09–1.47; fair TPI: HR = 1.32;  $P < 0.01$ , CI: 1.14–1.53) compared with the excellent TPI category (Figure 2A–D).

Parity of the dam ( $P = 0.06$ ), calving ease ( $P < 0.01$ ), birth month ( $P = 0.04$ ), calving assistance of

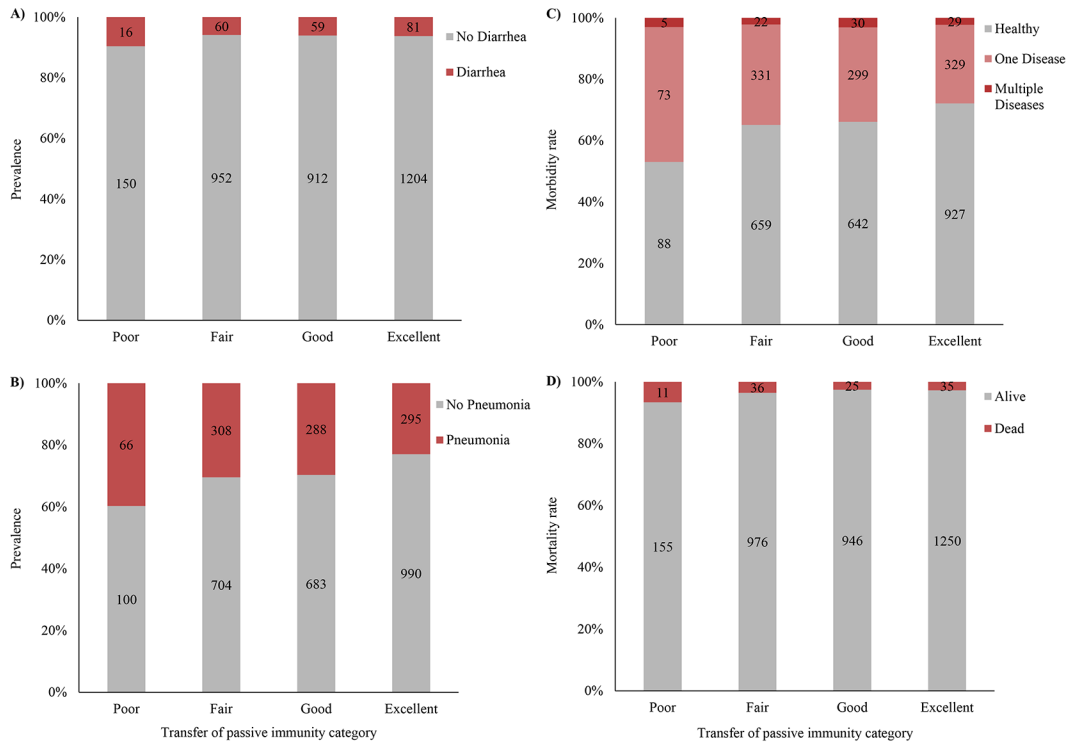
**Table 1.** Descriptive statistics and Pearson chi squared tests for the status of transfer of passive immunity (TPI) in % Brix of female Holstein Friesian calves considering parity ( $n = 3,434$ ), calving ease ( $n = 3,399$ ), ADG ( $n = 2,942$ ), and disease and survival prevalence during the first 90 d of life ( $n = 3,434$ );  $P$ -value adjusted using a Bonferroni correction in multiple comparisons

Item	n	%	Category of passive immunity <sup>1</sup>				%	Excellent TPI	%
			Poor TPI	Fair TPI	Good TPI	Excellent TPI			
Parity	3,434								
Parity 1	1,335	38.9	58 <sup>a</sup>	385 <sup>a</sup>	359 <sup>a</sup>	37.0	533 <sup>a</sup>	41.5	
Parity 2	807	23.5	35 <sup>a</sup>	227 <sup>a</sup>	237 <sup>a</sup>	24.4	308 <sup>a</sup>	24.0	
Parity $\geq 3$	1,292	37.6	73 <sup>a</sup>	400 <sup>a</sup>	375 <sup>a</sup>	38.6	444 <sup>a</sup>	34.6	
Calving ease <sup>2</sup>	3,399								
Score 1	2,189	64.4	77 <sup>a</sup>	615 <sup>b</sup>	637 <sup>b,c</sup>	66.2	860 <sup>c</sup>	67.5	
Score 2	628	18.5	39 <sup>a</sup>	200 <sup>a</sup>	172 <sup>a</sup>	17.9	217 <sup>a</sup>	17.0	
Score 3	582	17.1	50 <sup>a</sup>	181 <sup>b</sup>	153 <sup>b</sup>	15.9	198 <sup>b</sup>	15.5	
ADG (kg/d; mean $\pm$ SE)	2,942		0.86 $\pm$ 0.01 <sup>a</sup>	0.89 $\pm$ 0.01 <sup>b</sup>	0.92 $\pm$ 0.01 <sup>c</sup>		0.90 $\pm$ 0.01 <sup>b</sup>		
Diarrhea	216	6.3	16 <sup>a</sup>	60 <sup>a</sup>	59 <sup>a</sup>	6.1	81 <sup>a</sup>	6.3	
Pneumonia	957	27.9	66 <sup>a</sup>	308 <sup>a</sup>	288 <sup>a</sup>	29.7	295 <sup>b</sup>	23.0	
Omphalitis	31	0.9	1 <sup>a</sup>	7 <sup>a</sup>	1 <sup>a</sup>	1.2	11 <sup>a</sup>	0.9	
Morbidity	1,118	32.6	78 <sup>a</sup>	353 <sup>b</sup>	329 <sup>b</sup>	33.9	358 <sup>c</sup>	27.9	
Mortality	107	3.1	11 <sup>a</sup>	36 <sup>a,b</sup>	25 <sup>b</sup>	2.6	35 <sup>b</sup>	2.7	

<sup>a-c</sup>Rows with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Category of passive immunity: poor =  $<8.1\%$  Brix; fair =  $8.1$ – $8.8\%$  Brix; good =  $8.9$ – $9.3\%$  Brix; excellent =  $\geq 9.4\%$  Brix.

<sup>2</sup>Calving ease: score 1 = no calving assistance; score 2 = calving assistance by 1 person; score 3 = calving assistance by 2 persons.

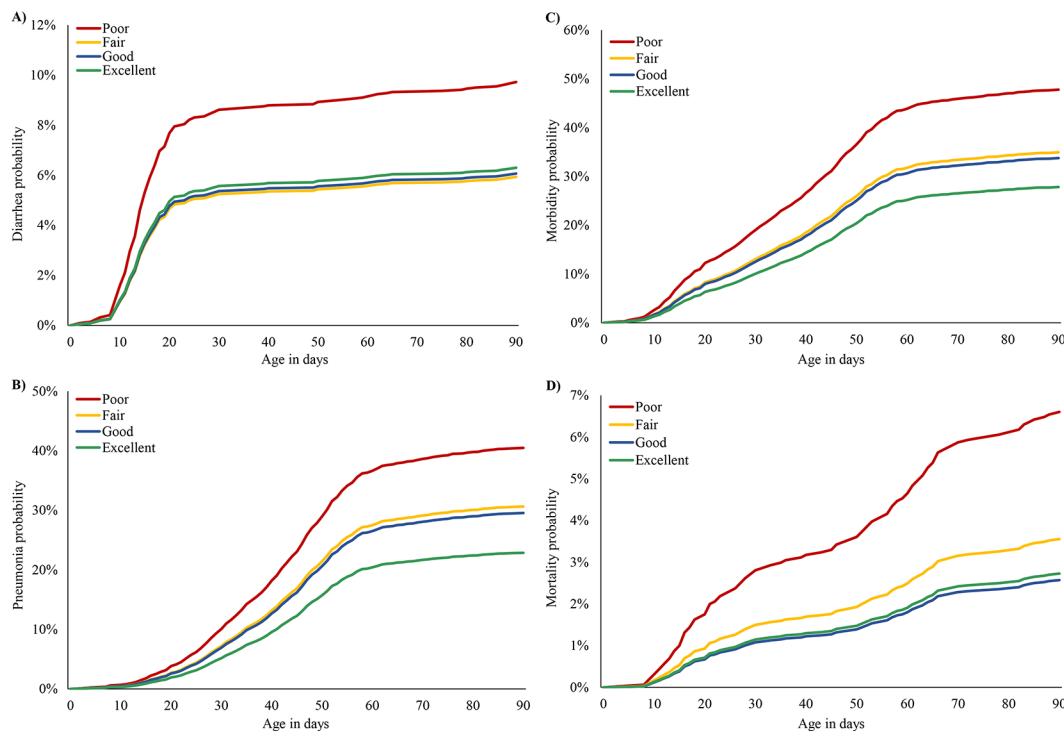


**Figure 1.** (A) Diarrhea prevalence (%) of female Holstein Friesian calves ( $n = 3,434$ ) with different transfer of passive immunity categories (poor:  $<8.1\%$  Brix,  $n = 166$ ; fair:  $8.1\text{--}8.8\%$  Brix,  $n = 1,012$ ; good:  $8.9\text{--}9.3\%$  Brix,  $n = 971$ ; excellent:  $\geq 9.4\%$  Brix,  $n = 1,285$ ) during the first 90 d of life. Neonatal diarrhea was defined as visibly loose feces of decreased consistency. (B) Pneumonia prevalence (%) of female Holstein Friesian calves ( $n = 3,434$ ) with different categories of transfer of passive immunity during the first 90 d of life. Pneumonia was defined as visibly detectable tachypnea with nasal discharge (serous, mucous, or purulent). (C) Morbidity rate (%) of female Holstein Friesian calves ( $n = 3,434$ ) with different categories of transfer of passive immunity during the first 90 d of life. Overall, 1,032 calves were suffering from 1 disease (30.1%), whereas 86 calves had multiple diseases (2.5%). (D) Mortality rate (%) of female Holstein Friesian calves ( $n = 3,434$ ) with different categories of transfer of passive immunity during the first 90 d of life.

different employees ( $P < 0.01$ ), and day of life for TPI assessment ( $P < 0.01$ ) were significantly associated with TS concentration in % Brix (Table 2). Calves from cows in lactation  $\geq 3$  had lower % Brix results in serum than calves from primiparous cows ( $-0.09 \pm 0.03\%$  Brix,  $P < 0.01$ ) or cows of second lactation ( $-0.06 \pm 0.03\%$  Brix,  $P = 0.06$ ). We found no statistical difference between calves from primiparous cows or cows of second lactation ( $P = 0.38$ ). Dystocia had a significant effect on the TS concentration. Calves requiring calving assistance had lower % Brix results (score 2:  $-0.08 \pm 0.03\%$  Brix,  $P = 0.01$ ; score 3:  $-0.14 \pm 0.03\%$  Brix,  $P < 0.01$ ) than calves that were born unassisted. No statistical difference occurred between calves that required calving assistance with 1 or 2 persons ( $P = 0.14$ ). Calves born in the month of April ( $-0.14 \pm 0.06\%$  Brix;  $P = 0.02$ ) had lower % Brix results than calves born in January. The employee providing calving assistance and neonatal care significantly affected the TS concentration (Table 2). Furthermore, the day of life for TPI assessment influenced TS concentration.

Calves sampled on d 2 had significantly higher % Brix results compared with the remaining sampling days ( $P < 0.01$ ; Table 2).

Average daily weight gain during the first 60 d of life was associated with TPI categories ( $P < 0.01$ ; Table 1 and 3). Calves with excellent and good TPI status had ADG of  $0.90 \pm 0.01$  kg/d and  $0.92 \pm 0.01$  kg/d (mean  $\pm$  SE), respectively. The ADG of calves with fair TPI status was  $0.89 \pm 0.01$  kg/d, and calves with poor TPI had  $0.86 \pm 0.01$  kg/d (Table 1). The ADG of calves with excellent or good TPI status were respectively  $0.04 \pm 0.01$  kg/d and  $0.06 \pm 0.01$  kg/d greater than that of calves with poor TPI (Table 3). Furthermore, the ADG of calves with fair TPI was  $0.03 \pm 0.01$  kg/d greater than that of calves with poor TPI (Table 3). Average daily weight gain differed in calves with poor TPI compared with the other categories (fair:  $P = 0.02$ ; good:  $P < 0.01$ ; excellent:  $P < 0.01$ ). Additionally, fair ( $P < 0.01$ ) and excellent TPI ( $P = 0.01$ ) differed from good TPI. No statistical difference was found between the TPI categories fair and excellent ( $P = 0.37$ ).



**Figure 2.** Kaplan-Meier survival analysis illustrating the association of category of transfer of passive immunity (poor: <8.1% Brix,  $n = 166$ ; fair: 8.1–8.8% Brix,  $n = 1,012$ ; good: 8.9–9.3% Brix,  $n = 971$ ; excellent:  $\geq 9.4\%$  Brix,  $n = 1,285$ ) and time to diarrhea (A), time to pneumonia (B), overall morbidity (C), and mortality (D) during the first 90 d of life. Neonatal diarrhea was defined as visibly loose feces of decreased consistency. Pneumonia was defined as visibly detectable tachypnea with nasal discharge (serous, mucous, or purulent). Morbidity included the following diseases: (1) neonatal diarrhea, which was defined as visibly loose feces of decreased consistency, (2) omphalitis, which was defined as inflammatory signs (redness, swelling, purulent discharge, or painful response to palpation) of the umbilical cord, in addition to fever or lack of appetite, and (3) pneumonia, which was defined as visibly detectable tachypnea with nasal discharge (serous, mucous, or purulent).

In the generalized linear mixed model TS concentration in % Brix had no significant association with diarrhea ( $P = 0.33$ ) and omphalitis ( $P = 0.59$ ; Table 1). Pneumonia ( $P < 0.01$ ), overall morbidity ( $P < 0.01$ ), and mortality ( $P = 0.03$ ) during the first 90 d of life were related to TS concentration in % Brix (Table 1). The estimated probability of developing diarrhea during the first 90 d of life was estimated including TS concentration in % Brix ( $P = 0.03$ ). Estimates were derived from the GENLIMIXED model, including % Brix ( $P = 0.03$ ) and the quadratic term of % Brix ( $P = 0.03$ ) as fixed effects. Calves with TS concentration of 8.0 to 10.5% Brix showed the lowest probability of developing diarrhea (Figure 3A). The probability of developing pneumonia during the first 90 d of life was estimated using the GENLIMIXED model and including TS concentration in % Brix ( $P < 0.01$ ) as fixed effect. The greater the % Brix results, the lower the probability of developing pneumonia (Figure 3B). Probability of mortality during the first 90 d of life was estimated using the GENLIMIXED model and including TS concentration in % Brix ( $P = 0.07$ ) as

fixed effect. The greater the % Brix results, the lower probability of mortality (Figure 3C).

## DISCUSSION

The overall objective of the study was to compare calf health, ADG, and calf mortality considering the proposed categories of TPI by the consensus report of Lombard et al. (2020) during the preweaning period. Furthermore, we looked at calving-related factors and their effects on TPI. The main findings of the present study were as follows: (1) the TPI status was associated with morbidity and mortality rate; (2) calves with poor TPI had greater hazards for diarrhea, pneumonia, or mortality during the weaning period; (3) the greater the TS concentration, the lower the probability of developing pneumonia or any disease, or of mortality; and (4) calves with excellent, good, and fair TPI status had greater ADG.

The TPI distribution of the farm in the present study did not entirely achieve the recommendations of Lombard et al. (2020) with a distribution of 4.8% FTPI

**Table 2.** Associations between parity, calving ease, calving assistance, transfer of passive immunity (TPI) assessment, birth month, and TS (in % Brix) of female Holstein Friesian calves (n = 3,339)

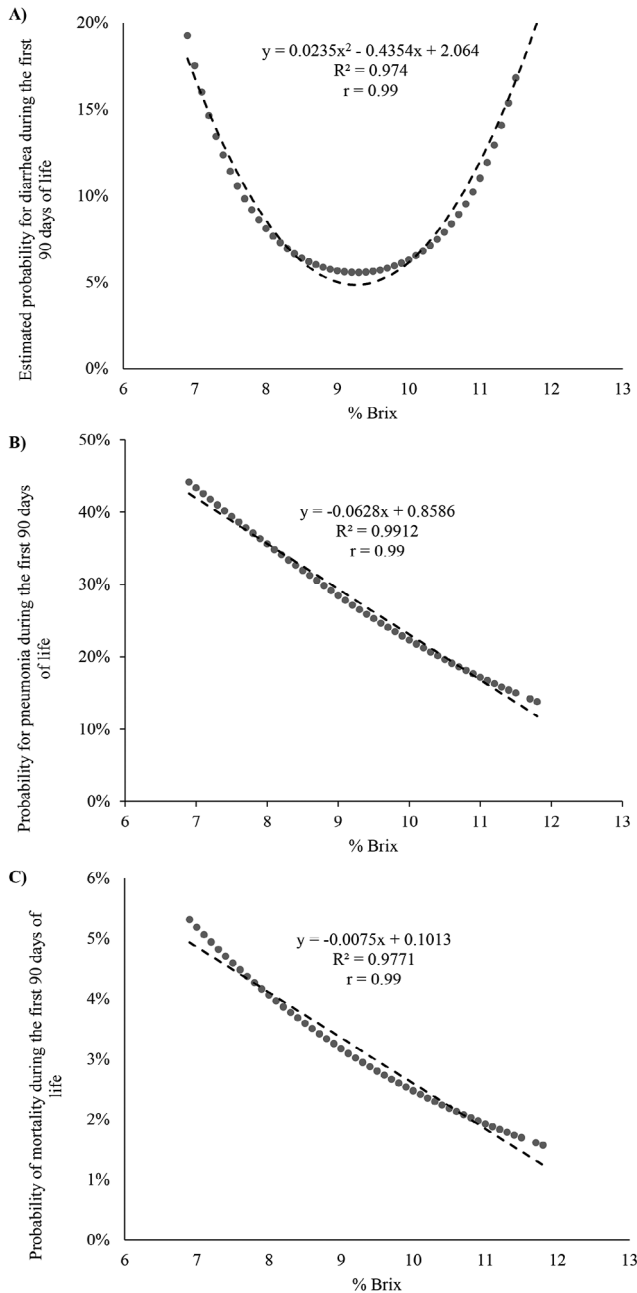
Variable	Estimate, % Brix	SE <sup>1</sup>	95% CI		P-value
			Lower bound	Upper bound	
Intercept	9.660	0.06	9.54	9.77	<0.001
Parity					
1	Referent				
2	-0.027	0.27	-0.087	0.033	0.376
≥3	-0.087	0.30	-0.140	-0.033	<0.001
Calving ease score <sup>2</sup>					
1	Referent				
2	-0.079	0.03	-0.139	-0.019	0.010
3	-0.138	0.03	-0.202	-0.073	<0.001
Birth month					
January	Referent				
February	0.024	0.06	-0.082	0.129	0.663
March	-0.091	0.06	-0.203	0.020	0.109
April	-0.136	0.06	-0.250	-0.022	0.019
May	-0.002	0.06	-0.117	0.112	0.967
June	0.003	0.06	-0.107	0.112	0.964
July	0.049	0.06	-0.064	0.162	0.397
August	-0.092	0.06	-0.201	0.017	0.099
September	-0.075	0.06	-0.185	0.034	0.177
October	-0.005	0.06	-0.113	0.103	0.929
November	-0.015	0.06	-0.123	0.093	0.786
December	0.031	0.05	-0.080	0.143	0.581
Calving assistance					
Employee 1	Referent				
Employee 2	-0.163	0.08	-0.316	-0.010	0.036
Employee 3	0.077	0.05	-0.014	0.169	0.098
Employee 4	-0.107	0.04	-0.180	-0.034	0.004
Employee 5	-0.125	0.04	-0.207	-0.043	0.003
Employee 6	-0.319	0.11	-0.532	-0.107	0.003
Employee 7	-0.016	0.06	-0.137	0.105	0.796
Employee 8	0.038	0.07	-0.095	0.170	0.557
Day of life for TPI assessment					
2	Referent				
3	-0.277	0.04	-0.363	-0.192	<0.001
4	-0.383	0.04	-0.468	-0.298	<0.001
5	-0.383	0.04	-0.467	-0.298	<0.001
6	-0.556	0.04	-0.639	-0.473	<0.001
7	-0.577	0.04	-0.660	-0.494	<0.001

<sup>1</sup>SE = standard error of the estimate.<sup>2</sup>Calving ease scores: 1 = no calving assistance; 2 = calving assistance by 1 person; 3 = calving assistance by 2 persons.**Table 3.** Associations of transfer of passive immunity (TPI) in % Brix on ADG of female Holstein Friesian calves (n = 2,942)

Variable	Estimate, kg/d	SE <sup>1</sup>	95% CI		P-value
			Lower bound	Upper bound	
Intercept	0.862	0.01	0.84	0.89	<0.001
Category of passive immunity <sup>2</sup>					
Poor TPI	Referent				
Fair TPI	0.031	0.01	0.005	0.058	0.022
Good TPI	0.057	0.01	0.031	0.084	<0.001
Excellent TPI	0.037	0.01	0.011	0.064	0.005

<sup>1</sup>SE = Standard error of the estimate.<sup>2</sup>Category of passive immunity: poor = <8.1% Brix; fair = 8.1–8.8% Brix; good = 8.9–9.3% Brix; excellent = ≥9.4% Brix.





**Figure 3.** (A) Estimated probability of diarrhea (%) during the first 90 d of life based on TS in serum (% Brix) for female Holstein Friesian calves ( $n = 3,434$ ) aged 2 to 7 d. Neonatal diarrhea was defined as visibly loose feces of decreased consistency. Estimates were derived from the GENLIMIXED model, including TS concentration in % Brix ( $P = 0.03$ ) and the quadratic term of TS concentration in % Brix ( $P = 0.03$ ) as fixed effects. (B) Probability of pneumonia (%) during the first 90 d of life based on TS in concentration (% Brix) for female Holstein Friesian calves ( $n = 3,434$ ) aged 2 to 7 d. Pneumonia was defined as visibly detectable tachypnea with nasal discharge (serous, mucous, or purulent). Estimates were derived from the GENLIMIXED model, including TS concentration in % Brix ( $P < 0.01$ ) as fixed effect. (C) Probability of mortality (%) during the first 90 d of life based on TS in serum (% Brix) for female Holstein Friesian calves ( $n = 3,434$ ) aged 2 to 7 d. Estimates were derived from the GENLIMIXED model, including TS concentration in % Brix ( $P = 0.07$ ) as fixed effect.

(<10%), 29.5% fair (20%), 28.3% good (30%) and 37.4% excellent (40%), respectively (values recommended by Lombard et al. are in parentheses). One reason for this might be that the farm management is still focused on avoiding FTPI, and the new recommendations have not been implemented into the calf health management.

Raboisson et al. (2016) reported an increased risk for mortality (2.12 times more likely) associated with poor TPI, as well as for pneumonia (1.75 times more likely), diarrhea (1.51 times more likely), and overall morbidity (1.91 times more likely). These findings are in accordance with the results of the Cox regression model of our study, showing greater hazards for calves with poor TPI regarding mortality (2.47 times more likely), pneumonia (2.00 times more likely), and overall morbidity (1.99 times more likely), and a tendency for greater hazard of diarrhea (1.57 times more likely) compared with excellent TPI, respectively. Calves with excellent TPI compared with the other TPI categories (fair, good, and poor) had lower probabilities of suffering from pneumonia ( $P < 0.01$ ) or overall morbidity ( $P < 0.01$ ). Crannell and Abuelo (2023) detected similar findings: calves with excellent TPI compared with inferior TPI categories had an increased risk of diarrhea (HR = 1.49), pneumonia (HR = 1.39), and mortality (HR = 4.29). Nevertheless, Crannell and Abuelo could not detect a significant difference of pneumonia and mortality risks between calves with fair or good TPI compared with excellent TPI. Based on our findings, we believe the new recommendations of the consensus report have the potential to reduce morbidity in calves.

In this study, the results from the generalized linear mixed model indicate that poor TPI was not associated with diarrhea or omphalitis. This might be due to the low number of cases of diarrhea ( $n = 216$ ; 6.3%) and omphalitis ( $n = 31$ ; 0.9%) detected in our study. This contrasts the findings of Crannell and Abuelo (2023), who detected a proportional increase in the risk of digestive disorders in calves as the quality of TPI decreases. Furthermore, a cross-sectional study also performed in Germany (Dachrodt et al., 2021) found associations, but with less-specific definitions of diarrhea (i.e., watery, liquid, or soft feces) and omphalitis (i.e., acute and chronic inflammatory cases of the external umbilical cord, classified as thickening, swelling, pain, or heat). Dachrodt et al. described much higher prevalence rates for diarrhea (18.5%) and omphalitis (20.9%), respectively. In the United States  $21.1 \pm 2.0\%$  (mean  $\pm$  SE) of preweaning heifer calves have been reported to have diarrhea, and  $1.7 \pm 0.3\%$  (mean  $\pm$  SE) had omphalitis (USDA, 2018), despite the observation that the prevalence of poor TPI had decreased from 41% in 1991 (USDA, 1993) to 13% in 2014 (Urie et al., 2018b). According to Raboisson et al. (2016), the

risk of diarrhea associated with poor TPI is low. This is related to the high prevalence of diarrheal diseases in calves despite poor TPI, which mathematically limits the value of the risk.

In this study 27.9% of the heifer calves ( $n = 957$ ) were diagnosed with pneumonia; the overall morbidity was 32.6% ( $n = 1,118$ ), and mortality was 3.1% ( $n = 107$ ). The prevalence of pneumonia is in accordance with the prevalence of respiratory diseases in the United States of  $12.0 \pm 1.4\%$  (mean  $\pm$  SE) in 2014 (USDA, 2018). The high mortality rates caused by diarrhea (56%) and respiratory diseases (24%) reported 2014 in the United States (USDA, 2018) indicate that it is important to reduce calves' susceptibility to diseases. Our results support the recommendations of Lombard et al. (2020) of greater TPI status on a herd level, as the likelihood of developing pneumonia or of mortality decreased with higher % Brix results. This contrasts the results of Crannell and Abuelo (2023), who found no significant differences in pneumonia risks between the TPI categories fair or good compared with excellent TPI.

Calves with excellent, good, and fair TPI had significant greater ADG compared with calves with poor TPI in the present study. However, no statistical difference was detectable between the TPI categories fair and excellent. The reason for this remains speculative. As described in literature, based on our results, we can confirm a difference in ADG of calves with poor TPI compared with calves with higher % Brix results. This is consistent with the findings of Robison et al. (1988) and Elsohaby et al. (2019), who observed a significant increase of ADG in dairy heifers with adequate TPI compared with those with poor. In contrast, Nocek et al. (1984) found no differences in weight gain of calves with poor TPI compared with calves with higher serum IgG concentrations at 45 d of age. Caldow et al. (1988) and Wittum and Perino (1995) observed that animals with poor TPI gained less weight than animals with adequate TPI but considered this to be an indirect effect of the increased morbidity observed in animals with poor TPI. According to Quigley et al. (1995) the differences in ADG are related to the management conditions of the farm rather than the TPI status of the calves. This finding was confirmed by Crannell and Abuelo (2023), who also found no difference in ADG within the 4 TPI categories. Average daily weight gain is an important measure for assessing calf management quality during the preweaning period (Breen et al., 2012). Greater ADG generates more profit for the dairy farmer. Soberon and Van Amburgh (2013) showed that calves with greater preweaning ADG were 2 times more likely to have greater milk yield in the first lactation (odds ratio = 2.09;  $P = 0.001$ ), whereas calves suffering from poor TPI have lower ADG and increased costs.

Raboisson et al. (2016) estimated the total costs per dairy calf with poor TPI to be €60 (€10–109). This is not only a severe economic loss but also the high disease prevalence represents an animal welfare issue. Therefore, the main goal for the dairy industry is to reduce morbidity and mortality during the preweaning period to achieve high-quality calf management. A greater number of calves with higher TPI status at herd level might help to reduce morbidity and mortality rates.

Furthermore, our data show that management factors (i.e., parity, calving ease, calving assistance, and day of life for TPI assessment) and environmental factors (birth month) were associated with TS concentration in % Brix. Calves from multiparous cows (parity  $\geq 3$ ) had lower % Brix results ( $P < 0.01$ ). The reason for this remains speculative. As the study farm implemented a standard operating procedure for the first feeding (4 L of pasteurized, pooled, colostrum, i.e.,  $\geq 22\%$  Brix fed via esophageal tube feeder), parity could be a subordinate factor. Therefore, the TS concentration might be an intrinsic characteristic of the calf, such as the individual abomasal emptying rate (Mokhber-Dezfooli et al., 2012) or lower calf birth weights of primiparous cows in relation to the standardized first feeding volume.

Calves experiencing calving assistance had lower TS concentration in % Brix ( $P < 0.01$ ). Vitality of calves born after dystocia is often compromised, and their attempts to stand are delayed (Riley et al., 2004; Barrier et al., 2012, 2013). Poor vigor has also been associated with a reduced absorption rate of IgG (Furman-Fratczak et al., 2011; Barrier et al., 2012) and therefore a greater risk of poor TPI.

The % Brix results were associated with the employee providing calving assistance and neonatal care ( $P < 0.01$ ). Neonatal calf care and calf rearing requires engagement and motivation. Benchmarking is effective in health care, farming, and the commercial industry, to engage and motivate employees (Jarrar and Zairi, 2001; Bogetoft, 2012). This offers the possibility to reflect on current practices and identify sources of error (Meade, 1994; Anand and Kodali, 2008). Atkinson et al. (2017) evaluated the effects of benchmarking at the farm level on the prevalence of poor TPI and ADG in preweaning calves in Canada and concluded that benchmarking motivated producers for management changes with the intention to improve performance.

The day of life for TPI assessment significantly affected the % Brix results ( $P < 0.01$ ). Calves sampled on d 2 had higher % Brix results compared with the remaining sampling days ( $P < 0.01$ ; range: 0.3–0.6% Brix). Blood sample collection for TPI assessment is possible from 24 h after the first colostrum feeding to

10 d of age (Wilm et al., 2018; Godden et al., 2019). However, the earlier the samples are collected, the more accurately the results reflect the true IgG absorption (Godden et al., 2019). Furthermore, the results are less likely to be influenced by IgG distribution or dehydration (Godden et al., 2019).

We note that this study was carried out on one dairy farm only. As trials conducted at a single location may not be representative of the variety of possible clinical situations (Sargeant et al., 2010), external validity is limited. Therefore, the results need to be validated with a multicentric study design. In addition, an objective parameter such as hematocrit was not considered to determine the hydration status of the calf, and only cases with treatment were regarded as diseased. This approach was chosen to increase compliance among farm employees. We are aware that mild cases of dehydration and disease were missed, and that the experience of the farm personnel might influence identification of clinical signs of disease, constituting a weakness of the study. This confirms the study of Baxter-Smith et al. (2022), addressing the problem of an accurate diagnosis of bovine respiratory diseases. Baxter-Smith et al. (2022) reported farmers underdiagnosing and misdiagnosing bovine respiratory disease: out of 53 actuated sick calves, only 13 were identified by the farmer, whereas out of the 294 healthy calves, 22 were treated.

## CONCLUSIONS

Our study provides further evidence for the validity of a passive immunity classification scheme based on 4 categories. Overall, calves with a higher TPI status had lower risks for pneumonia and overall morbidity as well as lower mortality rates during the preweaning period. Furthermore, calves with good or excellent TPI had greater ADG. The results should be validated in a multicentric study design to increase external validity.

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