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Management Practices to Prevent Neonatal Calf Diarrhoea: Effects on Mortality in Danish Dairy Calves

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Master Thesis (SASK14301E)

Author: Michelle Vikkelsø Phillip (ZWS281)

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Supervisors: Assistant professor, Line Svennesen & Teaching associate professor Dorte Bay Lastein

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Abstract

Neonatal calf diarrhoea (NCD) remains one of the most important health challenges in dairy calf production and is considered a multifactorial condition influenced by enteric pathogens, hygiene, and management-related factors. The aims of this study were to investigate associations between hygiene and management practices, herd-level occurrence of major enteropathogens, and calf mortality in Danish dairy herds, with the purpose of identifying potential management action points for reducing mortality associated with NCD.

A cross-sectional study was conducted in 31 Danish dairy herds. Data collection included a structured questionnaire on calf management and hygiene routines, visual hygiene scoring during herd visits, pooled faecal sampling from calves aged 5–21 days, and herd-level mortality data obtained from the Danish Dairy Management System (DMS). Pooled faecal samples were analysed using the Speed V-Diar 4 rapid test for detection of *Cryptosporidium parvum* (*C. parvum),* rotavirus, bovine coronavirus (BCoV), and *E. coli* F5. Herd-level mortality between 2 and 30 days of age was used as the primary outcome measure in case-control analyses of a subset of herds. Statistical analyses included descriptive statistics,

univariable screening analyses, multivariable logistic regression modelling, and comparisons between high- and low-mortality herds.

C. parvum was detected in 93.5% of herds, whereas rotavirus and BCoV were detected in 25.8% and 9.7% of herds, respectively. *E. coli* F5 was not detected in any herd. Considerable between-herd variation was observed in hygiene practices and cleaning routines, particularly regarding calving pen cleaning, calf pen cleaning protocols, and feeding equipment hygiene. However, only limited statistically significant associations with mortality were identified. Pathogen occurrence alone did not explain differences in herd-level calf mortality. Instead, the results suggested that hygiene- and cleaning-related management factors may contribute to differences in herd-level mortality. The case-control analyses indicated that calf pen cleaning protocols, calving pen cleaning frequency, and feeding equipment hygiene showed the strongest tendencies towards association with herd-level calf mortality.

Overall, the findings suggest that pathogen detection should be interpreted as a potential indicator of herd-level infection pressure rather than as a direct measure of disease severity or mortality risk. This was particularly evident for *C. parvum*, which was widespread across herds but had limited ability to explain differences in mortality. In contrast, detection of viral pathogens, particularly rotavirus and BCoV, showed stronger tendencies towards association with mortality than *C. parvum*. The study therefore suggests that preventive efforts aimed at reducing mortality associated with NCD should focus on reducing infection pressure through consistent hygiene and cleaning routines, rather than relying on pathogen elimination or single interventions alone. Further longitudinal studies with individual-level pathogen detection, clinical disease recording, and larger study populations are needed to strengthen evidence-based recommendations for prevention of NCD and associated calf mortality in dairy herds.

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Introduction

Background:

Neonatal calf diarrhoea (NCD) is one of the most common and economically significant health problems in dairy production worldwide and a leading cause of morbidity and mortality during the first month of life (Cho & Yoon, 2014). European studies show that despite decades of advisory focus, diarrhoea remains a major welfare and productivity challenge in young calves, strongly influenced by management, housing and environmental hygiene (Klein-Jöbstl, et al., 2014; Gulliksen, et al., 2009). Enteric pathogens are widely distributed in preweaned calves, often in co-infections (Gulliksen, et al., 2009; Uhde, et al., 2008; Naciri, et al., 1999; Torreggiani, et al., 2025).

In recent years, new large-scale data from Danish dairy herds have added an important national perspective. The “Robuste Kalve” project included investigations in more than 80 dairy herds and showed that diarrhoea is highly prevalent in Danish dairy calves, with approximately 30-50% estimated to experience diarrhoea during the first weeks of life (Nielsen, L. R., 2021a; Nielsen, L. R., 2021b).

Aetiology:

A range of infectious agents are associated with NCD, and their relative importance varies with age, management and local epidemiology. Among these, *Cryptosporidium parvum* (*C. parvum*) is repeatedly reported as highly prevalent in dairy calves across different production systems. Numerous European and international studies (Naciri, et al., 1999; Uhde, et al.,

2008; Torreggiani, et al., 2025) have found *C. parvum* in a high proportion of diarrhoeic calves and often also in clinically healthy animals, indicating both a high infection pressure and a potential for subclinical shedding (Naciri, et al., 1999). Danish data from the "Robuste Kalve" project and other national studies confirm that *C. parvum* is consistently reported as one of the most prevalent enteropathogens in calves between one and three weeks of age and frequently detected alongside rotavirus in this age group (Goecke, et al., 2021; Nielsen, L. R., 2021b).

Likewise, rotavirus A and bovine coronavirus (BCoV) are well-recognised contributors to early-life diarrhoea. They are commonly detected within the same age window as *C. parvum* and often occur in co-infections, which may be associated with more severe clinical disease and greater production losses (Torreggiani, et al., 2025; Cho & Yoon, 2014; Thanthrige-Don, et al., 2018; Renaud, et al., 2021; Garro, et al., 2021; Brandao, et al., 2007). While *C. parvum*, rotavirus and BCoV primarily affect calves during the first weeks of life, other pathogens are more relevant in the immediate neonatal period. Enterotoxigenic *Escherichia coli* expressing the F5/K99 (*E. coli* F5) fimbriae affects calves during the first days of life, when gut receptors for F5 are still expressed, and continues to play an important contributing role in NCD (De la Fuente, et al., 1998; Naciri, et al., 1999).

The global distribution of these agents is reflected in several international epidemiological studies (Cho, et al., 2013; Garro, et al., 2021) that documented widespread occurrence of rotavirus, BCoV, *C. parvum* and *E. coli* F5 in NCD across multiple continents. Taken together, the evidence suggests that NCD in most dairy systems should be regarded as a multifactorial syndrome rather than a disease with a single dominant cause.

Transmission, environmental hygiene and infection pressure:
Enteric pathogen transmission is closely linked to environmental hygiene and management. *C. parvum* oocysts are extremely resistant to environmental degradation, shed in very high quantities and capable of infecting calves at low doses (Fayer, 2004; Klein-Jöbstl, et al., 2014; Gulliksen, et al., 2009).

Field studies from different countries have consistently associated high diarrhoea prevalence and heavy pathogen shedding with poor bedding conditions, wet lying areas, accumulation of faeces in pens and inadequate cleaning routines between batches of calves (Uhde, et al., 2008; Garro, et al., 2021). Contaminated feeding equipment has also been identified as an important

transmission route, particularly for *C. parvum* and *E. coli* F5, when nipples, tubes and buckets are not cleaned and disinfected thoroughly between uses (Izzo, et al., 2011). In herds where calves are group-housed, high stocking density and frequent mixing of age groups can further exacerbate the risk, as susceptible neonates are exposed to pathogens shed by older calves that may already have developed subclinical infections (Uhde, et al., 2008).

In addition, several management practices related to hygiene, cleaning protocols, and calf handling represent important components of internal biosecurity, aiming to reduce pathogen transmission within the herd (Lorenz, et al., 2011).

Part of the Danish "Robuste Kalve" project (Nielsen, L. R., 2021a) adds important detail by linking specific hygiene-related practices to calf health outcomes. Herds with more systematic and proactive hygiene and colostrum routines – including regular Brix testing of colostrum, avoiding use of low-quality colostrum for heifer calves, ensuring colostrum administration within four hours after birth, consistent use of disposable gloves and frequent boot washing – had markedly lower mortality in the first 14 days of life than herds that did not implement such measures (Nielsen, L. R. 2022). These results support the concept that relatively simple, low-cost management changes can have a measurable impact on calf survival and the burden of NCD. Qualitative work from Danish herds suggests that farmers generally perceive hygiene and biosecurity as important, but implementation may be limited by barn design, workflow and time pressure (Nielsen, L. R. 2022). This suggests that the gap between knowledge and practice may be a key driver of continued high infection pressure in otherwise well-informed herds.

Immunity, colostrum management and susceptibility to NCD:
Calf immunity further modulates disease risk. Insufficient colostrum management – delayed feeding, low volume or poor immunoglobulin (IgG) concentration – is a major contributor to susceptibility to early-life infections. Studies showed strong associations between inadequate colostrum intake, low serum IgG concentrations and increased likelihood of diarrhoea (Beam, et al., 2009; Windeyer, et al., 2014). The Danish "Robuste Kalve" project data provided robust support for this association under local conditions. Across 83 dairy herds, measurements of serum IgG in 1-10-day-old calves showed that a large proportion of herds had more than 20% of calves below the target level of 10 g/L. When clinical outcomes were analysed, calves with poor immunisation had a 39% higher risk of diarrhoea (Nielsen, L. R., 2022).

Ensuring adequate colostrum intake and passive transfer is also considered part of tertiary biosecurity, as strengthening calf immunity may reduce susceptibility to infectious disease and improve resilience following pathogen exposure (Windeyer, et al., 2014).

These findings highlight colostrum management as a central component of NCD prevention (Nielsen, L. R, 2022; Windeyer, et al., 2014). They also highlight that immunity and hygiene interact. Even good hygiene may not fully compensate for a high proportions of calves with FPT, and conversely, good colostrum management may not be sufficient if the environmental infection pressure is very high (Windeyer, et al., 2014; Gulliksen, et al., 2009; Klein-Jöbstl, et al., 2014). Effective prevention of NCD is therefore likely to require simultaneous attention to both immunity and hygiene at herd level.

Importance of problem:

Beyond immediate morbidity and mortality, NCD leads to reduced growth, increased labour costs, antimicrobial use, delayed weaning, and poorer lifetime performance (Svensson, et al., 2003; Renaud, et al., 2021). The disease also has substantial welfare implications and remains a high-priority area in herd health advisory work.

In modern dairy systems, prudent antimicrobial usage has become a central priority (Cho & Yoon, 2014; Windeyer, et al., 2014). Reducing unnecessary antimicrobial use in calves requires robust preventive strategies that target the underlying drivers of infection pressure and susceptibility. In this context, NCD is both a welfare concern – because affected calves suffer pain, dehydration and malaise – and a strategic disease complex for improving sustainability and antimicrobial consumption patterns in the herd (Cho & Yoon, 2014; Nielsen, L. R. 2022).

For dairy producers, effective prevention requires understanding how management routines and hygiene influence pathogen load at herd level. Although Danish dairy production is highly specialised, with well-established management programmes, diarrhoea remains prevalent (Nielsen, L. R., 2021b; Goecke, et al., 2021).

What do we know and what don't we know:

The existing literature provides a strong foundation for understanding NCD, but important knowledge gaps remain, especially in a Danish context. Relatively few studies have combined detailed herd-level hygiene assessments with concurrent measurements of pathogen occurrence and calf mortality. Many epidemiological investigations have relied on individual

management variables or broader categorical assessments rather than structured multidimensional hygiene scoring approaches applied across both calving and calf housing environments (Gulliksen, et al., 2009; Klein-Jöbstl, et al., 2014; Uhde, et al., 2008). In Denmark, the "Robuste Kalve" project provided important insight into diarrhoea prevalence, FPT and mortality patterns, but knowledge remains limited regarding how everyday hygiene practices in calving and calf housing environments relate to herd-level pathogen occurrence and mortality (Nielsen, L. R., 2022; Goecke, et al., 2021).

Moreover, while associations between management factors and health outcomes have been identified, there is still a need for studies that not only quantify the relationships between hygiene, pathogen occurrence and mortality, but also identify the most promising and realistic management interventions under Danish production conditions.

The aim of the thesis was to assess biosecurity and hygiene related to calves aged 5-21 days in Danish dairy herds through a cross-sectional study, and to explore associations between hygiene-related management factors and herd-level calf mortality through a case-control component.
Research questions:

- How are calves in Danish dairy systems managed with regard to hygiene, housing, colostrum management, and other factors expected to influence the risk or resilience of NCD and *C. parvum* occurrence?
- What is the relationship between hygiene, management practices, and herd-level occurrence of major NCD-associated pathogens in Danish dairy herds?
- How are hygiene-related management practices associated with herd-level calf mortality, and which potential management action points can be identified?

Hypotheses:

- Poor hygiene in calving and calf housing environments (questionnaire + visual scoring) is associated with higher neonatal mortality (DMS data).
- Specific hygiene practices, particularly related to cleanliness, bedding management and feeding equipment (questionnaire + visual scoring), constitute potential management action points associated with lower herd-level calf mortality (DMS data).

Materials and Methods

Overall study design:

The thesis combined an observational field study in Danish dairy herds with a review of existing literature.

The empirical part was organised in two components:

1. Cross-sectional study
    - Convenience-sampled dairy herds from a single veterinary practice were evaluated using questionnaire-based management data, visual hygiene scoring, pooled faecal sampling tested for *C. parvum*, rotavirus, BCoV and *E. coli* F5, using the Speed V-Diar 4 test (Virbac, France), and herd-level calf mortality data retrieved from DMS.
2. Case-control study
    - Herds with high and low calf mortality were selected from the cross-sectional dataset to evaluate associations between mortality, hygiene, management factors, and pathogen occurrence.

All analyses were performed at herd level.

Herd recruitment and selection criteria:

Recruitment strategy:

Herds were recruited through a veterinary practice (Univet, Jernbanegade 10, 6650 Brørup, Denmark). Herd owners received an invitation letter about the project (Appendix 1+2) and were subsequently contacted by phone regarding participation and scheduling of herd visits. Written consent was obtained during the herd visit (Appendix 3).

Inclusion criteria:

Herds were considered eligible for inclusion if they met the following criteria:

- Operating as a dairy herd with at least five calves aged 5-21 days at the time of the visit
- Were willing to:
    - Allow a herd visit for visual scoring, faecal sampling and questionnaire
    - Permit access to calf mortality data from DMS

Exclusion criteria:

Herds were excluded if:

- The farmer did not consent to one or more of the data collection components (questionnaire, visit, sampling, or data access)
- On-farm test analysis failed (e.g. test failure, insufficient sample material)
- Essential herd-level data could not be obtained (calf mortality data)
- The herd situation changed substantially, so the study population no longer reflected the target group (e.g. disease outbreaks or management changes during the data collection period that compromise comparability)

Data collection:

For each participating herd, data were collected from the following main sources

1. Hygiene visual scoring (Appendix 4)
2. Management questionnaire (Appendix 5)
3. Herd-level pathogen status based on a pooled sample of 5 calves (Speed V-Diar 4 test)
4. Herd-level calf mortality data (DMS data via Univet)
5. Number of annual cows in same time frame as mortality (DMS data via Univet)
6. Whether the herd had one or more CHR numbers registered (DMS data via Univet)

Questionnaire on hygiene and management:

A structured questionnaire was developed based on the introductory literature review (Phillip, 2025) and additional relevant studies to capture hygiene- and management-related risk factors hypothesised to influence infection pressure and occurrence of NCD (Cho & Yoon, 2014; Godden, S., 2008; Lorenz, et al., 2011; Thamsborg, et al., 2022).

The questionnaire (Appendix 5) consisted of 10 core sections targeting areas such as:

- Colostrum management (timing, volume, quality assessment)
- Hygiene and cleaning routines in calving areas
- Type of housing and grouping of calves in the first weeks of life
- Cleaning and disinfection of calf pens and equipment between calves
- Treatment of diarrhoea

All questions were closed with predefined answer categories to facilitate coding and statistical analysis. The questionnaire was administered by the author and answered by the herd manager or the person responsible for calf routines during the herd visit.

Visual hygiene scoring:

A standardised visual scoring of hygiene and housing conditions in the calving and calf areas was performed by the author (Appendix 4). The scoring system was developed for this thesis and consisted of four categories targeting risk areas, which were evaluated on a 1-3 scale inspired by established extension guidelines on calf facility management from the University of Wisconsin Food Animal Production Medicine (FAPM) program's checklists (University of Wisconsin-Madison FAPM Program, 2020). The scoring protocol included:

- Cleanliness and bedding condition in calving pens in use at the time of the visit
- Cleanliness, dryness, and bedding condition in 5 calf pens housing calves aged 5-21 days
- Visible contamination of walls, floors, and equipment with manure in the calf housing
- General organisation and cleanliness of feeding equipment (bucket/bowl, tube feeder, milk taxa, teat bottle, colostrum equipment) for the calves in the age of 5-21 days

Observations were summarised into one herd-level score for each hygiene category. Each herd therefore received four scores, one for each hygiene category. In addition, the four hygiene categories were summed into an explanatory total hygiene score representing the overall observed hygiene level at herd level. Because the individual hygiene components may differ in biological importance, the total score should be interpreted as an overall descriptive indicator rather than a validated weighted scale.

Faecal sampling and cow-side testing:

In each herd, 5 calves aged 5-21 days were randomly selected for faecal sampling and analysed with the Speed V-Diar 4® rapid diagnostic test (Virbac, France). This cow-side immunochromatographic assay detects major enteric pathogens (*C. parvum*, rotavirus, BCoV, *E. coli* F5) involved in NCD. For the purposes of this study, the results for *C. parvum* were the focus, but all test results were recorded for supplementary analyses.

This age interval was chosen because the study primarily focused on *C. parvum*, which is most commonly associated with diarrhoea in calves during the first weeks of life, particularly from approximately one to three weeks of age (Naciri, et al., 1999; Garro, et al., 2021). The

selected age range was therefore considered relevant for detecting herd-level occurrence of *C. parvum*, while still allowing detection of other major NCD-associated pathogens such as rotavirus and BCoV. These data provided an indicator of pathogen presence that can be linked to hygiene and management practices.

Sampling procedure:
In each participating herd, five calves aged 5-21 days were selected for faecal sampling using random selection. The total number of calves within the eligible age group was recorded, and a random number generator website (CalculatorSoup, n.d.) was used to select five calves from the total population. The identification numbers of the selected calves were recorded based on their ear tag number.

For pseudo anonymisation, each selected calf was assigned a sample number (1-5), and the sample number was linked to the calf's identification number on a separate recording sheet. In addition, the herd identification number (CHR number) was recorded and assigned a corresponding herd test number (1-31). The link between CHR and test numbers was stored separate from the farm observations.

Individual faecal samples were collected directly from each selected calf by rectal sampling and placed in separate sterile 10 mL test tubes. Disposable gloves were worn during sampling and changed between each calf to minimise the risk of cross-contamination. A lubricating gel was applied to facilitate rectal sampling. Faeces were collected directly into the sample tubes to avoid environmental contamination. Each test tube was labelled with a unique code identifying both the calf and the herd (e.g. "3-17", indicating calf number 3 from herd number 17).

The individual samples were pooled by transferring an intended equal amount of faeces from each sample, using a wooden spatula marked at 1 cm, into a new sterile 10 mL test tube labelled with herd test number (Pool 1-31). The pool was mixed thoroughly with a sterile wooden spatula. Following the manufacturer's recommendations regarding minimum volume required for testing, the spoon from the test kit was used for collecting the appropriate amount of faeces from the pool to the test analysis.

Test procedure:
Testing was carried out according to the official instructions provided by Virbac (Virbac, France):

1. A scoop of faeces (premixed equally between the 5 calves in the pool) was mixed with the supplied dilution buffer to create a homogeneous sample solution
2. The dilution buffer tube was placed into the sample well of the test cassette and the well was closed
3. The cassette was left to develop at room temperature for 15 minutes, after which results were read
4. Each cassette contained an internal control line, which had to appear for the test to be considered valid
5. Separate test strips on the cassette indicated the presence or absence of the four pathogens

Results interpretation:
A test was considered positive for a pathogen if the relevant test line appeared together with the control line, following the manufacturer's interpretation guide. Results were recorded as positive/negative.

Pooled faecal samples were used to assess pathogen occurrence at herd level. This approach was considered appropriate due to both the biological characteristics of the investigated pathogens and the overall study design.

Supplementary real-time PCR analysis:
As supplementary diagnostic support, the pooled faecal samples were sent for intern laboratory analysing at the section for pathobiological Sciences at University of Copenhagen. They were analysed by real-time PCR for *Cryptosporidium* spp. DNA. DNA was extracted from approximately 0.2 g of each pooled faecal sample using the DNeasy PowerSoil Pro Kit (Qiagen). Real-time PCR was performed using primers, probes and cycling conditions based on Berg et al. (2021), with 2x QuantiNova Probe PCR Master Mix (Qiagen) on Rotor-Gene Q instruments. Results were reported as cycle threshold (Ct) values and interpreted descriptively, not as quantitative measures of pathogen load.

Calf mortality data from DMS:
Herd-level data on calf mortality was calculated based on data from DMS for each participating herd in the period of 12 months prior to the 16th of April 2026. The primary outcome was:

- Mortality of liveborn in the neonatal period (2-30 days of age)

Calculated as:

$$\frac{N\ dead\ between\ days\ 2-30}{(N\ born-N\ stillborn)-N\ deaths\ on\ days\ 0-1} * 100$$

Mortality was expressed as the proportion of calves dying within 2-30 days relative to the number of liveborn calves. These data were used both in the cross-sectional analyses and to define high- and low-mortality herds for the case-control component.

Case-control component:

After completing data collection and initial mortality calculations, a subset of herds was selected for a case-control analysis:

- Case herds: herds with relatively high calf mortality (>3% mortality in 2-30 days of age)
- Control herds: herds with relatively low calf mortality (<1% mortality in 2-30 days of age)

One herd with extreme mortality (>20%) was excluded from mortality-related analyses after assessment of its disproportionate influence on the mortality distribution and regression estimates.

Hygiene scores, questionnaire-based management variables, and pathogen status were evaluated using univariable logistic regression with herd-level mortality categorised as a dichotomous outcome (high vs. low mortality). The analysis aimed to identify associations between management-related factors and mortality category, thereby supporting the identification of potential management-related action points.

Data handling and statistical analysis:

All data were entered and managed in Microsoft Excel spreadsheets and subsequently imported into Rstudio (R version 2026.01.1+403 (2026.01.1+403); Posit Software, PBC, Rstudio, Boston, MA, USA) for statistical analysis. Descriptive statistics were used to explore, present and characterise hygiene, management, pathogen presence and mortality across herds. Associations between hygiene-related factors and pathogen occurrence or mortality were explored using univariable regression models at herd-level. Statistical analyses consisted of three main components: descriptive analysis, univariable screening, multivariable modelling with a dichotomous outcome.

Cross-sectional study:

Descriptive statistics:

Descriptive analyses were performed to summarise:

- Herd characteristics (number of annual cows in same time frame as mortality, one or more connected CHR numbers)
- Management practices (questionnaire responses)
- Hygiene level (visual scores, composite hygiene score)
- Prevalence of *C. parvum* (herd-level prevalence)
- Calf mortality distribution (2-30 days)

To facilitate interpretation, categorical management variables were classified into three levels of practice quality: good, moderate, or poor. The classification was based on biological relevance and existing knowledge of risk factors for NCD, with particular focus on *C. parvum*. In figures, colours were used to visualise this classification, with green indicating good practice, yellow moderate practice, and red poor practice. Continuous variables were summarised using means and standard deviations or medians and interquartile ranges, depending on distribution, while categorical and ordinal variables were summarised using frequencies and proportions.

A composite management score was constructed to describe the overall number of recommended practices implemented in each herd. Practices classified as good contributed one point, whereas moderate or poor practices contributed zero points. The score was analysed as both a continuous variable and a dichotomous variable, with herds divided into high- and low-management groups based on the median score. Associations between the composite score and mortality were explored using logistic regression, Fisher's exact test, and sensitivity analyses with continuous calf mortality.

Case-control study:

Univariable analyses:

Univariable analyses were used to evaluate one-to-one associations both between explanatory variables (management practices, visual hygiene scores, pathogen occurrence) and between explanatory variables and the main outcome (calf mortality). First, associations between management practices, visual hygiene scores, and pathogen occurrence were explored to

describe relationships between explanatory variables and to identify potential overlap before mortality modelling. Second, associations between explanatory variables and herd-level calf mortality were evaluated to identify potential predictors of mortality. Depending on variable type and distribution, Chi-square tests/Fisher's exact tests, Wilcoxon rank-sum tests and univariable logistic regression were used.

Mortality was primarily analysed as a dichotomous outcome in the case-control analyses. In addition, a sensitivity analysis using mortality as a continuous outcome, within the analytical dataset after exclusion of the mortality outlier, was made to assess whether major patterns changed when mortality was not categorised. This analysis included herds with intermediate mortality levels, and tested the same groups of explanatory variables, including management practices, visual hygiene scores, and pathogen occurrence, against continuous mortality.

Variables with $p < 0.20$ in the univariable screening were considered for inclusion in multivariable modelling.

Multivariable regression modelling:
Exploratory multivariable logistic regression models were used to evaluate associations between selected management-, hygiene-, and pathogen-related variables and herd-level mortality group, defined as high mortality (>3%) versus low mortality (<1%). The purpose of the multivariable modelling was to identify potential management-related action points associated with lower calf mortality.

First, candidate variables were identified from the univariable mortality screening. Variables with $p < 0.20$ were considered potential candidates for multivariable modelling. These variables were then evaluated based on biological relevance, conceptual overlap, and potential multicollinearity. Variables describing highly related management areas were not included simultaneously in the same model to reduce redundancy and model instability. In addition, time to first colostrum feeding and frequency of full mucking out in calving pens were retained as biologically relevant variables.

Secondly, based on the univariable screening and biological assessment, five variables were selected for the full mortality model: feeding equipment cleaning frequency, calf pen cleaning protocol, combined viral pathogen detection (rotavirus or BCoV), time to first colostrum

feeding, and frequency of full mucking out in calving pens. An initial full multivariable logistic regression model was then constructed using these five candidate variables.

Subsequently, model reduction was performed using backward stepwise selection based on Akaike's Information Criterion (AIC). Likelihood ratio tests were used to assess whether removal of variables worsened model fit. Reduced model alternatives were evaluated based on AIC, likelihood ratio test results, biological interpretability, and model stability. Models showing quasi-complete separation, non-estimable odds ratios, or extremely wide confidence intervals were considered unstable, even if they had a lower AIC.

Finally, due to the limited number of case and control herds, the number of predictors retained in the final model was restricted to reduce the risk of overfitting. The final model was selected as the most stable and biologically interpretable model, rather than solely as the model with the lowest AIC. Herd was explored as a random effect in supplementary models to account for potential clustering among management-related variables within herds. However, these models did not converge, likely due to the limited number of herds and sparse data structure and were therefore not used for interpretation. Effect estimates from the final model were reported as odds ratios (OR) with 95% confidence intervals.

Significance level and software:
Statistical significance for final inference was set at $p < 0.05$. A threshold of $p < 0.20$ was applied during univariable screening to identify candidate variables for inclusion in multivariable modelling. All analyses were carried out in RStudio using packages including dplyr, tidyr, ggplot2, broom, car, MASS, purrr, writexl, corrplot, and janitor.

Ethical considerations:
This study involved non-invasive on-farm data collection. Faecal sampling was performed by rectal collection and was considered minimally invasive, with no procedures expected to cause more than brief discomfort. Participation of dairy herds was voluntary, and herd owners were provided informed consent before inclusion. The project follows the ethical principles described in the Danish Code of Conduct for Research Integrity as well as the University of Copenhagen guidelines for responsible research practice. No personal or human data are included in this study, and therefore no formal ethical approval is required.

Data protection and confidentiality:

All collected data – including questionnaire responses, hygiene scores, pathogen test results and mortality data – were treated confidentially and stored securely in accordance with EU GDPR regulations and the University of Copenhagen's data protection guidelines. Herds were assigned a unique anonymised ID code, all identifying information was stored separately from the research dataset, only aggregated and anonymised data were presented in the thesis and any potential publications, and electronic data were stored on secure, password-protected university servers.

Use of generative AI tools:

Generative AI tools (ChatGPT, OpenAI) were used during the thesis process to support linguistic editing, text structuring, translation support, and assistance with development and troubleshooting of R code. All scientific decisions, methodological choices, data interpretation, statistical decisions, and final formulations/conclusions were made by the author. AI tools were not used to generate, analyse or manipulate empirical data or to produce final statistical results.

Results

The results are presented in two parts. First, the cross-sectional component describes herd characteristics, reported management practices, observed hygiene conditions, and herd-level pathogen occurrence. Second, the case-control component presents analyses of associations between management practices, hygiene scores, pathogen occurrence, and herd-level calf mortality, defined as high versus low mortality. In this study, management refers to questionnaire-based reported practices, whereas hygiene refers to observed environmental conditions assessed by visual hygiene scoring.

Cross-sectional study:

Inclusion of herds:

A total of 45 dairy herds were invited to participate in the study by email in week 4-2026. During weeks 7 and 8 all herds were contacted by telephone. Of these, 44 herds were reached by telephone, while one herd could not be contacted after five attempts. Among the 44 herds reached, 31 agreed to participate, corresponding to a participation rate of 70.5%. The most

commonly reported reasons for non-participation were an insufficient number of calves within the eligible age range (six herds) or lack of time during the data collection period (four herds). Three herds declined participation despite meeting the inclusion criteria. Following recruitment, participating herds were contacted in week 9 and 10 to schedule herd visits within week 11 and 12. Visit routes were planned with respect to *Salmonella Dublin* status of the herds.

The final study population therefore comprised 31 dairy herds. Herd size, measured as number of annual cows, varied considerably between herds (Figure 1). The distribution was right-skewed, reflecting a small number of very large herds. Approximately half of the herds were operated on a single site (17 herds; 54.8%), while the remaining herds had multiple locations.

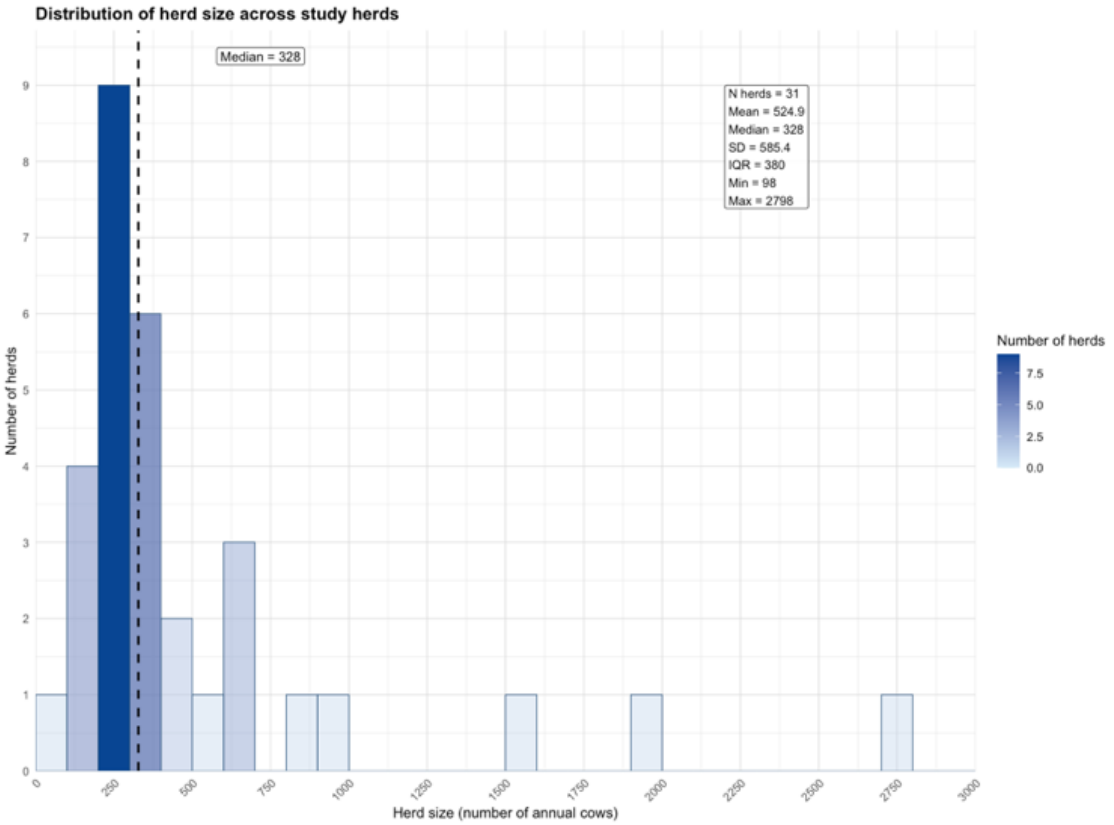

Figure 1: Distribution of herd size across the 31 participating dairy herds. Herd size was measured as the number of annual cows recorded in DMS. The dashed vertical line indicates the median herd size. The distribution was right-skewed, with most herds being small to medium-sized, while a few herds were substantially larger. The figure also includes descriptive statistics for the study population.

Management:

The full distribution of questionnaire responses is provided in Appendix 6. Selected management variables are presented in Figures 2-5, based on responses from all participating herds (n = 31).

Housing and calf responsibility:

The responses for prioritisation and housing of calves are shown in Figure 2. Most herds reported practices classified as moderate to good, while a smaller proportion reported practices classified as poor.

Most herds reported that calf care was prioritised equally with cow care. Responsibility for calf care also varied, with 45.2% of herds assigning calf care to a dedicated team, 35.5% to a single person, and 19.4% reporting shared responsibility.

Housing practices were relatively consistent across herds. The majority of herds housed calves individually during the first two weeks of life. Among herds practising group housing, age variation within groups was generally limited, and no herds reported age differences exceeding one week.

Overall, housing practices showed limited variation, whereas greater variation was observed in organisational aspects.

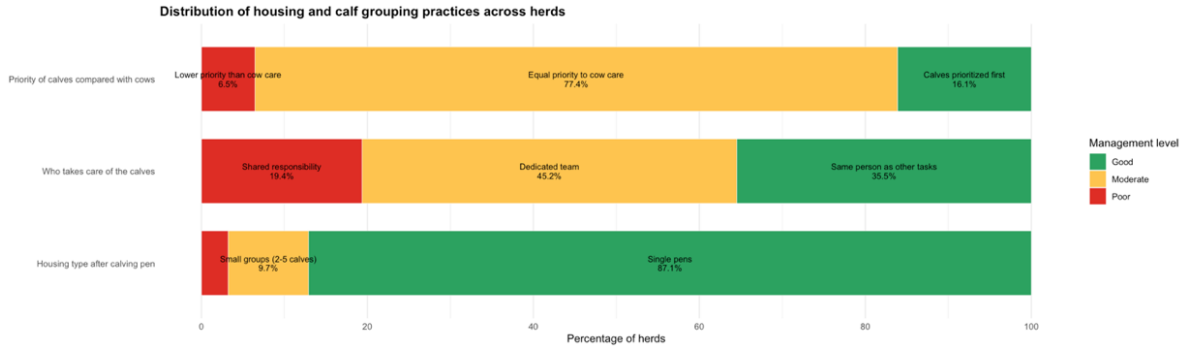

Figure 2: Distribution of selected calf management practices across herds. Each bar represents one management variable, and segments show the proportion of herds within each response category. Colours indicate the classification of each practice into good (green), moderate (yellow), or poor (red) based on expected impact on infection pressure and calf health.

Colostrum and early calf management:

Reported colostrum management practices are shown in Figure 3. Colostrum feeding practices were generally similar across herds, particularly regarding time to first colostrum feeding, volume administered, and method of administration. Greater variation was observed

in colostrum quality assessment, Brix threshold, and timing of calf removal from the calving pen.

Most herds reported practices classified as good for colostrum volume and timing. The majority of calves reportedly received more than 3 L of colostrum, and 80.6% of herds reported feeding colostrum within 2-6 hours after birth. Only a small proportion of herds reported delayed feeding beyond 6 hours.

In contrast, colostrum quality assessment varied between herds. Only 54.8% of herds reported measuring colostrum quality, while the remaining herds relied on visual or subjective assessment. Among herds measuring quality, the most commonly reported Brix threshold was 22-24%, followed by 18-21% (25.8%).

Methods of colostrum administration varied between herds. Tube feeding was reported as the most commonly used method, indicating that active colostrum administration was frequently used to ensure intake. Calves were most frequently reported to be moved after more than 12 hours, whereas fewer herds removed calves within 2-6 hours, 6-12 hours, or within 2 hours, respectively.

Overall, colostrum feeding practices were relatively uniform across herds, whereas quality control and timing of calf removal showed greater variation.

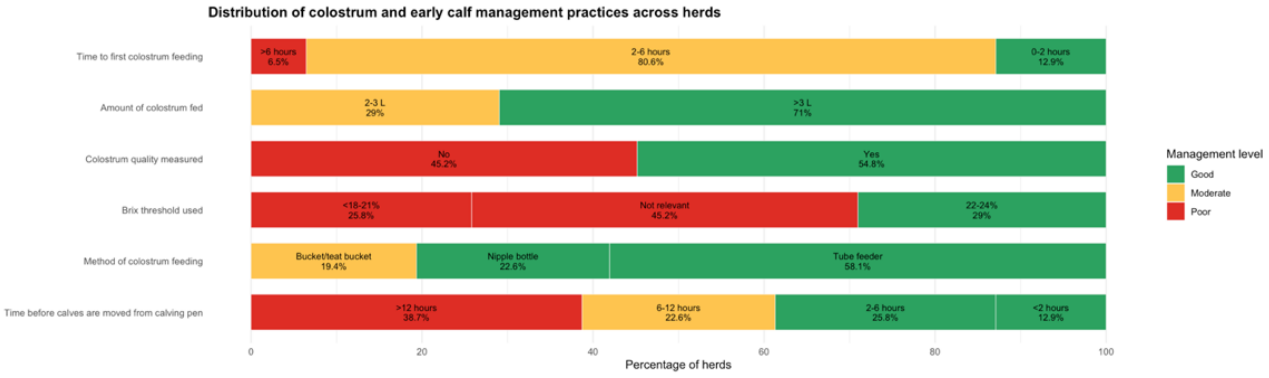

Figure 3: Distribution of colostrum management practices across herds. Each bar represents one practice, and segments show the proportion of herds within each response category. Colours reflect the classification of practices into good (green), moderate (yellow), and poor (red) according to their expected contribution to passive immunity and disease prevention.

Hygiene and cleaning routines:

Hygiene and cleaning routines showed substantial variation between herds (Figure 4). Bedding addition were generally frequent across herds. In calving pens, bedding was most

commonly added daily. Similarly, all herds reported adding of bedding in calf pens either daily or several times weekly.

In contrast, the frequency of full mucking out of calving pens varied considerably. Nearly half of the herds performed full mucking out after more than 10 calvings, whereas fewer herds cleaned more frequently.

Cleaning of feeding equipment also varied between herds. The most commonly reported routine was cleaning when a new calf entered a pen (29.0%) or daily cleaning (25.8%). Cleaning methods for feeding equipment included warm water above 60°C (96.8%), soap (71.0%), disinfection (25.8%), and cold water (12.9%). Some in combinations.

Cleaning methods also differed within calving pens and calf pens. In calving pens, all herds reported mucking out, while additional hygiene measures were less frequently reported: high-pressure washing (9.7%), soap (3.2%), drying (3.2%), and disinfection (25.8%). In calf pens, additional cleaning procedures were more common, including high-pressure washing (67.7%), drying (58.1%), disinfection (54.8%), and soap (48.4%). Also with some in combinations.

Overall, hygiene and cleaning practices varied most clearly in relation to cleaning frequency and the use of additional hygiene measures.

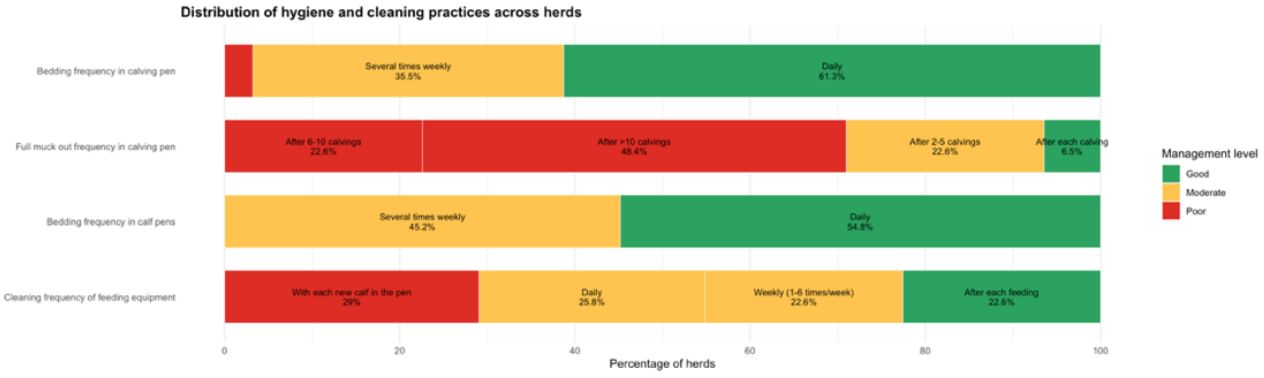

Figure 4: Distribution of hygiene and cleaning practices across herds. Each bar represents one practice, and segments show the proportion of herds within each response category. Colours indicate classification into good (green), moderate (yellow), and poor (red) practices, reflecting their expected influence on environmental contamination and pathogen transmission.

Disease management and biosecurity:

Disease management and preventive practices showed substantial variation between herds

(Figure 5). Structured monitoring was limited, with only 9.7% of herds reporting use of a fixed faecal scoring system. Diarrhoea was most commonly assessed based on visual clinical signs.

Isolation of sick calves was reported by 35.5% of herds. Basic hygiene measures also varied: separate tools for calf areas were used in 64.5% of herds, and personal hygiene measures such as handwashing or gloves were reported by 58.1%, whereas 32.3% reported no protective measures.

Preventive strategies specifically targeting *C. parvum* were limited, with only one herd reporting vaccination. Treatment approaches varied, and multiple responses were allowed. Electrolyte therapy was widely used (90.3%), and pain relief was reported by 71.0%, whereas specific antiparasitic treatments were rarely reported (9.7%).

Overall, disease management and preventive practices were heterogeneous, particularly regarding structured monitoring, isolation of sick calves, and targeted preventive measures.

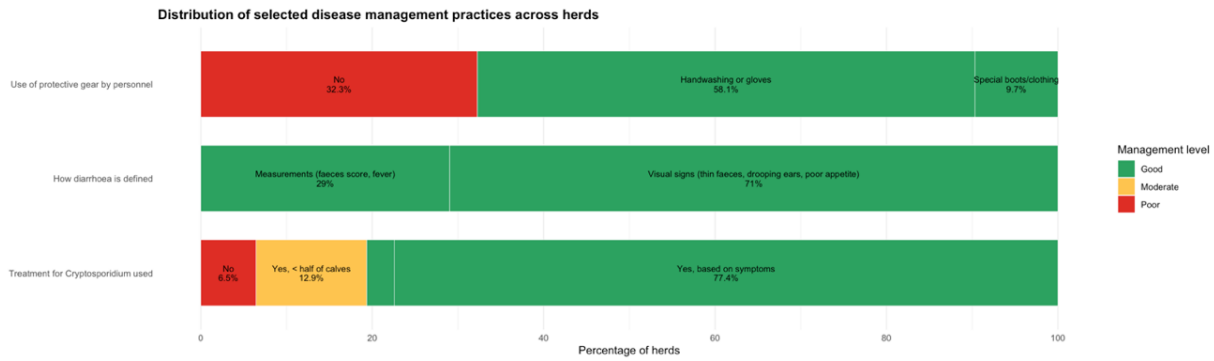

Figure 5: Distribution of selected disease management and biosecurity practices across herds. Each bar represents one practice, and segments show the proportion of herds within each response category. Colours represent classification into good (green), moderate (yellow), and poor (red) practices based on their expected role in disease detection, prevention, and control.

Hygiene:

The distribution of visual hygiene scores across the four assessed categories are presented in Figure 6, with detailed distributions and examples provided in Appendix 4+7.

Observed hygiene conditions varied across categories. Calving pens and overall manure contamination more frequently received higher (worse) scores compared to bedding quality of calf pens. Calf pen hygiene was generally good, with most herds receiving a score of 1 for

cleanliness, dryness, and bedding. In contrast, calving pen hygiene most commonly received a score of 2. Feeding equipment hygiene showed moderate variation, with most herds receiving scores of 1 or 2. Visible manure contamination was common, with most herds receiving a score of 2.

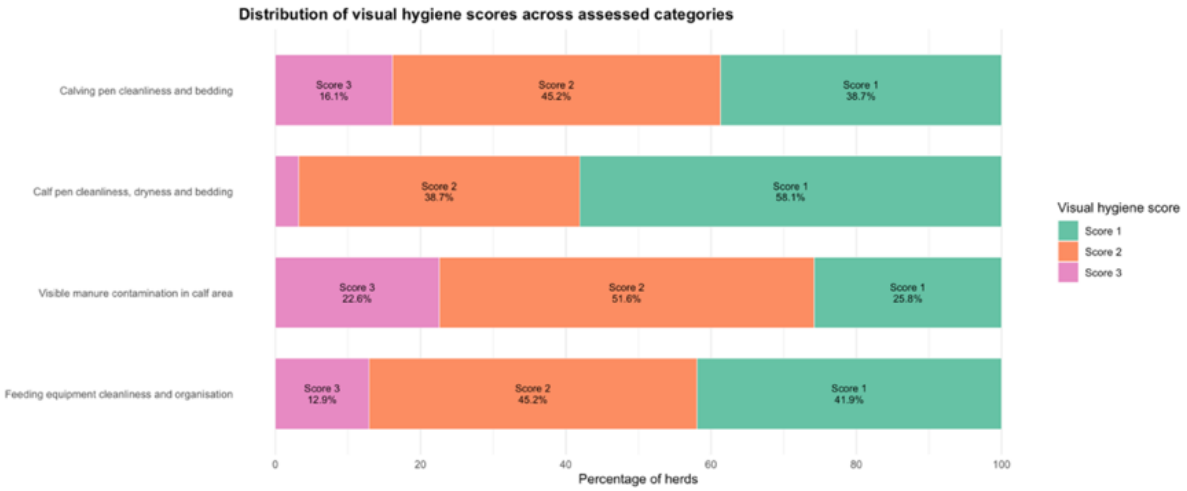

Figure 6: The figure shows the proportion of herds assigned hygiene scores from 1 (best) to 3 (poorest) across four assessed categories: calving pen cleanliness, calf pen cleanliness, manure contamination, and feeding equipment hygiene. Each bar represents one category, and colours indicate the distribution of scores.

The sum of visual scores is presented in Figure 7. The total visual hygiene score showed a concentration of herds around intermediate values. The heatmap (Figure 8) showed within-herd variation in scores across assessed areas, indicating that clearly suboptimal hygiene was present in at least one area in a substantial proportion of herds.

Overall, the visual hygiene assessment showed that hygiene conditions were not uniform across production areas, with the greatest challenges observed in calving pens and manure contamination.

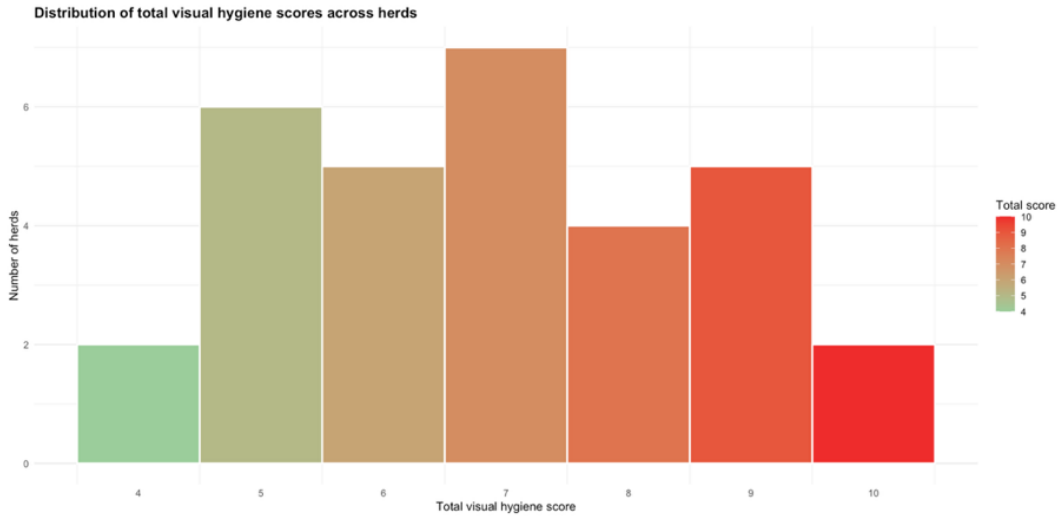

Figure 7: Distribution of total visual hygiene scores (sum of four categories) across 31 dairy herds. The total score ranged from 4 to 10, with higher scores indicating poorer hygiene. The distribution demonstrates moderate variation between herds, with most herds clustering around intermediate hygiene levels.

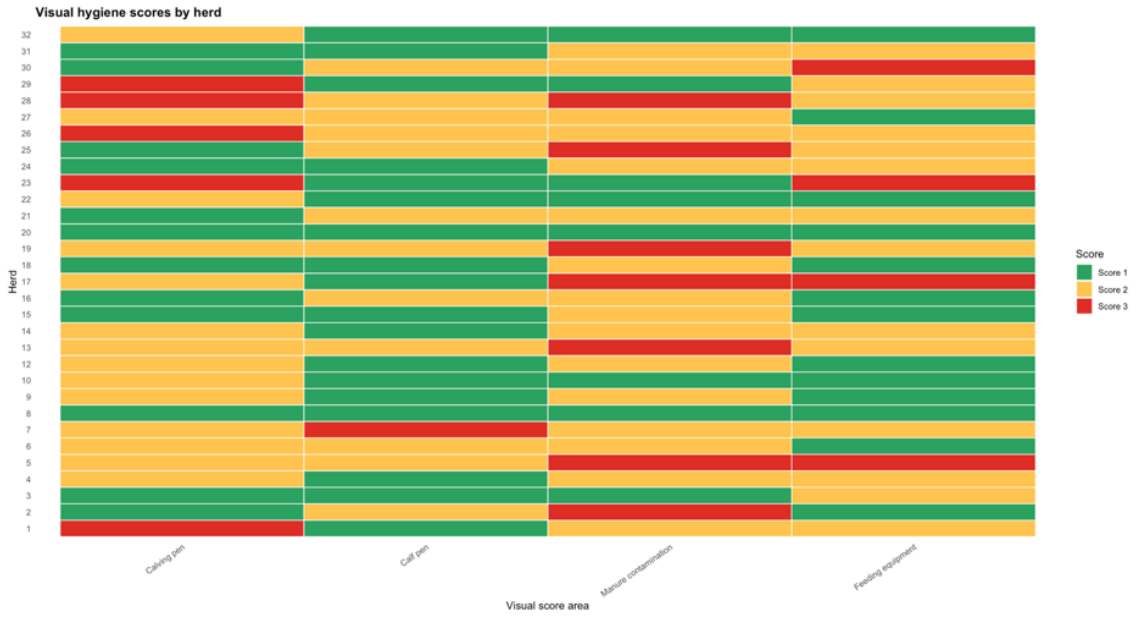

Figure 8: Heatmap illustrating herd-level visual hygiene scores across the four evaluated hygiene areas in the 31 participating Danish dairy herds (herd 11 is absent due to withdrawing from the study). Each row represents one herd, and each column represents one visual hygiene assessment area: calving pen, calf pen, manure contamination, and feeding equipment. Green indicates a score of 1 (good), yellow a score of 2 (moderate), and red a score on 3 (poor). The figure illustrates substantial variation in hygiene status between herds and between hygiene areas within herds.

Pathogen occurrence:

Herd-level enteric pathogen findings based on the 31 pooled tests are presented in Figure 9. The number of detected pathogens per herd ranged from 1 (71% of herds) to 2 (29% of herds). The most frequent finding was *C. parvum* alone, detected in 64.5% (20/31) of herds

(Figure 9). Among co-infections, the most common combination was *C. parvum* with rotavirus (19.4%, 6/31) (Figure 9), followed by *C. parvum* with coronavirus (9.7%, 3/31) (Figure 9). Rotavirus alone was detected in 6.5% (2/31) of herds. *E. coli* F5 was not detected in any herds.

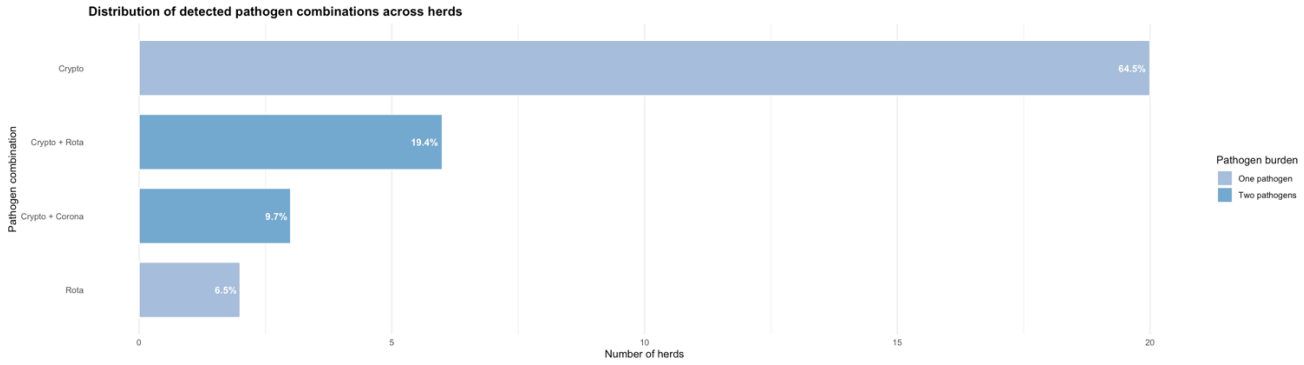

Figure 9: Herd-level occurrence of pathogen combinations detected using the Speed V-Diar 4 test in 31 Danish dairy herds. Combinations were dominated by co-infections involving C. parvum together with rotavirus or coronavirus.

Taken together, pathogen occurrence, based on the 31 tests, was dominated by *C. parvum*, with relatively limited variation between herds in pathogen presence.

Supplementary real-time PCR analysis supported the high occurrence of *Cryptosporidium* spp. DNA in the pooled faecal samples. Among the pooled herd samples, one sample was PCR-negative and corresponded to one of the two rapid test-negative samples. The other rapid test-negative sample was PCR-positive but had a comparatively higher Ct value than many of the positive pools, indicating a lower relative amount of *Cryptosporidium* DNA compared with the strongest positive samples.

Case-control study:

Given the high herd-level occurrence and limited between-herd variation in *C. parvum* status, the case-control analyses focused on management practices, visual hygiene scores, and viral pathogen detection in relation to herd-level calf mortality. *C. parvum* was not analysed as a separate explanatory pathogen variable in the mortality models because almost all herds tested positive.

Mortality:

Herd-level calf mortality from 2–30 days of age ranged from 0% to 20.98% in the full dataset, with a median of 1.45% (Figure 10). One herd with 20.98% mortality was excluded from

mortality-related analyses due to its disproportionate influence on the mortality distribution and regression estimates.

After exclusion of this outlier, mortality ranged from 0% to 8.9%, with a mean of 2.24% (SD = 2.10) and a median of 1.36% (IQR = 1.73) (Figure 11). Herds with mortality below 1% were classified as controls, whereas herds with mortality above 3% were classified as cases. Herds with intermediate mortality levels between 1% and 3% were excluded from the case-control comparison to increase contrast between groups. This resulted in 10 control herds and 7 case herds.

Overall, calf mortality varied considerably between herds, with most herds clustered at low mortality levels and a smaller number of herds showing higher mortality.

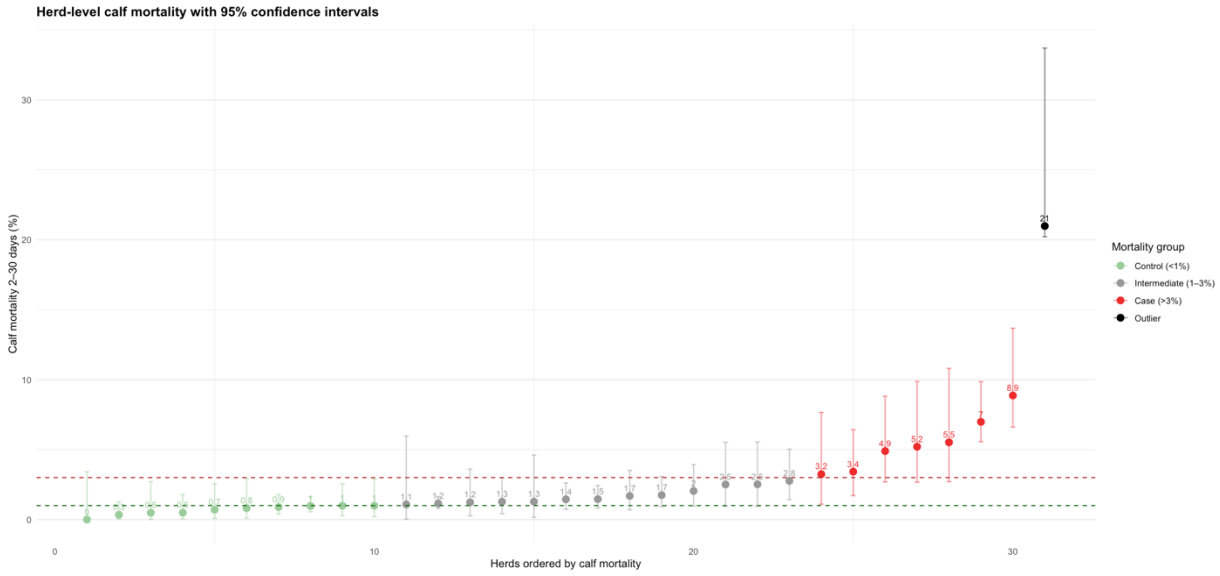

Figure 10: Herd-level calf mortality from 2–30 days of age (%) across the study population. Each point represents one herd, ordered by increasing mortality, with vertical error bars indicating 95% binomial confidence intervals. Dashed horizontal lines indicate the predefined mortality thresholds for low (<1%) and high (>3%) mortality. The figure illustrates substantial between-herd variation in calf mortality, with most herds clustered at low mortality levels and a small number of herds exhibiting markedly higher mortality including the outlier.

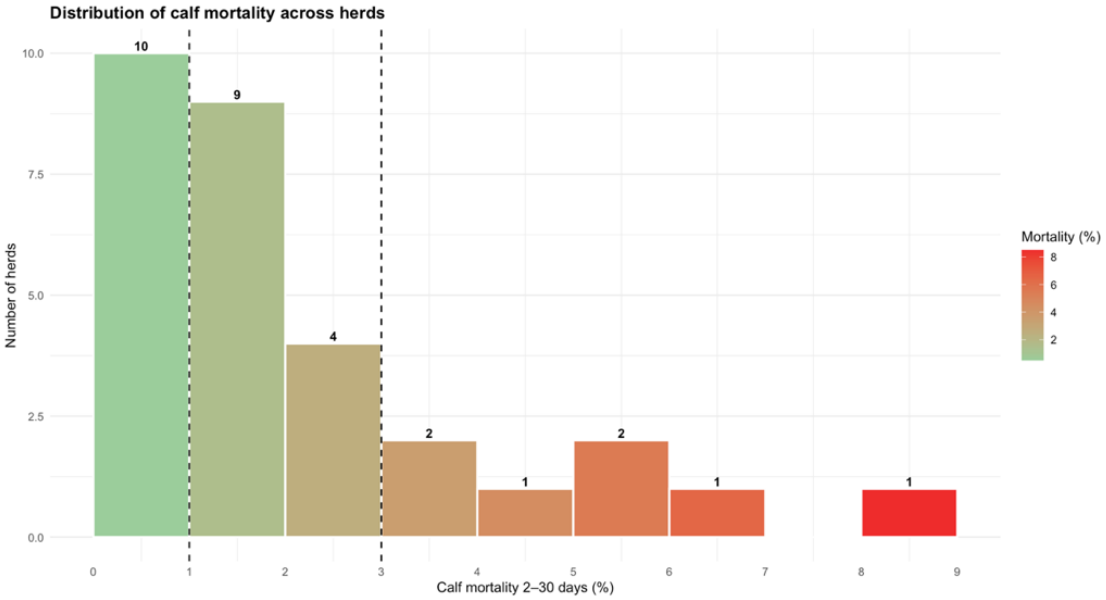

Figure 11: The histogram shows the distribution of calf mortality (%) across the 30 study herds. Most herds had low mortality levels, with the majority below 2%.

Conceptual framework and causal assumptions:

The conceptual causal diagram (directed acyclic graph; DAG) was constructed to clarify the assumed biological and epidemiological relationships between herd structure, management practices, hygiene, pathogen occurrence, NCD, and calf mortality (Figure 12). The DAG was used to support variable selection, identify potential confounding structures, and distinguish between upstream exposure variables and downstream intermediary variables located on the same biological pathway.

The arrows illustrate assumed causal relationships between variables based on biological plausibility and existing literature (Figure 12). Herd size was considered a potential upstream confounder, as it could influence both management-related factors and mortality outcomes. Cleaning routines and observed hygiene scores were assumed to influence environmental contamination and infection pressure, which in turn could affect pathogen occurrence. Colostrum management was assumed to influence calf immunity and resilience, thereby modifying disease severity and mortality risk. Pathogen occurrence was assumed to contribute to NCD severity, which could subsequently increase calf mortality. In addition, disease detection and treatment practices were assumed to influence mortality independently of pathogen occurrence by affecting the timeliness of intervention and supportive care.

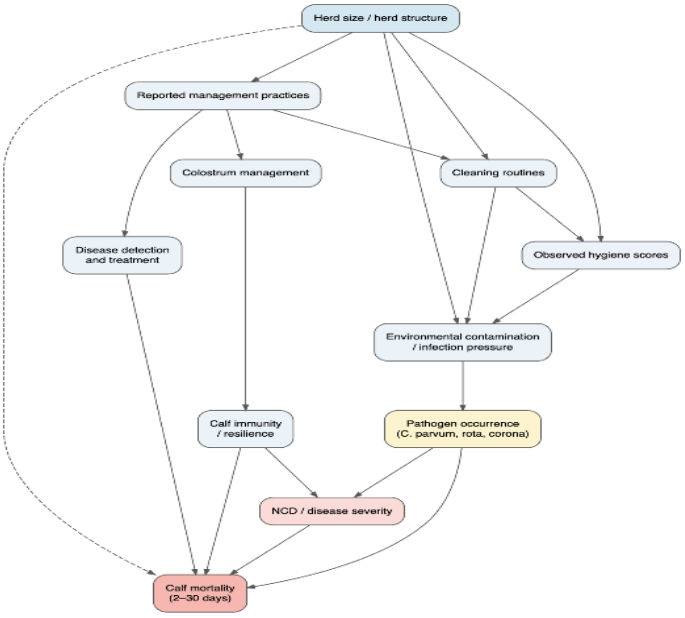

Figure 12: Conceptual causal diagram (directed acyclic graph; DAG) illustrating the assumed relationships between herd structure, management practices, hygiene, infection pressure, pathogen occurrence, calf immunity, neonatal calf diarrhoea (NCD), and calf mortality in the study population. The diagram was used to guide variable selection and interpretation of potential confounding and biological pathways in the univariable screening.

Univariable screening of associations:

The following sections present the results of the univariable screening analyses, including the correlation structure among management, hygiene, and pathogen variables, associations between explanatory variables, and associations with herd-level calf mortality. These analyses were used to evaluate overlap between explanatory variables, reduce redundancy before mortality modelling, and support interpretation of relationships between management, hygiene, pathogen occurrence, and mortality.

Correlation and structure of management variables:

Correlation analysis showed that most management variables were weakly to moderately correlated, although some strong correlations were observed (Appendix 9). The strongest correlations were found between measurement of colostrum quality and Brix threshold ($r = 0.90$), and between housing type and age variation within calf groups ($r = 0.88$). Strong correlations were also observed among cleaning-related variables describing washing, drying, and disinfection procedures. These correlations were expected, as several questionnaire items described related aspects of the same management area. Variables with little or no variation across herds were considered to have limited discriminatory value for further analyses.

Selection of management variables for further analyses:

To reduce redundancy, one variable from highly correlated or conceptually overlapping variable groups was retained based on interpretability and biological relevance. Brix threshold, age variation within calf groups, and drying during cleaning procedures were excluded due to overlap with measurement of colostrum quality, housing type, and soap use during cleaning, respectively.

Cleaning-related variables were further combined into simplified hygiene management variables. Calving pen hygiene was represented by a binary variable indicating use of disinfection, whereas calf pen cleaning protocol was retained as a three-level variable representing increasing cleaning intensity: muck out only, additional cleaning without disinfection, and additional cleaning with disinfection. Which includes the before mentioned variable soap use during cleaning. After variable reduction and regrouping, 31 management variables remained for further analyses.

Correlation and structure of hygiene variables:

Correlations between the visual hygiene scores were generally low to moderate (Figure 13). The strongest correlation was observed between calf pen hygiene and manure contamination ($r = 0.59$), whereas all other correlations were weak ($r < 0.34$). This suggests that the visual hygiene categories captured partly different aspects of hygiene rather than one uniform herd-level hygiene dimension.

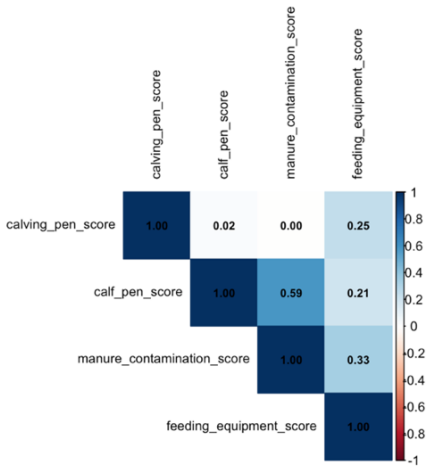

Figure 13: Correlation heatmap of visual hygiene scores across four assessed categories. Correlations were generally low to moderate, indicating that each score captured distinct aspects of hygiene rather than reflecting a single underlying dimension.

Correlation and structure of pathogen variables:

Among pathogen variables, *E. coli* F5 was excluded from further analyses due to complete detection absence in the dataset. Mixed infection was considered redundant with pathogen count, as it was directly derived from the number of detected pathogens (1 or 2 pathogens). A combined viral variable (Rotavirus or BCoV) was created due to the low occurrence of BCoV. Correlation between pathogen variables ranged from $r = 0.07$ to $r = 0.85$. The strongest correlation was observed between the combined viral variable and rotavirus ($r = 0.85$). All other correlations were < 0.5.

Associations between management and hygiene:

Several cleaning-related management practices were associated with visual hygiene scores ($p < 0.05$). Use of disinfection in both calving pens and calf pens was associated with lower (improved) hygiene scores, whereas use of cold water for cleaning feeding equipment was associated with poorer hygiene scores (Appendix 10).

Feeding equipment cleaning frequency, calf monitoring responsibility, and selected disease management practices showed weaker tendencies towards association with hygiene scores ($p < 0.20$).

Overall, visual hygiene scores were more closely associated with cleaning intensity and disinfection practices.

Associations between management and pathogen occurrence:

Few management variables were associated with pathogen occurrence at the predefined screening threshold of $p < 0.20$. For *C. parvum*, delayed colostrum feeding showed the clearest association ($p = 0.034$), while separate tools and structured faecal scoring showed weaker tendencies ($p = 0.10$ and $p = 0.19$, respectively).

For rotavirus and the combined viral outcome, calf movement and separation practices showed the clearest tendencies towards association, with p-values ranging from 0.06 to 0.11. For BCoV specifically, use of separate tools in calf areas and structured faecal scoring both showed weak tendencies ($p = 0.18$).

No clear associations were observed for co-infections, although prioritisation of calves relative to cow care showed a weak tendency towards association ($p = 0.11$). Overall, associations between management variables and pathogen occurrence were generally weak.

Associations between hygiene and pathogen occurrence:

The associations between visual hygiene scores and pathogen occurrence were generally weak. No hygiene variables were associated with *C. parvum,* rotavirus, BCoV, or co-infections at the predefined screening threshold ($p < 0.20$).

The only tendency towards association was observed between calving pen hygiene score and the combined viral outcome (rotavirus or BCoV) ($p = 0.15$).

Overall, the analyses did not identify strong direct relationships between visual hygiene assessments and pathogen occurrence at herd level, only the two mentioned variables showed weak tendencies meeting the screening threshold.

Associations with calf mortality:

Univariable associations between management variables and dichotomised herd-level calf mortality were generally weak ($p > 0.10$) (Table 1). Calf pen cleaning protocol ($p = 0.18$) and feeding equipment cleaning frequency ($p = 0.12$) showed the clearest tendency towards association with mortality. Calf management responsibility and use of separate tools showed weaker tendencies ($p > 0.20$). Confidence intervals were wide, indicating substantial uncertainty in the estimates.

Table 1: Distribution of selected management-related variables from the univariable mortality screening in low-mortality control herds and high-mortality case herds. Values are presented as number and proportion of herds within each mortality group. P-values were obtained from univariable logistic regression analyses with mortality group as the dichotomous outcome.

Variable	Level	Control n (%)	Case n (%)	P-value
Frequency of feeding equipment cleaning				0.122
	After every feeding	4 (40%)	1 (14.3%)	
	Daily	3 (30%)	1 (14.3%)	
	Several times weekly	2 (20%)	2 (28.6%)	
	Weekly or less	1 (10%)	3 (42.9%)	
Calf pen cleaning protocol				0.184
	Additional cleaning with disinfection	7 (70%)	2 (28.6%)	
	Additional cleaning without disinfection	2 (20%)	3 (42.9%)	
	Muck out only	1 (10%)	2 (28.6%)	
Responsibility for calf management				0.288
	Same person as other tasks	5 (50%)	2 (28.6%)	
	Dedicated team	2 (20%)	1 (14.3%)	
	Shared responsibility	3 (30%)	4 (57.1%)	
Use of separate tools in calf areas				0.318
	No	8 (80%)	4 (57.1%)	
	Yes	2 (20%)	3 (42.9%)	

Univariable associations between visual hygiene scores and mortality were weak ($p > 0.15$) (Table 2). No strong associations were found ($p < 0.05$), although poorer feeding equipment

hygiene scores and poorer calf pen hygiene scores showed tendencies towards association with higher mortality levels (p = 0.18 and p = 0.19, respectively).

Table 2: Distribution of visual hygiene scores in low-mortality control herds and high-mortality case herds. Visual hygiene scores were assessed on a three-level scale, where good, moderate and poor indicate increasing levels of suboptimal hygiene conditions. Values are presented as number and proportion of herds within each mortality group. P-values were obtained from univariable logistic regression analyses with mortality group as the dichotomous outcome.

Variable	Level	Control n (%)	Case n (%)	P-value
Visual feeding equipment score				0.176
	Good	5 (50%)	2 (28.6%)	
	Moderate	4 (40%)	2 (28.6%)	
	Poor	1 (10%)	3 (42.9%)	
Visual calf pen score				0.187
	Good	7 (70%)	3 (42.9%)	
	Moderate	3 (30%)	3 (42.9%)	
	Poor	0 (0%)	1 (14.3%)	

Among pathogen-related variables (Table 3), the combined viral variable (rotavirus or BCoV detection) showed the clearest tendency toward association with mortality (OR = 12.0; p = 0.056), although the estimate was imprecise due to the small number of herds.

Overall, the univariable mortality screening identified only weak and imprecise associations. The clearest tendencies were observed for selected cleaning-related management variables, visual feeding equipment score and combined viral pathogen detection.

Table 3: Distribution of pathogen-related variables in low-mortality control herds and high-mortality case herds. Pathogen detection was based on herd-level test results, and values are presented as number and proportion of herds within each mortality group. P-values were obtained from univariable logistic regression analyses.

Variable	Level	Control n (%)	Case n (%)	P-value
Rotavirus or BCoV detected				0.056
	Not detected	9 (90%)	3 (42.9%)	
	Detected	1 (10%)	4 (57.1%)	

In the sensitivity analysis using continuous calf mortality, the overall pattern was similar to the dichotomised mortality analyses. Frequency of full mucking out in calving pens showed the clearest association with continuous mortality (p = 0.021). Delayed colostrum feeding and the combined viral pathogen outcome showed weaker tendencies towards higher mortality (p = 0.18 and p = 0.09, respectively). Among visual hygiene scores, feeding equipment hygiene showed the clearest tendency towards increasing mortality (p = 0.12).

Selection of variables for further analyses:

Based on the univariable mortality screening, sensitivity analysis, and biological assessment, five variables were retained as primary candidates for multivariable mortality analyses: feeding equipment cleaning frequency, calf pen cleaning protocol, combined viral pathogen detection, time to first colostrum feeding, and frequency of full mucking out in calving pens (Table 4).

Feeding equipment cleaning frequency and calf pen cleaning protocol met the predefined screening threshold ($p < 0.20$) and appeared less favourable in high-mortality herds. The combined viral outcome showed the clearest pathogen-related association with mortality. Time to first colostrum feeding and frequency of full mucking out in calving pens were retained due to biological relevance and support from the sensitivity analysis. Overlapping cleaning and hygiene variables were not included simultaneously in multivariable models to reduce redundancy and improve interpretability.

Table 4: Distribution of selected candidate variables from the univariable case-control screening analyses comparing low-mortality control herds and high-mortality case herds. Variables included in the table met the predefined screening threshold of $p < 0.20$ in the univariable analyses and were therefore considered for inclusion in the subsequent multivariable logistic regression modelling or had biological relevance. Values are presented as number and proportion of herds within each mortality group. P-values were obtained from univariable logistic regression analyses.

Variable	Level	Control n (%)	Case n (%)	P-value
Frequency of feeding equipment cleaning				0.122
	After every feeding	4 (40%)	1 (14.3%)	
	Daily	3 (30%)	1 (14.3%)	
	Several times weekly	2 (20%)	2 (28.6%)	
	Weekly or less	1 (10%)	3 (42.9%)	
Calf pen cleaning protocol				0.184
	Additional cleaning with disinfection	7 (70%)	2 (28.6%)	
	Additional cleaning without disinfectio	2 (20%)	3 (42.9%)	
	Muck out only	1 (10%)	2 (28.6%)	
Visual feeding equipment score				0.176
	Good	5 (50%)	2 (28.6%)	
	Moderate	4 (40%)	2 (28.6%)	
	Poor	1 (10%)	3 (42.9%)	
Visual calf pen score				0.187
	Good	7 (70%)	3 (42.9%)	
	Moderate	3 (30%)	3 (42.9%)	
	Poor	0 (0%)	1 (14.3%)	
Rotavirus or BCoV detected				0.056
	Not detected	9 (90%)	3 (42.9%)	
	Detected	1 (10%)	4 (57.1%)	
Time to first colostrum feeding				0.996
	0-2 h	1 (10%)	0 (0%)	
	2-6 h	9 (90%)	5 (71.4%)	
	>6 h	0 (0%)	2 (28.6%)	
Frequency of full muck out in calving pen				0.997
	After every calving	1 (10%)	0 (0%)	
	After 2-5 calvings	0 (0%)	4 (57.1%)	
	After 6-10 calvings	3 (30%)	1 (14.3%)	
	After > 10 calvings	6 (60%)	2 (28.6%)	

Case-control comparison between high and low mortality rate herds:

The initial full candidate model included five variables representing colostrum management, calving pen cleaning, feeding equipment cleaning, calf pen cleaning protocol, and viral pathogen detection. The supplementary mixed effect model with herd as random effect did not converge, and standard logistic regression models were therefore used.

During backward selection, feeding equipment cleaning frequency and time to first colostrum feeding were removed because their removal did not worsen model fit and improved model stability. Two reduced candidate models were then evaluated.

The model with the lowest AIC included frequency of full mucking out in calving pens and combined viral pathogen detection. However, this model showed quasi-complete separation, resulting in implausible odds ratio estimates and extremely wide or non-estimable confidence intervals.

A second model including calf pen cleaning protocol and combined viral pathogen detection had a slightly higher AIC but showed better stability and interpretability. This model converged, and no further reduction improved AIC. It was therefore selected as the final exploratory multivariable model (Table 5).

In the final model, viral pathogen detection showed a tendency towards higher odds of belonging to the high-mortality group, whereas more extensive calf pen cleaning protocols showed a tendency towards lower odds. Confidence intervals remained wide, indicating substantial uncertainty. Likelihood ratio tests showed that removal of either retained variable worsened model fit.

Table 5: Exploratory multivariable logistic regression model evaluating associations between selected herd-level factors and belonging to the high calf mortality group (>3%) compared with the low mortality group (<1%). Candidate variables were selected based on the univariable screening analyses (p<0.20) and biological plausibility. Odds ratios (OR) with 95%

confidence intervals (CI) are presented for variables included in the final model. The table further includes retained variables in the final exploratory mortality model, model AIC, and likelihood ratio test (LRT) p-values for the retained predictors.

Model	Variable	Odds ratio	95% CI	P-value
Calf pen cleaning protocol + Rotavirus or BCoV detected	Additional cleaning without disinfection	0.25	0.00-6.56	0.423
Calf pen cleaning protocol + Rotavirus or BCoV detected	Additional cleaning with disinfection	Not reliably estimable	-	0.996
Calf pen cleaning protocol + Rotavirus or BCoV detected	Rotavirus or BCoV detected	6.29×10^8	Not reliably estimable	0.996

Model	Retained variables	AIC
Final exploratory mortality model	Calf pen cleaning protocol + rotavirus or BCoV detection	19.5

Variable	LRT p-value
Calf pen cleaning protocol	0.030
Rotavirus or BCoV detection	0.003

Combined effect of management practices:

The composite management score ranged from 9 to 19 implemented good practices (median = 13). Control herds had a slightly higher average number of implemented good practices compared with case herds (mean = 13.8 vs. 12.4 practices, respectively). However, the difference was not statistically significant (Wilcoxon rank-sum test, p = 0.37).

In logistic regression analysis, an increasing number of implemented good management practices was associated with lower odds of belonging to the high-mortality group (OR = 0.78, 95% CI: 0.45–1.18, p = 0.28). Although the estimated effect suggested a potential protective tendency, the confidence interval was wide and included unity.

When herds were categorised into high- and low-management groups based on the median score, mean calf mortality was similar between management groups (2.79% vs. 2.53%). No statistically significant association was observed using Fisher's exact test (p = 1.00; OR = 0.90; 95% CI = 0.09 – 9.77) or logistic regression analysis (OR = 0.89; 95% CI: 0.12 – 6.69; p = 0.91).

Overall, these findings did not demonstrate a clear association between the combined management score and herd-level calf mortality.

Sensitivity analysis using continuous mortality outcome:

Sensitivity analyses using continuous mortality did not identify a clear association between the composite management score and herd-level mortality. Correlation and regression estimates suggested weak negative associations, consistent with the direction observed in the primary case-control analyses.

Discussion

Summary of key findings:

Taken together, the results demonstrated substantial between-herd variation in management practices, hygiene conditions and calf mortality, whereas pathogen occurrence showed a different pattern. Colostrum management practices were generally consistent across herds, while hygiene- and cleaning-related routines varied considerably, particularly regarding calf pen cleaning, calving pen cleaning and feeding equipment hygiene.

Pathogen findings were dominated by a consistently high occurrence of *C. parvum*, with 93.5% of herds testing positive. This limited the ability of *C. parvum* presence to differentiate between herds with different mortality levels. In contrast, rotavirus and BCoV were detected less frequently, and the combined viral outcome showed clearer tendencies towards association with herd-level mortality.

Across the analyses, cleaning-related management practices appeared more consistently than broader indicators of overall management level. However, few statistically significant associations were identified, and most estimates were imprecise. The results should therefore be interpreted as exploratory herd-level tendencies rather than causal effects.

Interpretation of pathogen occurrence and effects:

The limited number of statistically significant associations identified in this study should be interpreted in relation to both the biological complexity of NCD and the structure of the dataset. Several variables showed biologically plausible tendencies, particularly related to hygiene- and cleaning-related management practices and viral pathogen occurrence, but most estimates were characterised by wide confidence intervals and limited statistical precision.

A central finding was the high herd-level occurrence of *C. parvum,* with 93.5% of herds testing positive. Because almost all herds were classified as exposed, binary *C. parvum* status had limited ability to differentiate between high- and low-mortality herds. The high occurrence should therefore be interpreted as evidence that *C. parvum* was widespread in the calf environment of the study population, rather than as a factor that clearly explained

variation in mortality between herds. This interpretation is consistent with previous studies showing that *C. parvum* is frequently detected in young calves and may occur in both diarrhoeic and non-diarrhoeic animals, underlining the distinction between pathogen detection, infection pressure and clinical disease expression (Gulliksen, et al., 2009; Naciri, et al., 1999; Garro, et al., 2021).

The supplementary real-time PCR results supported this interpretation. One rapid test-negative herd was also PCR-negative, whereas the other rapid test-negative herd was PCR-positive but with a comparatively higher Ct value than many of the positive pools. In contrast, rapid test-positive pools generally showed strong PCR positivity. This suggests that rapid antigen detection may primarily identify herds with higher pathogen loads, whereas low-level shedding may be missed. Consequently, herd-level pathogen status based on pooled rapid testing should be regarded as a practical indicator of infection pressure rather than a quantitative measure of pathogen burden.

Although *C. parvum* is consistently recognised as an important contributor to NCD, disease severity is unlikely to be determined by its presence alone. Clinical outcomes are influenced by infection dose, shedding intensity, environmental contamination, calf immunity, co-infections and disease management (Cho & Yoon, 2014; Windeyer, et al., 2014; Klein-Jöbstl, et al., 2014). Previous studies have also shown that co-infections can increase both the likelihood and severity of diarrhoea (Cho, et al., 2013; Thanthrige-Don, et al., 2018; Renaud, et al., 2021). In the present study, co-infections were less frequent than reported in several previous studies, but the combined viral outcome showed clearer tendencies towards association with mortality than *C. parvum* alone. This may indicate that viral pathogen detection identified a smaller subset of herds with additional enteric pathogen pressure. However, given the small number of positive herds and the limited case-control dataset, this finding should be interpreted cautiously.

The absence of strong statistical associations is also likely to reflect limited contrast and statistical power. Several management practices, particularly colostrum-related routines, showed relatively little variation between herds, reducing their ability to separate high- and low-mortality herds. In addition, the small number of case and control herds increased uncertainty around effect estimates. Under these conditions, only large effects would be

expected to reach statistical significance, while moderate but biologically relevant associations may remain undetected.

Mortality should also be considered a relatively crude and non-specific outcome for evaluating NCD-related risk factors. It reflects only the most severe endpoint of disease and may be influenced by treatment practices, euthanasia decisions, concurrent disease, disease detection, calf movement and general herd management. Effects on diarrhoea incidence, pathogen shedding or disease severity may therefore not necessarily translate into detectable differences in herd-level mortality. The sensitivity analysis using continuous mortality produced patterns broadly similar to the primary case-control analyses, suggesting that the limited number of statistically significant findings was not explained solely by dichotomisation of mortality.

Overall, the pathogen results indicate that herd-level calf mortality was unlikely to be explained by pathogen presence alone. Instead, differences between herds were more likely shaped by the combined effects of pathogen load, environmental contamination, calf immunity, co-occurring pathogens and disease management (Schroeder, et al., 2012; Klein-Jöbstl, et al., 2014; Windeyer, et al., 2014). This supports the interpretation of NCD-related mortality as a multifactorial herd-level outcome and emphasises the importance of reducing infection pressure rather than focusing solely on pathogen presence or complete pathogen elimination.

Management, hygiene and immunity-related practices:

The observed tendencies in this study suggest that specific hygiene- and cleaning-related practices may be more informative than broad indicators of overall management level when evaluating differences in herd-level calf mortality. Calf pen cleaning protocol, frequency of full mucking out in calving pens, and feeding equipment hygiene repeatedly appeared among the most relevant variables across analyses. These practices represent different but connected routes of faecal-oral exposure, including the calf's immediate housing environment, the calving area, and equipment used for milk or colostrum feeding. This is consistent with previous studies identifying hygiene, housing conditions, cleaning routines and disease management as relevant, although context-dependent, risk factors for calf diarrhoea (Gulliksen, et al., 2009; Klein-Jöbstl, et al., 2014; Garro, et al., 2021).

The lack of a clear association between the composite management score and mortality supports the interpretation that simply counting the number of "good" practices may be too crude to capture biologically important differences between herds. Individual practices are unlikely to contribute equally to NCD prevention, and their effects probably depend on both their biological relevance and their consistency of implementation. A herd may therefore report several good practices while still having weaknesses at critical contamination points, such as calf pen cleaning, calving pen hygiene or feeding equipment hygiene. Conversely, a herd with fewer recorded good practices may have strong routines in the most important exposure pathways. This suggests that targeted evaluation of high-risk routines may be more useful than broad assessment of general management level alone.

Both questionnaire-based management data and visual hygiene scoring should be interpreted with caution. Self-reported routines may be affected by recall bias and social desirability bias, while visual hygiene scoring reflects conditions at only one herd visit. Hygiene may vary with season, workload, recent calvings, staffing, bedding availability and timing of cleaning. In addition, classification of practices as good, moderate or poor may oversimplify complex management systems, as the effectiveness of a practice depends on how consistently and correctly it is performed. Nevertheless, the combination of reported routines and observed hygiene conditions strengthened the interpretation compared with questionnaire data alone.

Some early calf management practices should also be interpreted in relation to Danish legislation and herd-specific disease control strategies. Timing of calf removal from the calving pen is one example. In the present study, calves were most frequently reported to be moved after more than 12 hours, which is consistent with Danish legislation requiring calves to remain with the dairy cow in a single calving pen for at least 12 hours after calving (Ministry of Food, Agriculture and Fisheries of Denmark, 2024). However, earlier separation may occur under specific herd health circumstances. For example, *Salmonella Dublin* control may justify an exemption from the 12-hour requirement when documented by a veterinary certificate and regularly reassessed (Danish Veterinary and Food Administration, 2020). Variation in calf removal practices may therefore reflect not only management preferences, but also regulatory requirements and herd-specific disease control strategies.

Colostrum management represents a related but biologically different component of NCD prevention, as it primarily influences calf immunity and resilience rather than environmental

infection pressure. In the present study, colostrum-related variables did not appear as clear explanatory factors for differences in herd-level mortality. This should not be interpreted as evidence that colostrum management is unimportant, but rather as a reflection of limited contrast between herds and the indirect nature of the questionnaire-based measures. Timing, volume and method of colostrum administration do not necessarily reflect achieved passive transfer. Direct measures such as serum IgG or total protein would have provided a more precise assessment of calf immunity. Previous studies have shown that adequate colostrum intake and passive transfer are central for reducing morbidity and mortality in dairy calves (Godden, S., 2008; Beam, et al., 2009; Windeyer, et al., 2014).

The reported use of tube feeding for colostrum administration illustrates a practical dilemma. Tube feeding may help ensure intake of a known volume of colostrum shortly after birth, which is relevant for passive transfer and calf resilience (Godden, S., 2008; Windeyer, et al., 2014). However, Danish legislation states that routine tube feeding of colostrum is not permitted and should only be used for sick calves (Ministry of Food, Agriculture and Fisheries of Denmark, 2024). This highlights the need to interpret reported colostrum routines in relation to both biological relevance, legislation and practical on-farm constraints. At the same time, the reporting of tube feeding despite this sensitive context may support the credibility of the questionnaire data. If farmers were willing to report a practice that may be perceived as legally or ethically sensitive, this may indicate that their responses were not solely shaped by social desirability bias. This does not remove the risk of reporting bias, but it suggests that at least some respondents answered openly about practical routines.

Overall, these findings suggest that management and hygiene influence calf health through combined and context-dependent effects rather than through single identifiable risk factors. Hygiene-related practices are likely to influence infection pressure, whereas colostrum management influences calf resilience following exposure. Effective prevention of NCD-related mortality therefore requires attention to both sides of this interaction: reducing environmental contamination while ensuring that calves have sufficient immunity to tolerate unavoidable pathogen exposure (Godden, S., 2008; Windeyer, et al., 2014; Klein-Jöbstl, et al., 2014). Future studies should include direct measures of passive transfer together with repeated assessments of hygiene and management routines to better evaluate how infection pressure and immunity interact in the development of NCD and associated mortality.

Methodological considerations:

The methodological design of this study has important implications for the interpretation of the findings. The study provided useful herd-level insight into management practices, hygiene conditions, pathogen occurrence and calf mortality under commercial field conditions, but several design-related factors limited the ability to identify clear statistical associations.

First, all analyses were conducted at herd level. This was appropriate for evaluating management and hygiene routines, as these practices are generally implemented at herd level and were central to the aim of the study. Moreover, veterinary advisory work in dairy herds is most often based on herd-level observations, comparisons between herds and identification of management routines that can be translated into practical recommendations. In this context, the inclusion of 30 Danish dairy herds represents a considerable field-based dataset for individually conducted data, particularly because all visits, questionnaire interviews, visual hygiene assessments and sample collected were performed by the same observer. This may have reduced inter-observer variation and strengthened the internal consistency of the management and hygiene assessments.

Nevertheless, calf diarrhoea, pathogen shedding, immunity, treatment response and mortality occur at individual calf level and may vary substantially between calves within the same herd. By aggregating pathogen occurrence and mortality to herd level, individual-level variation may have been averaged out, potentially obscuring associations that could have been detected in calf-level analyses. The findings should therefore be interpreted as herd-level tendencies rather than direct individual-level risk estimates (Gulliksen, et al., 2009; Schroeder, et al., 2012). However, this herd-level approach also increases the practical relevance of the results, as the identified tendencies reflect the level at which most preventive management decisions are made in commercial dairy herds.

The selected sampling age of 5–21 days should also be considered when interpreting the pathogen results. This age range was chosen because the study focused primarily on *C. parvum*, which is particularly relevant during the first weeks of life. Consequently, the sampling strategy may have increased the probability of detecting *C. parvum* at herd level, while pathogens typically associated with the first days of life, such as *E. coli* F5, may have been underestimated. The pathogen occurrence reported in this study should therefore be

interpreted as occurrence within the selected age group, rather than as a complete estimate of pathogen occurrence across the entire neonatal period.

The mortality outcome also introduced important limitations. Mortality from 2–30 days was calculated as a herd-level proportion based on DMS data. This allowed comparison between herds but did not account for individual calf-level time at risk. This is particularly relevant because both bull and heifer calves were included, and bull calves may leave the herd during the defined mortality period. If calves leave the herd before 30 days of age, they no longer contribute time at risk, which may lead to underestimation of mortality and complicate comparisons between herds. Ideally, mortality would have been analysed as an incidence rate or time-to-event outcome using individual-level data on birth date, exit date, disease events, treatment and death.

Structural differences between herds, including herd size, farm organisation, number of CHR locations and calf management systems, may also have contributed to variation in mortality outcomes. Larger or more complex production systems may involve increased animal movement and management variability, which can influence disease dynamics, although such effects are often context-dependent (Klein-Jöbstl, et al., 2014; Godden, S., 2008; Windeyer, et al., 2014).

The cross-sectional design further limited the ability to establish temporal relationships between exposures and outcomes. Management practices, hygiene conditions and pathogen occurrence were measured during a single herd visit, whereas calf mortality was calculated over a 12-month period. Conditions recorded at the visit may therefore not fully reflect the management routines, infection pressure or disease dynamics present throughout the mortality period. This is particularly relevant for hygiene and pathogen occurrence, which may vary with season, calving intensity, staffing, bedding availability and timing of cleaning routines. The results should therefore not be interpreted as evidence that the observed management or hygiene conditions directly caused differences in mortality.

Diagnostic misclassification may also have influenced the results. Herd-level pathogen status was based on pooled faecal samples from five calves and analysed using a cow-side rapid antigen test. This approach was practical and relevant under field conditions but may reduce the probability of detecting low-prevalence or low-shedding infections within herds. A pooled

sample represents only the selected calves at the time of sampling and does not capture temporal variation in shedding or pathogen occurrence among all calves in the herd. In addition, rapid antigen tests have imperfect sensitivity and specificity, and non-differential misclassification would be expected to attenuate associations between pathogen occurrence and explanatory variables.

The relatively small sample size and sparse data structure further limited statistical power and model stability, particularly in the case-control component. The limited number of case and control herds increased the risk of wide confidence intervals, quasi-complete separation and unstable odds ratio estimates in multivariable models. This was observed during model selection, where the model with the lowest AIC showed quasi-complete separation and non-estimable or extremely imprecise estimates. The final exploratory model was therefore selected based on stability and biological interpretability rather than AIC alone. A supplementary mixed-effects model, with herd as random effect, was explored, but did not converge, likely due to the limited number of herds and sparse data structure. Standard logistic regression models were therefore used.

Generalisability should also be considered. Herds were recruited through a limited veterinary practice network, and the study population may therefore not fully represent the general Danish dairy population. Participating herds may have differed systematically from non-participating herds in management level, calf health status or motivation to engage in calf health improvement. Herds with poorer calf management or more severe calf health problems may have been less willing to participate, particularly because the study involved on-farm visits, visual hygiene assessment and evaluation of calf management routines. Consequently, the study population may have been biased towards herds with greater interest in calf health and possibly better-than-average management practices.

Despite these limitations, the study has several strengths. It was conducted under field conditions in commercial dairy herds, which increases the practical relevance of the findings. The combination of questionnaire-based data, visual hygiene scoring, pathogen detection and DMS-derived mortality data allowed management practices, observed environmental conditions, pathogen occurrence and mortality to be evaluated within the same study population. This integrated approach is particularly relevant when investigating a multifactorial disease complex such as NCD, where outcomes are unlikely to be explained by

a single factor alone (Schroeder, et al., 2012). In addition, the inclusion of both reported management routines and observed hygiene conditions strengthened interpretation compared with questionnaire data alone. Sensitivity analyses using continuous mortality produced findings broadly similar to the primary case-control analyses, suggesting that the limited number of statistically significant associations was unlikely to be explained solely by dichotomisation of mortality.

Overall, the absence of clear statistical associations should be interpreted in the context of the study design and data structure. The findings reflect real-life herd conditions, but they also illustrate the challenges of identifying specific risk factors for complex diseases using herd-level cross-sectional and case-control approaches. Future studies would benefit from prospective longitudinal designs with repeated recording of management practices, hygiene conditions, pathogen occurrence, diarrhoea events, treatments, mortality and individual calf-level time at risk. Such designs would allow more precise evaluation of whether specific exposures precede disease or death, and how infection pressure, immunity and disease management interact over time.

Practical implications:

The practical value of this study lies in identifying realistic and biologically plausible areas for herd-level improvement. Given the widespread occurrence of *C. parvum*, control of NCD-related mortality should focus on reducing infection pressure rather than attempting to eliminate specific pathogens completely. This places particular emphasis on management routines that reduce faecal-oral transmission in the everyday calf environment, including calf pen cleaning, removal of organic material from calving areas, hygiene of feeding equipment and reduction of visible manure contamination. These action points are consistent with previous studies highlighting the importance of environmental contamination, pathogen shedding, hygiene and cleaning routines in the epidemiology and prevention of calf diarrhoea (Garro, et al., 2021; Gulliksen, et al., 2009; Klein-Jöbstl, et al., 2014).

The findings also support the view that effective prevention of NCD should be approached as a multifactorial herd-level strategy rather than as a set of isolated interventions, as disease outcomes are shaped by interactions between pathogens, host immunity, management and environmental conditions (Cho & Yoon, 2014; Schroeder, et al., 2012; Windeyer, et al.,

2014). Broad indicators of overall management level may be less useful for advisory purposes than targeted evaluation of specific high-risk routines. Herd health advisory work should therefore focus not only on whether recommended routines are present, but also on whether they are implemented consistently, correctly and at the most relevant points of pathogen exposure. In this study, the most relevant practical action points were calf pen cleaning protocols, frequency of full mucking out in calving pens and hygiene of feeding equipment. Although these findings should be interpreted as exploratory rather than causal, they identify relevant areas for advisory focus and further investigation.

Colostrum management remains an essential part of this approach. Adequate passive transfer does not prevent exposure to enteric pathogens, but it may improve calf resilience and reduce the severity of disease following exposure. Preventive strategies should therefore combine reduction of environmental infection pressure with continued focus on timely colostrum administration, sufficient colostrum volume and colostrum quality control (Godden, S., 2008; Beam, et al., 2009; Windeyer, et al., 2014). Good hygiene and good immunity should be considered complementary rather than interchangeable components of NCD prevention.

Diagnostic findings should also be interpreted within the broader herd context. Detection of *C. parvum* or other enteropathogens indicates exposure or infection pressure but does not necessarily explain disease severity or mortality risk in a given herd. Diagnostic results should therefore be evaluated together with calf age, clinical signs, hygiene conditions, colostrum routines and disease management practices, particularly in high-prevalence settings where pathogens may also be detected in clinically healthy calves or as part of co-infections (Cho & Yoon, 2014; Garro, et al., 2021; Renaud, et al., 2021).

Overall, prevention of mortality associated with NCD should prioritise consistent hygiene routines in calf pens and calving areas, improved hygiene of feeding equipment, reduction of visible manure contamination, continued focus on colostrum management and early disease detection. These should be regarded as targeted management action points rather than definitive evidence that individual interventions alone will reduce mortality.

Conclusion

This study assessed biosecurity, hygiene-related management practices, herd-level pathogen occurrence and calf mortality in Danish dairy herds, with particular focus on calves aged 5–21 days. The cross-sectional part of the study demonstrated substantial between-herd variation in hygiene- and cleaning-related management practices, visual hygiene conditions and calf mortality. In contrast, colostrum management practices were relatively consistent across herds and generally aligned with commonly recommended standards.

With regards to herd-level management practices, calves in the participating Danish dairy herds were generally managed with a high degree of consistency in relation to early colostrum provision, whereas greater variation was observed in housing, cleaning routines, disinfection practices and hygiene-related biosecurity. This suggests that potential differences in herd-level NCD risk and calf resilience may be more closely linked to variation in environmental hygiene and cleaning implementation than to broad differences in colostrum routines alone.

Regarding herd-level pathogen occurrence, *C. parvum* was detected in the vast majority of participating herds, indicating widespread herd-level occurrence within the study population. Rotavirus and BCoV were detected less frequently, whereas *E. coli* F5 was not detected in any herd. Co-detection of pathogens mainly involved *C. parvum* in combination with viral pathogens. However, due to the uniformly high occurrence of *C. parvum*, pathogen presence alone had limited ability to explain differences between herds. The findings therefore suggest that pathogen detection should be interpreted as an indicator of herd-level infection pressure rather than as a direct measure of clinical disease severity or mortality risk.

When evaluating potential association with herd-level calf mortality, the case-control analyses identified only few clear statistical associations between individual management practices, pathogen occurrence and herd-level calf mortality. Nevertheless, hygiene- and cleaning-related practices appeared repeatedly across analyses, particularly calf pen cleaning protocol, calving area hygiene and feeding equipment hygiene. In addition, combined viral pathogen detection showed a tendency towards association with higher herd-level mortality. These findings suggest that specific management routines at key points of pathogen exposure may be more informative than broad indicators of overall management level when evaluating differences in herd-level calf mortality.

For the hygiene-related hypothesis, the assumption that poor hygiene in calving and calf housing environments is associated with higher neonatal mortality, was only partly supported. The results did not provide strong statistical evidence for a consistent association between overall visual hygiene scores and mortality. However, repeated tendencies involving calf pen cleaning, calving area hygiene and manure contamination suggest that hygiene-related conditions may still contribute to differences in mortality between herds.

For the proposed management action points, the hypothesis that specific hygiene practices related to cleanliness, bedding management and feeding equipment constitute potential management action points associated with lower herd-level calf mortality, was supported at an exploratory level. The findings point towards cleaning between calves or batches, removal of organic material from calving areas, hygiene of feeding equipment, reduction of visible manure contamination and continued focus on colostrum management as biologically plausible areas for herd-level prevention.

Overall, herd-level calf mortality was not explained by pathogen presence alone. Instead, the results support the interpretation of NCD and associated mortality as multifactorial outcomes influenced by the interaction between infection pressure, hygiene routines, calf resilience and disease management. Due to the limited sample size, sparse data structure, wide confidence intervals and cross-sectional design, the findings should be interpreted as exploratory herd-level tendencies rather than causal effects. This study therefore cannot draw conclusions about clinical diarrhoea occurrence or individual calf infection risk. However, it identifies realistic and practically relevant management action points that may guide herd health advisory work and future longitudinal studies on prevention of NCD-related mortality in Danish dairy herds.

Perspectives

The findings of this study support the growing understanding that neonatal calf diarrhoea should be approached as a complex herd-level condition rather than as a disease explained by the presence or absence of a single pathogen. The high occurrence of *C. parvum* across participating herds, together with the detection of viral pathogens in a smaller subset of herds, suggests that future prevention strategies should focus less on pathogen elimination and more on reducing infection pressure and improving calf resilience.

From a practical perspective, this means that calf health advisory work should prioritise routines that influence faecal-oral transmission in the everyday calf environment. Particular attention should be directed towards cleaning between calves or batches, hygiene of feeding equipment, removal of organic material from calving areas, bedding management, and reduction of visible manure contamination. These areas are directly linked to pathogen exposure and are realistic intervention points under commercial dairy farm conditions. However, they should be implemented as part of a broader herd-level strategy that also includes colostrum management, early disease detection, supportive treatment routines and evaluation of calf housing.

The findings also highlight the need for future studies using prospective longitudinal designs. Repeated recording of hygiene conditions, management routines, clinical diarrhoea, pathogen shedding, treatment events, passive transfer status and mortality at individual calf level would allow a more precise evaluation of how infection pressure, immunity and disease management interact over time. Such studies would be particularly valuable for distinguishing between pathogen presence, clinically relevant infection, disease severity and mortality risk.

Future diagnostic strategies may also benefit from more comprehensive and quantitative approaches. Rapid cow-side tests are useful for practical herd screening, but quantitative PCR or repeated sampling may provide a more nuanced understanding of pathogen load, co-infections and clinically relevant infection pressure. Combining diagnostic results with clinical observations and herd-level management data could strengthen decision-making in calf health advisory work and support more targeted prevention, treatment and antimicrobial stewardship.

Finally, the consistently high occurrence of *C. parvum* observed in this and several previous studies indicates that improved preventive strategies against cryptosporidiosis remain highly relevant. Because effective control of *C. parvum* is challenging due to environmental persistence and limited treatment options, future research should focus on practical methods for reducing environmental contamination, improving hygiene implementation, strengthening calf resilience and evaluating new preventive tools under field conditions in Danish dairy herds.

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Appendices

Appendix 1: Information letter

DYRLÆGERNE UNIVET

PROJEKT CRYPTO – Vil du være med?

Projektet om kalvediarré sker i samarbejde med Dyrlægerne Univet v. dyrlæge Dorte Bay og Virbac, der sponsorerer testkit.

Projektet undersøger, hvordan hygiejne og management i kælvningsafsnit og kalvestalden påvirker kalvedødelighed og hænger sammen med udskillelsen af Cryptosporidier. Målet er at komme med anbefalinger til danske malkekvægsbesætninger, der kan reducere diarré og dødelighed.

❖ **Hvem kan deltage?**

Besætninger der:

- Har både kælvninger og kalve op til 14 dage i besætningen
- Har minimum 4 kalve i alderen 5-21 dage på de fleste dage af året.
- Er villige til at deltage i en times besætningsbesøg med et kort spørgeskema og dataudlevering (adgang via UNIVET/DMS).

❖ **Hvad indebærer det for jeres besætning?**

Hvis I takker ja til at deltage, indebærer det følgende:

Jeg kommer på besøg i jeres besætning (ca. 45-60 minutter). Vi aftaler en tid, der passer dig.

- Jeg udfylder et spørgeskema om kalve-management, mens vi går en tur rundt i besætningen
- Jeg laver en visuel scoring
- Jeg indsamler gødningsprøver fra 4 kalve i alderen 5-21 dage og udfører en test for diarré-patogener (gratis).

Adgang til DMS-data:
For at kunne sammenligne management med kalvedødelighed, har jeg brug for tilladelse til at hente følgende data fra DMS for de seneste 12 mdr. Det foregår via Univets dataadgang.:

- Kalvedødelighed 0-30 dage
- Antal kalve født
- Antal kalve i alderen 0-30 dage

❖ **Fortrolighed**

Alle data og oplysninger behandles anonymt og udelukkende til forskningsformål.
I rapporten fremgår ingen besætningsnavne, adresser eller persondata.

❖ **Vil I være med?**

Jeg ringer til dig i januar-februar angående deltagelse.

Michelle Vikkelsø Phillip
Kandidat studerende, Animal Science, Københavns Universitet
Mail: zws281@alumni.ku.dk
Telefon: +45 50537301

1.Vejleder: Line Svennesen, Københavns Universitet
2.Vejleder: Dorte Bay Lastein, Københavns Universitet og Dyrlægerne Univet

Appendix 2: Recruitment email

Subject: DELTAG I PROJEKT CRYPTO via Univet – Har dine kalve nogensinde diarré?

I samarbejde med Univet får du nu muligheden for at deltage i et projekt om kalvediarré med fokus på Cryptosporidier.

Mit navn er Michelle Phillip, og jeg læser kandidat i Animal Science på Københavns Universitet.
Før jeg begyndte at læse, arbejdede jeg 5 år i malkekvægsbesætninger – først som skolepige og senere fuldtid.

Det var her, jeg opdagede min store interesse for arbejdet med køer og kalve. Jeg vidste, at dette skulle være min levevej. Her opdagede jeg også, hvor stor en forskel godt management kan gøre i hverdagen.

Nu skriver jeg min masteropgave om kalve diarré forårsaget af Cryptosporidier.

Jeg søger derfor malkekvægsbesætninger, som vil være med i et projekt, der skal afdække, hvordan I bedst forebygger diarré gennem praktiske realistiske tiltag i den daglige kalvepasning.

Hvis du er nysgerrig, så læs videre i den vedhæftede beskrivelse.
Jeg ringer direkte til dig i januar/februar 2026 og hører om du har lyst til, at deltage.

Med Venlig Hilsen
Michelle Phillip (og vejleder Dorte Bay, Univet/Københavns Universitet)

DYRLÆGERNE UNIVET

Appendix 3: Consent form

Samtykke til behandling af besætningsdata og deltagelse i Crypto-projekt.

Crypto-projektet er min hovedopgave ved Animal Science studiet på Københavns Universitet (KU). Formålet med Crypto-projektet er at opnå højere forståelse for sammenhænge mellem kalvepasning/management/hygiejne og forekomsten af Cryptosporidier hos småkalve. Jeg ønsker ligeledes at undersøge, hvorvidt der er sammenhæng mellem forekomsten af Cryptosporidier, kalvepasning og kalvedødelighed.
Projektet laves i samarbejde med vejlederne Line Svennesen, KU, og Dorte Bay, Univet/KU. Diagnostiske tests er sponsoreret af Virbac.

Som deltager i projektet bidrager besætningen med data fra DMS samt ét besætningsbesøg. Ved besætningsbesøget svarer den kalvepasningsansvarlige på spørgsmål vedrørende kalvepasning. Den studerende indsamler gødningsprøver fra 5 kalve i alderen 5-21 dage, som undersøges for diarré patogener. Den studerende laver desuden en vurdering af kalve- og kælvningsfaciliteterne. Forventet varighed af besøget er 45-60 minutter.

Ved at samtykke til deltagelse gives der tilladelse til, at jeg må tilgå besætningsdata i DMS via Dyrlægerne Univet. Fra DMS ønskes adgang til data for de seneste 12 måneder. Som eksempel vil jeg udtrække data om kalvedødelighed i alderen 0-30 dage.

Lagring og håndtering af data foregår i fuld overensstemmelse med EU's GDPR-lov (EU-forordning 2016/679). Oplysningerne behandles fortroligt og vil udelukkende blive brugt til videnskabelige formål. Under projektet vil indsamlede data kun være tilgængelige for mig og mine vejledere. Oplysningerne opbevares på en pseudo-anonymiseret måde, hvor dine personlige oplysninger (navn, efternavn, adresse, email, telefonnummer, CHR) vil blive opbevaret adskilt fra svarene, og alle besætninger vil få tildelt et anonymiseret ID-nummer i projektet. Personlige oplysninger indsamlet ved besøget vil kun blive brugt til at kontakte dig igen, hvis yderligere oplysninger er nødvendige. Hvis de indhentede oplysninger offentliggøres, vil det ske i anonymiseret form. Det betyder, at der ikke vises nogen data, som kan bruges til at identificere dig som person eller din besætning, undtaget dit forhold som tilknyttet Dyrlægepraksis Univet.

Deltagelse i projektet er frivillig, og samtykket kan til enhver tid tilbagekaldes med fremadrettet virkning. Samtykket tilbagekaldes ved at kontakte Adjunkt ved Københavns Universitet, Line Svennesen (line.svennesen@sund.ku.dk).

Har du spørgsmål til Crypto-projektet, er du til enhver tid velkommen til at kontakte den projektansvarlige:

Michelle Vikkelsø Phillip
+45 50537301
Zws281@alumni.ku.dk

Navn:	
Adresse:	
CHR:	
Dato og Underskrift:	

Jeg ønsker at deltage i projektet beskrevet på side 1:

Vi gør opmærksom på at data både lagres og anvendes af Michelle Vikkelsø Phillip og indgår i master projektet med deltagelse fra flere landmænd.

Hvis du vælger "NEJ", afsluttes besøget.

☐ JA

☐ NEJ

Nøgletal fra DMS må anvendes i projektet i anonymiseret form, og indhentes via Dyrlægerne Univets adgang:

☐ JA

☐ NEJ

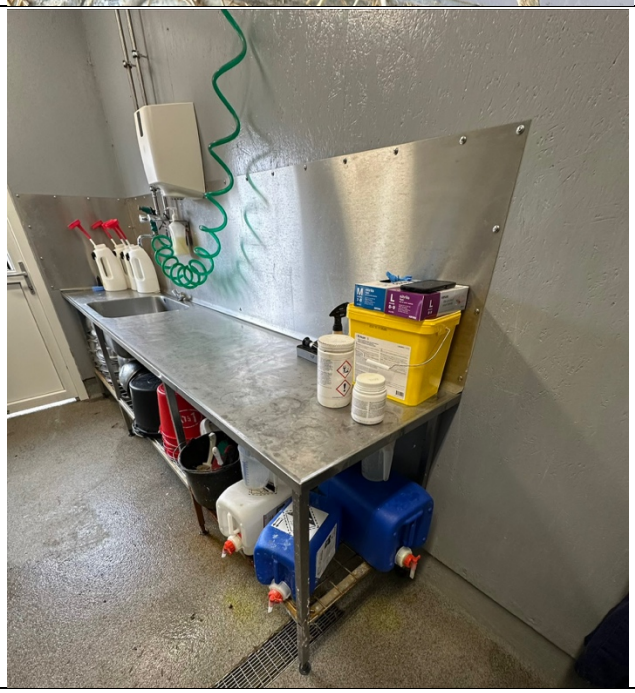
Appendix 4: Visual scoring chart

Area assessed	Visual Score	Description	Example image
Calving pen cleanliness and bedding	1	Clean, dry, good amount of fresh bedding	
	2	Some contamination, partly wet, moderate bedding	

	3	Visible contamination, wet and inadequate amount of bedding	
Calf pen cleanliness, dryness and bedding	1	Dry boxes, good bedding, minimal contamination	

	2	Some humid areas, small contamination, moderate bedding	
	3	Wet areas, visible contamination, inadequate amount of bedding	

Visible manure contamination in calf area	1	No visual manure	
	2	Minor manure contamination	

	3	Clearly visible manure on floor, walls, equipment	
General organisation and cleanliness of feeding equipment	1	Clean and organised, no visible milk contamination	

			
	2	Some milk contamination, minor dirty equipment	

	3	Dirty equipment, dried old milk, unorganised, visible manure	

Appendix 5: Questionnaire

Spørgeskema: Kalvehygiejne og Management

Formål med projektet:

Besætningsnummer: _____ Besvaret af: _____

1. Fokus

1.1 Hvor er kalvepasning prioriteret på listen over opgaver i denne besætning?

☐ Først ☐ På lige fod med pasning af køer ☐ Lavere end pasning af køer

1.2 Hvem passer kalvene?

☐ Den samme ☐ Fælles opgave ☐ Fast team

2. Råmælkshåndtering

2.1 Indenfor hvilket tidsrum vil du tro at 75% af kalvene får råmælk?

☐ 0–2 t ☐ 2–6 t ☐ >6 t

2.2 Hvor stor udfodrings mængde af råmælk benyttes?

☐ <2 L ☐ 2–3 L ☐ >3 L

2.3 Måles råmælkskvaliteten?

☐ Ja ☐ Nej

2.3.1 Hvis ja, hvad er Brix grænsen for den råmælk som udfodres til nyfødte kalve?

☐ <18-21% ☐ 22–24% ☐ >24%

2.4 Hvordan udfodres råmælk som regel?

☐ Sonde ☐ Skål/suttespand ☐ Sutteflaske

3. Hygiejne i kælvningsboks

3.1 Hvor ofte strøes der i kælvningsboksen når den er i brug?

☐ Dagligt ☐ Flere gange ugentligt ☐ Ugentligt ☐ Sjældnere

3.2 Hvor ofte udføres fuld udmugning af kælvningsboksen?

☐ Efter hver kælvning ☐ Efter 2-5 kælvninger ☐ Efter 6-10 kælvninger

☐ Efter >10 kælvninger

3.3 Hvordan rengøres kælvningsboksen/ene? (flere krydser tilladt)

☐ Udmugning ☐ Skylles/højtryksrens ☐ Vask med sæbe ☐ Tørring

☐ Desinfektion

3.4 Benyttes desinfektionsmidler med bevist effekt på Cryptosporidium (Neopredisan®, KENO^{TM}COX, Brintoverilte)

☐ Ja ☐ Nej

4. Flytning af kalve

4.1 Hvor lang tid går der inden kalven flyttes fra kælvningsboksen/koen i gennemsnit?

☐ <2 t ☐ 2–6 t ☐ 6–12 t ☐ >12 t

5. Kalveopstaldning (de første 2 leveuger)

5.1 Hvordan huses kalven efter flytning fra kælvningsboksen?

☐ Enkeltbokse ☐ Små grupper (2–5) ☐ Store grupper (>5)

5.1.1 Hvis grupper, hvad er aldersspredningen i kalvegrupperne de første 2 leveuger?

☐ 1-2 dage ☐ 3-4 dage ☐ 5-7 dage ☐ >1 uge

6. Rengøring af kalvebokse

6.1 Hvordan rengøres bokse mellem hver kalv? (Flere krydser tilladt)

☐ Udmugning ☐ Skylles/højtryksrens ☐ Vask med sæbe ☐ Tørring

☐ Desinfektion

6.2 Benyttes desinfektionsmidler med bevist effekt på Cryptosporidium (Neopredisan®, KENO^{TM}COX, Brintoverilte)

☐ Ja ☐ Nej

6.3 Hvor ofte strøes der I kalveboksene?

☐ Dagligt ☐ Flere gange ugentligt ☐ Ugentligt ☐ Sjældnere

6.4 Bruges separate redskaber (greb, kost, skovl) til kalvestald?

☐ Ja ☐ Nej

7. Hygiejne af fodringsudstyr

7.1 Hvornår rengøres fodringsudstyr (spand/sutteflaske/skål)?

☐ Efter hver fodring ☐ Dagligt ☐ Ugentligt (1-6 gange/uge) ☐ Ved ny kalv i boksen

7.2 Hvilken rengøringsmetode benyttes? (Flere krydser tilladt)

☐ Sæbe ☐ Varmt vand (+60 grader) ☐ Koldt vand ☐ Desinfektion

8. Personalets hygiejne

8.1 Bruger personalet følgende i kalvestalden?

☐ Særlige støvler/tøj ☐ Støvledesinfektion ☐ Håndvask eller handsker

☐ Nej

9. Definition af diarré

9.1 Anvendes et fast fæces score system?

☐ Ja ☐ Nej

9.2 Hvornår defineres en kalv, som diarré kalv?

☐ Målinger (fæces score, feber) ☐ Visuelt (tynd afføring, hænger med ørerne, ingen appetit)

10. Behandling

10.1 Isoleres syge dyr i enkeltbokse (hvis i fællesbokse)?

☐ Ja ☐ Nej

10.2 Anvendes vaccination mod crypto (bovilis cryptium)?

☐ Ja ☐ Nej

10.3 Behandler I mod crypto?

☐ Ja, alle ☐ Ja, < halvdelen ☐ Ja, > halvdelen ☐ Ja, på symptomer

☐ Nej

10.3.1 Hvis ja, hvad behandler I med? (Flere svar tilladt)

☐ Halocur ☐ Paromomycin (grabbovet multi)

☐ Væske/elektrolyt opløsning ☐ Smertestillende

Appendix 6: Questionnaire responses

Variable_label	Response	N	%
Halocur	Not selected	30	96,8
Halocur	Selected: Halocur	1	3,2
Paromomycin	Not selected	29	93,5
Paromomycin	Selected: Paromomycin	2	6,5
Electrolytes	Not selected	3	9,7
Electrolytes	Selected: Electrolyte fluids	28	90,3
Pain relief	Not selected	9	29
Pain relief	Selected: Pain relief	22	71

Variable_label	Response	N	%
Use of disinfectant with proven effect against Cryptosporidium in calving pen	No	28	90,3
Use of disinfectant with proven effect against Cryptosporidium in calving pen	Yes	3	9,7
Use of disinfectant with proven effect against Cryptosporidium in calf pen	No	29	93,5
Use of disinfectant with proven effect against Cryptosporidium in calf pen	Yes	2	6,5
Separate tools used in calf stable	No	11	35,5
Separate tools used in calf stable	Yes	20	64,5
Use of protective gear by personnel	Special boots/clothing	3	9,7
Use of protective gear by personnel	Boot disinfection	0	0
Use of protective gear by personnel	Handwashing or gloves	18	58,1
Use of protective gear by personnel	No	10	32,3
Structured faecal scoring system used	No	28	90,3
Structured faecal scoring system used	Yes	3	9,7
How diarrhoea is defined	Measurements (faeces score, fever)	9	29
How diarrhoea is defined	Visual signs (thin faeces, drooping ears, poor appetite)	22	71
Isolation of sick calves from groups	No	20	64,5
Isolation of sick calves from groups	Yes	11	35,5
Use of Bovilis Cryptium vaccination	No	30	96,8
Use of Bovilis Cryptium vaccination	Yes	1	3,2
Treatment for Cryptosporidium used	Yes, all calves	0	0
Treatment for Cryptosporidium used	Yes, < half of calves	4	12,9
Treatment for Cryptosporidium used	Yes, > half of calves	1	3,2
Treatment for Cryptosporidium used	Yes, based on symptoms	24	77,4
Treatment for Cryptosporidium used	No	2	6,5

Variable_label	Response	N	%
Mucking out (calving pen)	Selected: mucking out	31	100
High-pressure cleaning (calving pen)	Not selected	28	90,3
High-pressure cleaning (calving pen)	Selected: rinse/high-pressure wash	3	9,7
Soap cleaning (calving pen)	Not selected	30	96,8
Soap cleaning (calving pen)	Selected: washed with soap	1	3,2
Drying (calving pen)	Not selected	30	96,8
Drying (calving pen)	Selected: drying	1	3,2
Disinfection (calving pen)	Not selected	23	74,2
Disinfection (calving pen)	Selected: disinfection	8	25,8
Mucking out (calf pen)	Selected: mucking out	31	100
High-pressure cleaning (calf pen)	Not selected	10	32,3
High-pressure cleaning (calf pen)	Selected: rinse/high-pressure wash	21	67,7
Soap cleaning (calf pen)	Not selected	16	51,6
Soap cleaning (calf pen)	Selected: washed with soap	15	48,4
Drying (calf pen)	Not selected	13	41,9
Drying (calf pen)	Selected: drying	18	58,1
Disinfection (calf pen)	Not selected	14	45,2
Disinfection (calf pen)	Selected: disinfection	17	54,8
Soap (feeding equipment)	Not selected	9	29
Soap (feeding equipment)	Selected: soap	22	71
Warm water >60°C	Not selected	1	3,2
Warm water >60°C	Selected: warm water (+60°C)	30	96,8
Cold water	Not selected	27	87,1
Cold water	Selected: cold water	4	12,9
Disinfection (feeding equipment)	Not selected	23	74,2
Disinfection (feeding equipment)	Selected: disinfection	8	25,8

Variable_label	Response	N	%
Frequency of bedding in calving pen	Daily	19	61,3
Frequency of bedding in calving pen	Several times weekly	11	35,5
Frequency of bedding in calving pen	Weekly	1	3,2
Frequency of bedding in calving pen	Less often	0	0
Frequency of full muck out in calving pen	After each calving	2	6,5
Frequency of full muck out in calving pen	After 2-5 calvings	7	22,6
Frequency of full muck out in calving pen	After 6-10 calvings	7	22,6
Frequency of full muck out in calving pen	After >10 calvings	15	48,4
Frequency of bedding in calf pen	Daily	17	54,8
Frequency of bedding in calf pen	Several times weekly	14	45,2
Frequency of bedding in calf pen	Weekly	0	0
Frequency of bedding in calf pen	Less often	0	0
Frequency of cleaning feeding equipment	After each feeding	7	22,6
Frequency of cleaning feeding equipment	Daily	8	25,8
Frequency of cleaning feeding equipment	Weekly (1-6 times/week)	7	22,6
Frequency of cleaning feeding equipment	With each new calf in the pen	9	29

Variable_label	Response	N	%
Time to first colostrum feeding	0-2 hours	4	12,9
Time to first colostrum feeding	2-6 hours	25	80,6
Time to first colostrum feeding	>6 hours	2	6,5
Amount of colostrum fed	<2 L	0	0
Amount of colostrum fed	2-3 L	9	29
Amount of colostrum fed	>3 L	22	71
Colostrum quality measured	No	14	45,2
Colostrum quality measured	Yes	17	54,8
Brix threshold used	Not relevant	14	45,2
Brix threshold used	<18-21%	8	25,8
Brix threshold used	22-24%	9	29
Brix threshold used	>24%	0	0
Method of colostrum feeding	Tube feeder	18	58,1
Method of colostrum feeding	Bucket/teat bucket	6	19,4
Method of colostrum feeding	Nipple bottle	7	22,6
Time before calves are moved from calving pen	<2 hours	4	12,9
Time before calves are moved from calving pen	2-6 hours	8	25,8
Time before calves are moved from calving pen	6-12 hours	7	22,6
Time before calves are moved from calving pen	>12 hours	12	38,7

Variable_label	Response	N	%
Priority of calves compared with cows	Calves prioritized first	5	16,1
Priority of calves compared with cows	Equal priority to cow care	24	77,4
Priority of calves compared with cows	Lower priority than cow care	2	6,5
Who takes care of the calves	Same person as other tasks	11	35,5
Who takes care of the calves	Shared responsibility	6	19,4
Who takes care of the calves	Dedicated team	14	45,2
Housing type after calving pen	Single pens	27	87,1
Housing type after calving pen	Small groups (2-5 calves)	3	9,7
Housing type after calving pen	Large groups (>5 calves)	1	3,2
Age spread within group-housed calves	Not relevant	27	87,1
Age spread within group-housed calves	1-2 days	1	3,2
Age spread within group-housed calves	3-4 days	2	6,5
Age spread within group-housed calves	5-7 days	1	3,2
Age spread within group-housed calves	>1 week	0	0

Appendix 7: Visual score distribution

	Mean	Median	SD	IQR	Min	Max
Calving pen score	1.77	2	0.717	1	1	3
Calf pen score	1.45	1	0.568	1	1	3
Manure contamination score	1.97	2	0.706	0.5	1	3
Feeding equipment score	1.71	2	0.693	1	1	3
Visual total	6.90	7	1.72	2.5	4	10

category	score	n	percent
<chr>	<dbl>	<int>	<dbl>
Calf pen cleanliness, dryness and bedding	1	18	58.1
Calf pen cleanliness, dryness and bedding	2	12	38.7
Calf pen cleanliness, dryness and bedding	3	1	3.2
Calving pen cleanliness and bedding	1	12	38.7
Calving pen cleanliness and bedding	2	14	45.2
Calving pen cleanliness and bedding	3	5	16.1
Feeding equipment cleanliness and organisat…	1	13	41.9
Feeding equipment cleanliness and organisat…	2	14	45.2
Feeding equipment cleanliness and organisat…	3	4	12.9
Visible manure contamination in calf area	1	8	25.8
Visible manure contamination in calf area	2	16	51.6
Visible manure contamination in calf area	3	7	22.6

Appendix 8: Pathogen proportions and combinations

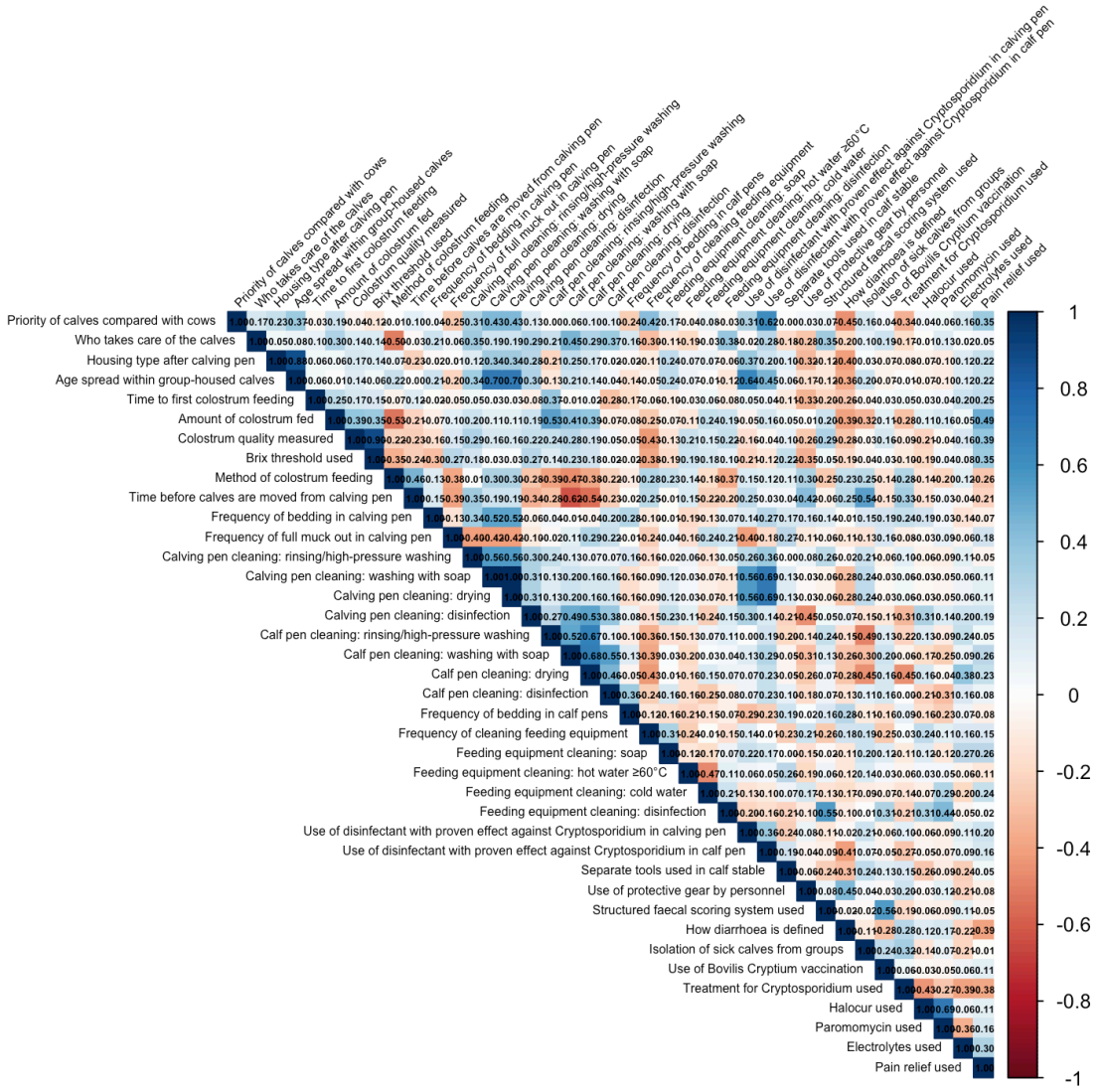
pathogen	proportion	percent
<chr>	<dbl>	<dbl>
Crypto	0.935	93.5
Rota	0.258	25.8
Corona	0.0968	9.7
E. coli	0	0

mean	median	sd	IQR	min	max
<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1.29	1	0.461	1	1	2

pathogen_combination	n	percent
<chr>	<int>	<dbl>
Crypto	20	64.5
Crypto + Rota	6	19.4
Crypto + Corona	3	9.7
Rota	2	6.5

infection_type	n	percent
<fct>	<int>	<dbl>
Single infection	22	71
Mixed infection	9	29

Appendix 9: Associations between management, hygiene and pathogen occurrence



Appendix 10: Associations between management and hygiene

Variable	Test	P-value
Cold water feeding equipment	Wilcoxon rank-sum	0.01
Calving desinfection protocol	Wilcoxon rank-sum	0.03
Preventive measures in calf stable	Kruskal-Wallis	0.04
Calf cleaning protocol	Kruskal-Wallis	<0.05
Who takes care of the calves	Kruskal-Wallis	0.07
Frequency of cleaning feeding equipment	Kruskal-Wallis	0.08
Use of separate tools	Wilcoxon rank-sum	0.17
Frequency of bedding in calving pens	Kruskal-Wallis	0.18
Isolation of sick calves	Wilcoxon rank-sum	0.22
Bedding frequency in calf pens	Wilcoxon rank-sum	0.24
Priority of calves compared to cows	Kruskal-Wallis	0.25
Use of vaccination against C. parvum	Wilcoxon rank-sum	0.27
Frequency of muck out in calving pen	Kruskal-Wallis	0.28
Soap usage feeding equipment	Wilcoxon rank-sum	0.34
Housing type of calves	Kruskal-Wallis	0.40
Electrolytes treatment	Wilcoxon rank-sum	0.42
Measurement of colostrum quality	Wilcoxon rank-sum	0.46
Definition of diarrhoea	Wilcoxon rank-sum	0.46
Method of colostrum administration	Kruskal-Wallis	0.47
Warm water feeding equipment	Wilcoxon rank-sum	0.52
Use of faeces score system	Wilcoxon rank-sum	0.58
Treating C. parvum	Kruskal-Wallis	0.61
Halocur treatment	Wilcoxon rank-sum	0.64
Paromomycin treatment	Wilcoxon rank-sum	0.64
Painkiller treatment	Wilcoxon rank-sum	0.65
Amount of colostrum administered	Wilcoxon rank-sum	0.67
Use of specific C. parvum disinfectants calving pen	Wilcoxon rank-sum	0.80
Use of specific C. parvum disinfectants calf pen	Wilcoxon rank-sum	0.89
Disinfectants feeding equipment	Wilcoxon rank-sum	0.92
Timing of moving from calving pen	Kruskal-Wallis	0.93
Timing of colostrum administration	Kruskal-Wallis	0.98

AI declaration

Declaration of using generative AI tools (for students)

☒ **I/we have used generative AI as an aid/tool** *(please tick)*

☐ **I/we have NOT used generative AI as an aid/tool** *(please tick)*

List which GAI tools you have used and include the link to the platform (if possible):

ChatGPT (OpenAI): https://chatgpt.com

Describe how generative AI has been used in the exam paper:

1) Purpose (what did you use the tool for?)

I used ChatGPT as a support tool for academic dialogue, sparring and quality assurance during the preparation of my master thesis. The tool was used to discuss and refine the overall structure and red thread of the thesis, clarify whether the research questions were answered, identify repetitions or unclear passages, improve language and readability, and support the presentation of methods, results and discussion. ChatGPT was also used for sparring on my explanations of the study design, case-control part, variable selection, univariable screening, multivariable modelling, sensitivity analyses, figure/tables and conclusions to reduce repetition and ensure sharpness. In addition, I used ChatGPT to help check consistency in terminology, formatting and troubleshooting in RStudio.

2) Work phase (when in the process did you use GAI?)

I used ChatGPT throughout the writing and revision process after I had developed the project idea, collected the data, performed the analyses and drafted major parts of the thesis. The use was especially concentrated in the later phases of the project: revising the methods and discussion, shortening repeated text, strengthening the connection between results, discussion and research questions, checking the clarity of tables/figures. I also used ChatGPT during literature work as a reading and organisation aid, for example to discuss how published studies could be used to support specific scientific points. The scientific articles, data interpretation and final selection of references were assessed by me.

3) What did you do with the output? (including any editing of or continued work on the output)

The output from ChatGPT was used as suggestions and feedback, not as an independent source of scientific evidence. I critically evaluated all suggestions and either rejected them, revised them substantially, or used them as a starting point for my own wording. All final decisions regarding study design, analysis choices, interpretation of results, discussion points and conclusions were made by me. Any factual or scientific claims in the thesis were checked against the original source, my own data and supervisor feedback, and the cited references in the thesis refer to the original source, not to ChatGPT. I did not use ChatGPT to generate data, conduct the statistical analyses independently, or replace my own academic assessment. Materials shared with ChatGPT consisted of my own draft text, anonymized or aggregated project material, and scientific literature used for academic sparring and revision.

Please note: Content generated by GAI that is used as a source in the paper requires correct use of quotation marks and source referencing. Read the guidelines from Copenhagen University Library at KUnet.