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Management of calves in commercial dairy farms in Mecklenburg-Western Pomerania, Germany and its impact on calf mortality and prevalence of rotavirus and *Cryptosporidium parvum* infections in pre-weaned calves

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A B S T R A C T

In a cross-sectional study, impact of management in dairy farms on calf mortality rates and prevalence of rotavirus and *Cryptosporidium parvum* in feces of calves was investigated. Sixty-two commercial dairy herds in Mecklenburg-Western Pomerania, Germany, were stratified selected in 2019. We performed in-person interviews and fecal specimens in samples of all-female calves of age 7 up to 21 days. Management data were documented on farm level. A Multiscreen Ag-ELISA was performed to determine rotavirus and *Cryptosporidium parvum*. Associations between two calf mortality rates, detection of *C. parvum* and rotavirus, and predictors were examined with GLM models. In farms with routine vaccination against respiratory diseases, 31-days mortality rate was 4.2% +/-1.26 compared to 7.6% +/-0.97 ($p = 0.040$) on non-vaccinating farms. Six-months mortality was lower in farms that continued feeding milk to calves during periods of diarrhea compared to farms that did not (6.9% +/-0.8 vs. 12.4% +/-2.3). In case of a routine shifting of calves from the calving box into calf boxes less *C. parvum* was detected compared to an individual moving of calves (33.3% +/-2.6 vs. 19.6% +/-5.3; $p = 0.024$). Our model confirms a positive association between occurrence of aqueous feces and frequency of detection of *C. parvum* (45.4% +/-23.6 vs. 21.4% +/-18.7; $p < 0.001$). Frequency of detection of rotavirus was lower in farms that reported a defined amount of applicated colostrum per calf than in farms that presented a range of colostrum instead of a defined amount. This study indicates the potential for mitigation of risk factors for mortality in calves.

Introduction

Animal welfare and health is of high potential interest to farmers, consumers and politicians (Amon et al., 2014). High rates of morbidity and mortality lead to economic losses, are at odds with animal welfare and food safety (Tautenhahn, Merle & Müller, 2020). High rates of morbidity effects an increase of application of antibiotics. That leads to a rise in antimicrobial resistance (World Health Organization, 2014). The adjustment of the "New Common Animal Health Strategy 2007–2013" includes a „One Health" strategy. The European Union perceives human health, animal health and animal welfare as an inseparable complex. The purpose of the strategy is to enhance animal health and reduce morbidity by improving husbandry conditions (European Commission, 2007).

Calf mortality is of great economic importance and an indicator of livestock welfare (Bähler et al., 2012). Effective management of pre-weaned dairy calves, as well as their survival, is an important factor affecting the performance of calves and dairy farms (Renaud, Kelton, LeBlanc, Haley & Duffield, 2018).

Calf mortality in dairy farms is influenced by region, production systems, and the considered period. Annual calf mortality rates are often reported. A recent Chinese study reported an annual mortality rate of 5.5% from day 3 up to day 60 (Zhang et al., 2019). The mortality rates for pre-weaned calves range from 2.6% (day 0 – 90 in Sweden; Olsson, Viring, Emanuelsson & Jakobsson, 1993), 3.3% (calves < 14 d in a Dutch study; Santman-Berends, Schukken & van Schaik, 2019) to 7.8% in the United States (NAHMS, 2007), and up to 10% in Swiss dairy farms (1 to 21 days of age, Swiss animal and movement database, Tierverkehrsdatenbank, TVD; Bähler et al., 2012).

The highest risk for mortality in dairy calves is during their first three weeks (Wells et al., 1996). The risk of mortality in pre-weaned dairy calves is affected by calving management, colostrum management, feeding, and housing (Renaud et al., 2018). Gastrointestinal disorders are one of the most important risk factors for mortality in pre-weaned calves (Torsein et al., 2011; Wells et al., 1996; Windeyer et al., 2013; Yong-il & Kyong-Jin, 2014; Zhang et al., 2019). Other risk factors for

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high mortality are an inadequate passive transfer of colostral immunoglobulins (Wells et al., 1996), a colostral meal more than 3 h after calving (Zucali, Bava, Tamburine, Guerci & Sandrucci, 2013), restricted feeding (Zucali et al., 2013), group housing in the 1st month (vs. single boxes, Olsson et al., 1993, Zucali et al., 2013), routine antibiotic treatment of calf diarrhea (Lance et al., 1992), and less intensity of calf care (> 50 calves per person; Zucali et al., 2013). Herd size was not observed to be a risk factor for preweaning mortality (Zucali et al., 2013). An Irish study did not find any association between colostrum and calf management practices with a 28-day calf mortality rate (Barry et al., 2019).

Multiple pathogens are known or postulated to cause or contribute to calf diarrhea development. Other factors, including both the environment and management practices, influence disease severity and outcomes (Gomez & Weese, 2017; Yong-il & Kyong-Jin, 2014). Several infectious agents have been implicated in calf diarrhea. Rotavirus and *C. parvum* are recognized as major pathogens and causative agents of diarrhea in calves (Yong-il & Kyong-Jin, 2014). The within-herd prevalence of *C. parvum* shedding was associated with calving management (Trotz-Williams et al., 2008).

The objective of our investigation was the detection of management factors in dairy farms on herd level that are associated with calf mortality, the prevalence of rotavirus, and *Cryptosporidium* in feces of calves of a random sample.

Material and methods

Selection of farms

We enrolled a total of 62 dairy herds in the study. For logistical reasons farms had to be located in Mecklenburg-Western Pomerania, Germany. The population of dairy farms in Western Pomerania houses 370 dairies on average with a yearly milk yield of 9669 kg and 275.000 cells per ml (DHI 2018). Our study farms houses 432 dairy cows on average with a yearly milk yield of 8998 kg and 232.000 cells per ml (DHI 2018). Our study population is consistent with the population of the DHI herd in Mecklenburg Western Pomerania. A high percentage of contracted employees and "getting in a bit long" husbandry facilities are characteristic for the average commercial farm in this region.

Inclusion criteria for the study were an average herd size of ≥ 50 dairy cows and the availability of calves for sampling in the study period from June up to December 2019.

A stratified sample of dairy herds in Mecklenburg-Western Pomerania, Germany was selected. We prepared a list of all eligible dairy herds, including data on average 31-days mortality from 2016 up to 2018. We assigned the herds in three strata: herd with low mortality (</= 2.0%), medium mortality (> 2.0% up to 6.0%), and high mortality (> 6.0%). We selected 30 herds per stratum using a randomization table. The participation of the farms in the project was voluntary. Therefore, we contacted the farms via telephone call for information. The farms were enrolled after they accepted the conditions. We included 21 farms with low mortality, 19 farms with medium mortality, and 22 farms with high mortality in the study.

Each farm was visited once in the study period from June 2019 up to December 2019. We performed structured in-person interviews with the herdsmen and/or owner of the farm and fecal samples of calves were sampled during this visit for diagnostic tests.

Questionnaire

A questionnaire was prepared to address farm demographic data and management practices related to calving management. The questionnaire was generated respecting literature (Lorenz et al., 2011; Tautenhahn et al., 2020) focusing the hypotheses. The questionnaire was pre-tested using in-person interviews with 15 dairy farmers and 10 veterinarians and adapted afterwards. The five-page questionnaire comprised 48 questions and one statement in five sections: herd demographics, prophylactic measures, colostrum, measures around calving of cows and pre-weaned calves – feeding, hygiene, routines. Questions were always asked in the same way. They were explained if the respondent asked for further clarification. The manuscript includes a list of potential risk factors included in the models, their categorization and observed frequencies at herd level is presented in the manuscript (Table 1) which represents the content of the questionnaire. The whole questionnaire (in German) is available from the corresponding author upon request.

Sampling and sample size per farm

We obtained fecal specimens in randomized samples of all-female calves from ages 7 up to 21 days on the day of the farm visit. We selected the calves from a randomization list. All available calves with these properties without consideration of health status were enrolled. The health status of the calves was not considered. The consistency of fecal specimens was judged immediately after removal from the calves. We used a 1–5 ordinal scale ("firm", "pasty", "pulpy", "mushy", "watery") The result was documented in a form (identity of the animal, date of sampling and evaluation of fecal consistency). We collected and documented the availability of concentrates, hay and water for every single calf in the sample. These two variables were dichotomous (Yes/No).

The sample size was defined as seven calves in small farms (up to 200 animals > 24 months), 10 in medium farms (200 up to 400 animals per farm), and 15 in large farms (> 400 animals).

A single fecal specimen was collected per rectum from each calf. The material was stored in clean, labeled boxes with leakproof screw caps. The samples were transferred within two days stored at 4 °C to the laboratory (LALLF Rostock, Mecklenburg-Western Pomerania, Germany).

Testing of fecal samples

A Multiscreen Ag-ELISA (Bio-X Diagnostics, Rochefort, Belgium) was performed to determine Rotavirus and *C. parvum*.

The fecal samples were barcoded with an accompanying identification document and stored in leakproof plastic cups, and transferred at 4 °C to the laboratory (LALLF, Rostock, Germany). The samples were recorded in the laboratory-specific software laboratory information system, and data about animal identification, sampling person, sample receipt date were recorded.

The samples were prepared for examination on the day of their receipt. They were mixed well, homogenized, and diluted at 1:2 with the buffer solution from the test kit. The minimum quantity of prepared sampling material was 800 μL. The prepared samples were examined immediately after preparation or stored at 4 °C. The samples were examined 65 h post receipt on average.

The ELISA was performed following the instructions of the manufacturer information.

All ingredients were brought to room temperature and filled in the sinkings of the test kit plate. All samples and controls were incubated for 3 h at 21°. After incubation, the plates were washed three up to four times with washing buffer. Then 100 μL of the conjugates (Anti-*C. parvum* MAK and Anti-Rotavirus MAK) were filled in the sinkings and incubated 60 min at 21 °C. Then, this was washed and a second washing was performed. Then substrate (TMB) was filled and incubated for 10 min at 21 °C. After the stop solution was added immediately after incubation, optical density (OD) was measured with a plate photometer at a wavelength of 450 nm. Relative OD values were calculated as per the test manual.

Definition of calf mortality

We considered two mortality rates in this study:

Table 1
List of potential risk factors included in the models, their categorization and observed frequencies at herd level.

Variable	Category	Herds (n)	Missing
Herd demographics			
Cows in milk per farm	metric	61	1
Average daily milk yield per cow	metric	61	1
Annual average somativ cell count	metric	60	2
Evaluation health status of calves			0
	good	31	
	good-medium	4	
	medium	24	
	medium-poor	2	
	Poor	1	
Use of vaccination Rota, Coronavirus, *E. coli* F5			0
	Yes	26	
	No	35	
	irregular	1	
Prophylactic measures			
Supplementation of calves (Iron)			0
	Yes	26	
	No	36	
Supplementation of calves (Selenium)			0
	Yes	10	
	No	52	
Treatment of calves against *C. parvum*			0
	Yes	18	
	No	44	
Supplementation of calves (Iron)			4
	All	36	
	individual	22	
Supplementation (Vitamines, Immunglobulins)			0
	Yes	21	
	No	41	
Implementation of diagnostic methods in supervising health status of calves			0
	Yes	39	
	No	23	
Vaccination of calves (only respecting respiratory diseases)			0
	Yes	24	
	No	38	
Colostrum management			
Milking of colostrum at time of milking or exclusive after calving			2
	Milking	52	
	Exclusive	8	
Evaluation of colostrum			0
	No Evaluation	37	
	Evaluation "under special circumstances"	4	
	Evaluation of a sample	5	
	Evaluation of every colostrum portion	16	
Transportion of calves from the calving box			0
	Routinely	12	
	Depending on individual calving time	50	
1st colostrum application (Suckeling)			0
	Yes	24	
	No	38	
1st colostrum application (Time)			2
	Up to 2 hrs post calving	21	
	Up to 4 hrs post calving	31	
	> 4 hrs post calving	4	
	Suckeling (exclusive)	4	
Monitoring of calving			0
	Yes	25	
	No	37	
Documentation of colostrum application			0
	Yes	14	
	No	48	
			0
Evaluation of 1st colostrum	Yes	25	
	N	37	
1st colostrum from mother cow			1
	Yes	25	
	No	7	
	Sometimes	29	
1st colostrum (>3 Liters)			5
	Yes	36	
	No	21	
Documentation of colostrum quality determination			37
	Yes	10	
	No	15	
First colostrum exklusively frozen (no fresh colostrum)		62	0
	Yes	4	
	No	58	
First colostrum - defined amount or range?			2
	Defined	39	
	Range	21	
First colostrum, amount (minimum in l)			8
	2	19	
	3	19	
	4	15	
	5	1	
First colostrum intake – Dokumentation			0
	Yes	14	
	No	48	
Measures around calving			
Calving (heifers and cow seperated)			3
	Yes	41	
	No	18	
Monitoring of calving 24 h a day			0
	Yes	25	
	No	37	
Who monitors calving?			0
	All	46	
	Special stuff	16	
Documentation of calving monitoring?			0
	Yes	11	
	No	51	
Calving box - using of lime for disinfection			1
	Yes	21	
	No	40	
Calving box - disinfection (all disinfectants)			0
	Yes	14	
	No	48	
Feeding, hygine and routines in pre-weaned calves			
Calves (n) per calving pen in until day 14			0
	$N = 1$	56	
	$N > 1$	6	
Cleaning calf pens in the 1st 14 days with water			0
	No water	10	
	Cold	30	
	Hot	22	
Cleaning calf pens			0
	Only removing manure	10	
	More intense cleaning	52	
Disinfection calf pens			0
	Yes	51	
	No	11	
Exclusive stuff for pre-weaned calves			0
	Yes	14	
	No	48	
Number of calfes per person	Metric	58	4
Calf feeder (youngest calves)			2
	Whole milk	48	
	Replacer	12	
Feeder temperature (youngest calves)			2
	Cold	15	
	Warm	45	
Feeder (youngest calves) – Consistency			6
	Yes	45	
	No	11	
Numbers of feed sections (pre-weaned calves))			0
	One	9	
	Two	39	
	Three	14	
Feeding profiles			1

(*continued on next page*)

Table 1 (*continued*)

Variable	Category	Herds (n)	Missing
	Milk replacer	9	
	Whole milk	9	
	Whole milk or Milk replacer	43	
Desinfection feeding equipment (youngest calves)			0
	Yes	15	
	No	47	
Offer of elektrolytes in case of diarrhea in pre-weaned calves			0
	Yes	54	
	No	8	
Reduction of milk portions in case of diarrhoe in pre-weaned calves			13
	Yes	29	
	No	20	
Continuation of milk feeding in case of diarrhea			0
	Yes	55	
	No	7	
Treatment protocol for diarrhea			0
	Yes	34	
	No	28	
Treatment protocol for diarrhea (in written form)			0
	Yes	21	
	No	41	
Starting hard feed in calves (in days)	metric		5
Offer of drinking water (calves)		61	1
	Yes	61	
	No	0	
Constant offer of drinking water			1
	Yes	56	
	No	5	
Characteristics of feces in the fecal sample and availability of potable water and hard feed			
hard feed for calves in the sample (7–21 days)	Metric	62	0
Hay for calves in the sample (7–21 days)	Metric	62	0
Water for calves in the sample (7–21 days)	Metric	62	0
Fecal consistency - watery (%)	Metric	62	0
Fecal consistency - mushy (%)	Metric	62	0
Fecal consistency - pulpy (%)	Metric	62	0
Fecal consistency - pasty (%)	Metric	62	0
Fecal consistency - firm (%)	Metric	62	0
Percentage of *C. parvum* in Calf sample	Metric	62	0
Percentage of Rotavirus in Calf sample	Metric	62	0

- Average 31-days mortality from 2016 up to 2018
- six-months mortality of 2019 per farm

The number of calves in the different periods was calculated based on the National Identification and Registration database (HI Tier, Germany) and Tschindi (Tschindi.org, Germany).

Statistical analysis

Data collection and processing were carried out with Microsoft Excel (Microsoft Corp., 2010). For analyzing the dataset, the software SPSS 26.0 (Chicago IL, USA) was used with herds considered as a statistical unit.

Descriptive analyses of all data at herd level were performed. Continuous not normally distributed data was dichotomized or categorized. Associations between average 31-days mortality from 2016 up to 2018 (V1), frequency of detection of *C. parvum* in the sample of calves per farm (V2), frequency of detection of rotavirus in the sample of calves per farm (V3), 6-months mortality in 2019 per farm (V4, dependent variables), and predictors (independent variables n = 63 for V1, n = 61 for V2, n = 60 for V3, and n = 62 for V4) were examined with generalized linear mixed models with an identity link after pre-screening for variable selection in univariable analysis. The independent variables derived from the questionnaire were subjected to univariable analyses. Variables with a P-value < 0.3 were considered to be potentially associated with the different outcome variables and were entered in the multivariable model.

The multivariable analysis was performed using a backward stepwise selection and elimination procedure. After each run, the variable with the highest P-value was excluded from the model until all variables had $P \leq 0.05$. The most optimal model was evaluated using the Akaike information criterion (AIC), where an AIC closest to zero was deemed the best model (Akaike, 1974). The confounding variable was monitored by the change in the coefficient of a variable after removing another variable from the model. If the change in the estimates exceeded 25% or 0.1 when the value of the estimate was between −0.4 and 0.4, the removed variable was considered a potential confounder and was re-entered in the model. The remaining potential risk factors as well as categories and observed frequencies are summarized in Table 1. In the final models, all biological credible two-way interactions were tested. Model fit was evaluated by checking the normality of the residuals. Estimated marginal means from the model were calculated. A P-value < 0.05 indicated a statistically significant difference.

Results

Descriptive statistics

Herd demographics

We enrolled 62 commercial dairy farms from June up to December 2019 in the study. The interview partners in the survey were owners in 38 farms (61.2%) and the herdsmen in 24 farms (38.7%). The average 31 d mortality of the calves per farm from 2016 – 2018 was 6.3% +/−6.1. The farms housed 439 +/−389 animals with a daily milk yield of 29.8 *l* +/−4.3 and a somatic cell count of 232,000 cells per mL +/−65,000. Sixty-one farms were conventional farms, and one facility was an organic farm. The interview partners assessed the health status of the pre-weaned and weaned calves to be good (31 farms, 50.0%), medium-good (4 farms, 6.5%), medium (24 farms, 38.7%), medium-poor (2 farms, 3.2%), and poor (1 farm, 1.6%).

Within herd prevalence of diarrhea pathogens (Cryptosporidium parvum, rotavirus)

In total, we collected fecal samples from 529 calves in 62 farms. The sample size depended on farm size and available calves at the time of sampling. The average sample size was 8.5 (+/−4.0) calves per farm.

In 51 out of 62 farms, we detected *C. parvum* antigen in at least one sample (82.3%). The within-herd prevalence of *C. parvum* ranged from 0%−100% (Fig. 1). The within-herd prevalence of rotavirus was lower than that of C. *parvum*; out of 529 samples, 50 were positive. In 27 of 62 farms, we detected one positive sample (43.5%). The within-herd prevalence ranged from 0%−50%.

Mortality (average 31-days mortality from 2016 up to 2018)

We defined mortality of the herds in three strata: 21 herds with low mortality (</= 2.0%), 19 herds with medium mortality (>2.0% up to 6.0%), and 22 herds with high mortality (>6.0%). The mortality was 1.24% +/−0.54 (mean and standard deviation) in the herds with low mortality, 4.31% +/−1.28 in the herds with medium mortality, and 12.62% +/−5.53 in the herds with high mortality.

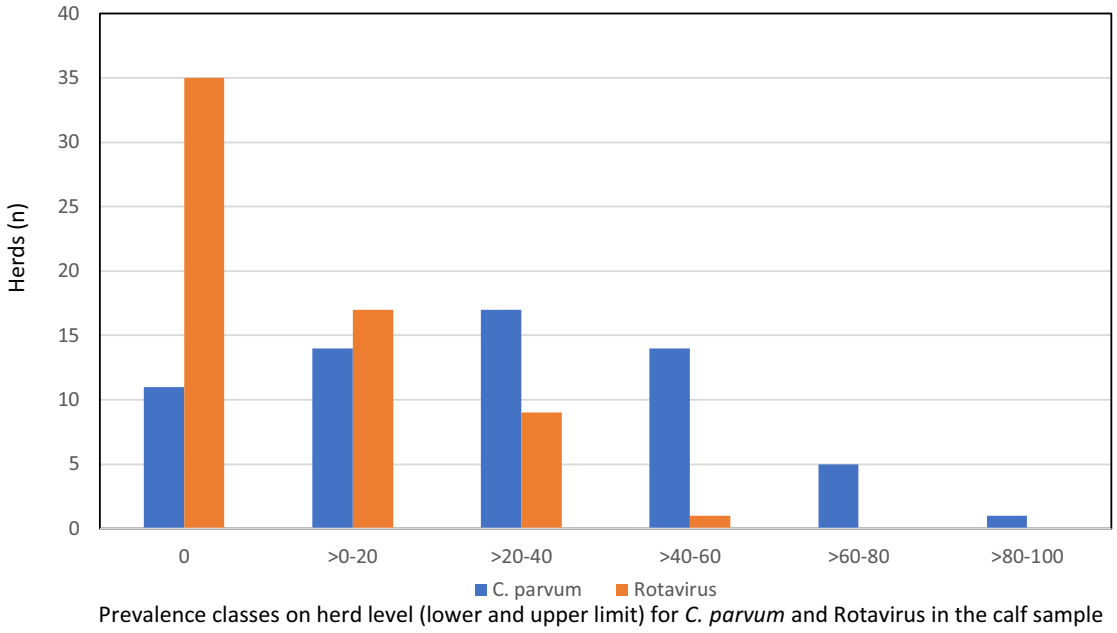

Fig. 1. Distribution of within-herd prevalence of *C. parvum* and Rotavirus in calves of an age of 7–21 days in 62 dairy farms in Mecklenburg-Western Pomerania, Germany.

Results of multivariable analyses

The final models are represented in Table 2. No evidence of confounding was observed during the model-building procedure. We calculated models with four different dependent variables.

Thirty-one-days mortality from 2016 up to 2018 (V1)

We tested a total of 63 risk factors. Our model describes the impact of the implementation of vaccination against respiratory diseases in farm animals. In farms with routine vaccination, the herd mortality rate was determined to be 4.2% +/−1.3 (estimated marginal mean and standard error), while non-vaccinating farms have a mortality rate of 7.6% +/−0.9 (p = 0.040; Table 2 and Table 3 estimated marginal means).

Frequency of detection of Cryptosporidium parvum in the fecal samples of calves per farm (V2)

We tested 61 risk factors. The model determined two risk factors for the detection of *C. parvum* in the fecal samples of the calf sample per farm. In the case of a routine moving of calves (at defined times), less *C. parvum* was detected compared to an individual moving of the calves with respect to already occurred calving (33.3% +/−2.6 vs. 19.6% +/−5.3; Table 3; p = 0.024).

Our model confirmed a positive association between the occurrence of aqueous feces in the calf sample and the frequency of detection of *C. parvum* (45.4% +/−23.6 vs. 21.4% +/−18.7, $p < 0.001$, estimated marginal means).

Frequency of detection of rotavirus in the sample of calves per farm (V3)

We tested 60 risk factors. The model determined two risk factors (*p*

Table 2
Results of multivariable analysis of 31-days mortality from 2016 up to 2018 (V1), frequency of detection of C. parvum in the sample of calves per farm (V2), frequency of detection of rotavirus in the sample of calves per farm (V3) and 6-months mortality of 2019 per farm (V4).

Model term	Coefficient	Std. Error	t	P	95% confidence interval	
					Lower	Upper
average 31-days mortality from 2016 up to 2018 (V1)						
Intercept	4.218	1.2612	3.344	0.001	1.692	6.743
Vaccination_No	3.35	1.5927	2.103	0.04	0.16	6.539
Vaccination_Yes	0	.	.	.	.	.
frequency of detection of *C. parvum* in the calf sample (V2)						
intercept	10.574	5.46	1.937	0.058	−0.352	21.499
feces_aequous	1.449	0.2467	5.875	0	0.956	1.943
Routine moving of calves_no	13.78	5.9377	2.321	0.024	1.898	25.661
Routine moving of calves_yes	0	.	.	.	.	.
frequency of detection of rotavirus in the calf sample (V3)						
Intercept	17.403	3.3341	5.22	0	10.727	24.08
Defined amount of applicated colostrum_no	−6.149	3.06	−2.01	0.049	−12.277	−0.022
Defined amount of applicated colostrum_yes	0	.	.	.	.	.
Percentage of availability of hard feed (yes_no)	−0.079	0.0353	−2.248	0.028	−0.15	−0.009
6-months mortality in 2019 per farm (V4)						
Intercept	6.886	0.8104	8717	0	5.264	8.507
Continuing milk feeding during periods with diarrhea_no	6.484	2.3924	2.704	0.009	1.697	11.271
Continuing milk feeding during periods with diarrhea_yes	0	.	.	.	.	.

Table 3
Estimated marginal means describing differences between farm-level variables with 31-days mortality from 2016 up to 2018 (V1), frequency of detection of C. parvum in the sample of calves per farm (V2), frequency of detection of rotavirus in the sample of calves per farm (V3) and 6-months mortality of 2019 per farm (V4).

Variable	Mean	Standard error	Confidence interval 2,5%	Confidence interval 97,5%
average 31-days mortality from 2016 up to 2018 (V1)				
Vaccination (only considering respiratory disease vaccinations)				
No	7.567	0.973	5.620	9.515
Yes	4.218	1.261	1.261	6.743
frequency of detection of c. parvum in the calf sample (v2)				
feces_watery				
No	33.34	2.608	28.122	38.558
Routine				
Routine	19.560	5.330	8.895	30.226
frequency of detection of rotavirus in the calf sample (V3)				
Defined amount of Colostrum				
Exact	6.548	1.807	2.930	10.165
Range	12.697	2.464	7.762	17.632
6-months mortality in 2019 per farm (V4)				
Continuing milk feeding during periods with diarrhea				
No	13.370	2.251	8.866	17.874
Yes	6.886	0.810	5.264	8.507

< 0.05).

The rotavirus detection rate was lower in farms that reported a defined amount of applied colostrum at the first colostrum meal per calf than in farms that referred a range instead of a defined amount of colostrum (6.6% +/−1.8 vs. 12.7% +/−2.5; $p = 0.016$; estimated marginal means). The second influencing factor was the percentage of availability of hard feed (hay, concentrates, TMR) in the calf sample. A low availability was associated with a lower percentage of rotavirus in the calf sample ($p = 0.028$).

Six-months mortality of calves per herd in 2019 (V4)

We tested a total of 62 risk factors. We constituted a risk factor regarding farm-specific routine in the management of diarrhea in calves. The six-month mortality is lower in herds that continue milk feeding during periods with diarrhea then herds that did not practice it (6.9% +/−0.8 vs. 12.4% +/−2.3; estimated marginal means).

Discussion

Multivariable models for risk factors associated with the 31-days mortality from 2016 up to 2018 and with the six-month mortality in 2019 on herd level

Differences in definitions of mortality have been observed to have a significant effect on the magnitude of mortality (Santman-Berends et al., 2019). Particularly, different mortality rates have been reported in different studies; that complicate or even makes comparisons impossible (Compton et al., 2017).

Our GLM model detected only one risk factor for the average 31-days mortality - the performance of a vaccination against respiratory diseases on herd level, The implementation of this prophylactic measure is negatively associated with this early calf mortality rate ($p = 0.040$).

There are no reports on the specific association between mortality of calves and immunization activity against respiratory diseases in calves. Vaccination for endemic bovine infections is described as a herd-level risk factor for the mortality of dairy cows (McConnel, Lombard, Wagner, Kopral & Garry, 2015; Reimus, Alvåsen, Emanuelson, Viltrop & Mõtus, 2020; Reski-Weide, 2013). However, recent studies have described associations between management practices that focus on strengthening the immune system. Interestingly, Reimus et al. (2020) suggested that the prophylactic administration of vitamins to all calves as a management tool increases the risk of high mortality of calves in the first 90 days. The vaccination carried out possibly indicates dedicated animal health work with the aim of prophylaxis.

A German cross sectional study describes two risk factors for high mortality in calves (Tautenhahn et al., 2020). A routine halofuginon lactate administration on herd level had a higher calf mortality than farms that did not perform routine use of this drug. Another risk factor was the failure of passive transfer of colostrum (FPT). FPT of more than 25% of the neonatal calves in a herd was associated with high calf mortality (Tautenhahn et al., 2020). In our study FPT was not considered. The use of halofuginon lactate was covered in our questionnaire but the variable was not selected for the final model.

Just as in the other model, only one parameter proved as an associated factor for the six-month mortality in 2019 on herd level. It is lower in farms that continue milk feeding during periods with diarrhea than in farm that do not.

Herd size as a risk factor for mortality is discussed, although it is debatable (no: Zucali et al., 2013, yes: Alvåsen, Jansson Mörk, Hallén Sandgren, Thomsen & Emanuelson, 2012, Reimus et al., 2020). In our study, herd size did not affect the two different considered mortality rates in calves. This is in accordance with Zucali et al. (2013), who collected data on management and mortality in 28 commercial dairies in Italy. This group detected feeding of first colostrum more than 3 h after birth, group housing of calves before 30 days of age, feeding daily less than 5 L of milk or milk replacer as risk factors for higher early calf mortality of >10.0% (Zucali et al., 2013). Extensive recent work from an Estonian working group involved 214 farms with verified herd size as a risk factor for high mortality (Reimus et al., 2020). The average Estonian herd size was 152 cows per farm in 2015 (Koelemann, 2017). In our study the average herd size is higher (439 +/−389 cows per herd).

Frequency of detection of Cryptosporidium parvum in the sample of calves per farm (herd-level prevalence; V2)

The herd-level prevalence of *C. parvum* in the presented study ranges from 0% up to 100% of once sampled calves aged 7–21 days. In 17.8% of farms, we found no *C. parvum*, in accordance with earlier reports (Garber, Salman, Hurd, Keefe & Schlater, 1994; Sischo, Atwill, Lanyon & George, 2000; Trotz-Williams, Jarvie, Martin, Leslie & Peregrine, 2005, 2007, 2008).

Various management risk factors on herd level for *C. parvum* infection of dairy calves have been described earlier. Promoting factors include the feeding of milk replacer in the 1st week (Trotz-Williams et al., 2008), while the limiting factors include the use of calf scour prophylaxis for cows and calves (Trotz-Williams et al., 2008) and concrete flooring (Castro-Hermida, Gonzalez-Losanda & Ares-Mazas, 2002). The potential risk factors include large herd size, multi-cow maternities, a long calving season, and intense contact of calves with other calves (Atwill, Johnson & Pereira, 1999; Brainard et al., 2020; Garber et al., 1994).

In this study, we observed that moving the newborn calves from the calving box into the calf boxes has an impact on the farm level prevalence of *C. parvum*. In the case of a routine moving of calves (at defined times), less *C. parvum* is detected compared to an individual moving of the calves with respect to the already occurred $p = 0.024$). The type of maternities (single or multi-cow) did not influence the within-herd prevalence of *C. parvum*, as reported by Trotz-Williams et al. (2007).

The data of our study indicate a positive association between the aqueous feces in the calves and the frequency of detection of *C. parvum* (45.4% +/−23.6 vs. 21.4% +/−18.7, $p < 0.001$). These findings are in accordance with a Canadian study (Mawly et al., 2015; Trotz-Williams et al., 2007), wherein calves shedding *C. parvum* oocysts had an Odds ratio of 5.3 (95% CI 4.4, 6.4) for diarrhea in comparison to non-shedding calves, controlling other factors included in the final multivariable model.

Frequency of rotavirus detection in the sample of calves per farm (V3)

In our study in 43.5% of farms rotavirus was detected. This is lower than that reported in another recent work (Mawly et al., 2015, 70.1%). The within-herd prevalence in our work ranged from 0%−50%. This is in accordance with the findings of a study in Australia (Abuelo, Havrlant, Wood & Hernandez-Jover, 2019), wherein the fecal samples from 23 farms were examined, and a range of rotavirus prevalence from 0%−63.4% was described.

Considering the management of the application of colostrum, the rate of rotavirus detection was lower in farms with a defined amount of applied colostrum per calf than in farms that applied a range of colostrum instead of a defined amount. Colostrum management is most important management factor in determining calf health and survival (Godden, Lombard & Woolums, 2019). It is recommended that calves be fed 10% to 12% of their body weight (BW) of colostrum at first feeding (3–4 L for a Holstein calf; Godden et al., 2019). Serum IgG levels in calves were determined to be significantly higher in calves fed 4 L of colostrum at 0 h and a further 2 L at 12 h (serum IgG = 31.1 g/L) compared with calves fed only 2 L of high-quality colostrum at 0 h and a further 2 L at 12 h (serum IgG = 23.5 g/L; Morin, McCoy & Hurley, 1997). However, the absolute volume of colostrum could not be confirmed as a predictor in our investigation. A reduced intake of colostrum is a risk factor for higher severity of the disease caused by a rotavirus infection (Dhama et al., 2009).

Data of our study indicate a lower within-herd prevalence of rotavirus when concentrates are provided for young calves ($p = 0.028$). Free availability of concentrates and water for calves facilitates optimal growth of the forestomach`s digestion (Lorenz et al., 2011).

Other findings

Calves are born without immunoglobulins (IG) due to the morphology of the bovine placenta. For a functional immune defense in calves, passive transfer of maternal IG from a first colostrum meal is the absolute precondition (Godden 2008). The key factors of a suitable passive transfer are the quality of the first colostrum meal, the calf`s ability to absorb IG, and the volume ingested (Lorenz et al., 2011).

We generated data on the production of the first colostrum meal. In most farms (52 farms, 83.9%) the colostrum is milked at fixed milking times, two farms (3.2%) don`t generate colostrum servings, and the calves get their colostrum by suckling, eight farms (12.9%) milk their colostrum with no regard to the usual milking times. Colostrum IG concentration decreases by 3.7% each hour post-calving (Lorenz et al., 2011; Morin et al., 2010). Although the production of the first colostrum meal during the routine milking process practiced under the conditions of our study is economically and process-efficiently advantageous, we recognize a bottleneck for the quantity of IG in the first colostrum meal for calves.

Written documentation of dairy farms is rare (Falkenberg, Krömker, Heuwieser & Fischer-Tenhagen, 2019; Hesse, Bertulat & Heuwieser, 2017). In this survey, we asked exemplary for the documentation of the practiced colostrum testing and the initial colostrum treatment of the neonatal calves. Only 10 farms (40.0% of testing farms, 16.1% of all farms) practice written documentation of colostrum testing, and only 14 farms (22.6%) have written documentation on colostrum application. Furthermore, written documentation of monitoring of calving is rare (11 farms, 17.7%). From our perspective, this is a lack in calf management with a lot of potential for improvement.

Currently, the application of 3–4 L colostrum in 2–4 h post-calving is recommended to ensure the coverage with IG in neonatal calves (Chigerwe et al., 2008; Chigerwe et al. 2009; Lorenz et al. 2011). We asked for time intervals relating to the calving event. We are aware that this is a reflection of the condition of the respondent. Of course, these data cannot be compared with measured data. The farms referred to in this study provide the calves their first colostrum meal within 2 h after calving (21 farms, 33.9%), within 4 h after calving (31 farms, 50.0%), later than 4 h after calving (4 farms, 6.5%). Our interpretation of this data is: most farmers in our study have a good awareness of the importance of an early calving-associated application of the first colostrum meal. The design of our study does not allow an estimation of time intervals between the birth of calves and colostrum application, as well as IG supplementation on-farm and calf levels.

Strength and limitations of our study

A limitation of the presented study is its regionality and the voluntariness of participation of the farms. We enrolled farms in one federal state in the northeast of Germany (Mecklenburg-Western Pomerania). Comparing farm demographic data of the study population with German dairy farm demographics (ADR 2016) revealed that milk yield was higher (9.0289 kg vs. 8.453 kg) and farms size (439.6 vs. 58.5) was greater in our study.

We enrolled farm size in three classes (low, medium, high average calf 31 days mortality) to demonstrate potential differences in management similar to a recent study on management practices related to mortality rates in calves (Tautenhahn et al., 2020). The selection of herds in our study is not really random, but stratified. This allows including a sufficient number of herds with low and high calf mortality in your study. It precludes a response bias caused by the voluntary agreement step.

The outcomes of this cross-sectional study are associative. A case-control study would be required to demonstrate causal effects.

Statistical methods

The used statistic procedure considered data challenges such as multicollinearity, confounding and interaction effects (Dohoo et al., 1997; Tautenhahn et al., 2020). We applied procedures to select variables with high explanatory power before inserting them into one of our models. Confounding and interaction effects were considered, too.

Conclusion

The results of our study indicate the potential for reducing calf mortality and the within herd prevalence of pathogens associated with diarrhea in pre-weaned calves. Areas for improvement are routine management of the newborn calf and feeding concepts for calves with diarrhea.

According to our data, insufficient written documentation of routines on dairy farms is another weakness in calf management on dairy farms. In our view, this is a deficiency in calf management with much potential for improvement.

Ethical approval

All examinations and sample collections were performed in line with legal regulations on the performance of experiments on animals (Bundesministerium, 2010).

Declaration of Competing Interest

The authors declare no conflicts of interest.

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