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Master Thesis

Clara Marie Paulsen

Assessment of immunisation of calves in cow-calf systems with prolonged cow-calf contact

Supervisor: Mette Bisgaard Petersen
Co-supervisor: Kirstin Dahl-Pedersen
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Name of department:	Department of Veterinary Clinical Science
Faculty:	Faculty of Health and Medical Sciences, University of Copenhagen
Author:	BSc. med. vet. Clara Marie Paulsen (shr400)
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PREFACE

This paper is a 30 ECTS master thesis written by Clara Marie Paulsen at the University of Copenhagen, concluding my master’s degree in veterinary medicine. The master is based on a cross-sectional study that seeks to explore the subject of prolonged cow-calf contact and its impact on the calves' immunisation.

I would like to thank my academic advisors, Mette Bisgaard Petersen and Kirstin Dahl-Pedersen, for their support and guidance in the making of this master's thesis, as well as a special thanks to the farmers who contributed to this study and let me into their daily routines.

At last, thank you to those who read the thesis and gave great input.

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ABSTRACT

Prolonged cow and calf contact (CCC) is gaining the attention of both the public and the dairy industry. The practice is diverse, both in duration, hours of contact each day and intervention by the calf staff. The knowledge is therefore limited with few studies describing the positive and negative physiological impact of prolonged CCC, some describe a positive impact on the behaviour and weight gain but an increased risk of FTPI.

This study aims to describe the health of the calves in the sampled herds and compare their immunisation to that of conventional herds with standard CCC, at last, the study aims to compare the Brix% of the dam's colostrum with that of the calf.

Four Danish organic herds were sampled and a total of 126 calves aged 1-8 days were sampled; 83 of these aged 3-7 days, and 50 corresponding colostrum samples were included in the statistical analysis. The calves were clinically assessed on 6 parameters and for 82 out of 83 calves' rectal temperature was measured. A digital Brix refractometer was used to measure serum Brix% as well as colostrum Brix%, and Total Serum Protein was measured on a refractometer.

The results of this study showed healthy calves in the sampled herds with a prevalence of disease at 7.23%, the majority presenting with gastric symptoms, the mean Serumbrix% was 9.63%, where only 2.4% of the sampled calves had FTPI.

There was a significant difference between the immunisation of the calves in this study and those in Conventional when applying the Chi-squared test and T-test, with the calves in this study being significantly better immunised. Out of the 572 calves in Conventional 34.4% had FTPI and the mean Serumbrix% was 8.46%.

No significant correlation was found between the Colostrumbrix% of the dam and the Serumbrix% of the corresponding calf when using linear regression. There was a source of error in the sampling being inconsistent decreasing the credibility.

To conclude, the health of the calves was better than recommended and a low percentage of calves had FTPI, and the immunisation was better for the calves within this study than the compared calves of Conventional. The risk of FTPI was greatest for calves in Conventional compared with the calves with prolonged CCC. Further studies are needed to compare the different methods of prolonged CCC and to argue for the change from standard practice to that of prolonged CCC.

RESUME

Forlænget ko- og kalv kontakt (CCC) vinder opmærksomhed fra både offentligheden og mælkeindustrien. Praksissen er alsidig, både i varighed, kontakttid i løbet af døgnet og intervention fra kalvepersonalet, og videnskabelig evidens er derfor begrænset. Der er få studier, der beskriver den positive og negative fysiologiske påvirkning af langvarig CCC. Studier beskriver en positiv indvirkning på adfærd og vægtøgning for kalven, men en øget risiko for failure of transfer of passive immunity (FTPI).

Dette studie havde til formål at beskrive kalvenes sundhed i de undersøgte besætninger og sammenligne deres immunisering med den for konventionelle besætninger med standard CCC. Endelig havde studiet til formål at sammenligne Brix% af moderens råmælk med kalvens.

Der indgik fire danske økologiske besætninger, hvoraf i alt 126 kalve i alderen 1-8 dage blev undersøgt, 83 af disse i alderen 3-7 dage blev inkluderet i den statistiske analyse og 50 tilsvarende råmælksprøver blev inkluderet. Alle kalve blev klinisk vurderet på 6 parametre og rektal temperatur blev målt for 82 af de 83 kalve. Et digitalt Brix refraktometer blev brugt til at måle serum Brix% samt colostrum Brix% og totalt serumprotein blev målt på et refraktometer.

Resultaterne af specialet viste, at de undersøgte kalve i besætningerne var sunde med en sygdomsprævalens på 7,23 %, hvoraf størstedelen havde gastrointestinale symptomer. Den gennemsnitlige Serumbrix% var 9,63%, hvor kun 2,4% af de undersøgte kalve havde FTPI.

Ud af de 572 kalve i Konventionel havde 34,4% FTPI og den gennemsnitlige Serumbrix% var 8,46%. Der var en signifikant forskel mellem immuniseringen af kalvene i denne undersøgelse og dem i Konventionel ved anvendelse af Chi-i-anden-test og T-test, hvor kalvene i denne undersøgelse var signifikant bedre immuniserede.

Der blev ikke fundet nogen signifikant sammenhæng mellem Colostrumbrix% af moderen og Serumbrix% af den tilsvarende kalv ved brug af lineær regression. Der var en fejlkilde i, at prøvetagningen var inkonsekvent, hvilket mindskede troværdigheden.

Det kunne konkluderes at kalvenes helbred var bedre end det anbefalede, en lav procentdel af kalvene havde FTPI, og immuniseringen var bedre for kalvene i denne undersøgelse end de sammenlignede kalve af Conventional. Risikoen for FTPI var størst for kalvenr i Conventionel sammenlignet med kalvene med forlænget CCC. Yderligere undersøgelser er nødvendige for at kunne sammenligne de forskellige former for forlænget CCC og for at argumentere for skiftet fra standardpraksis til forlænget CCC.

ABBREVIATIONS

CCC	Cow and calf contact
FTPI	Failure of transfer of passive immunity
IgG	Immunoglobulin G
IQR	Interquartile range
SD	Standard deviation
THS	Total Health Score
TPI	Transfer of passive immunity
TSP	Total serum protein

1. INTRODUCTION

1.1 Prolonged cow-calf contact

The topic of animal welfare is gaining increasingly more attention (Ministeriet for Fødevarer, Landbrug og Fiskeri, 2023), and studies have shown that consumers would prefer for the calf to stay with the cow for a longer period of time, to gain more natural conditions in production as reviewed by Meagher et al. (2019).

Under Danish law (BEK 1743 §89) the cow and calf must stay together for a minimum of 12 hours after birth, while for organic herds the industry requires a minimum of 24 hours of contact (Økologisk Landsforening, 2022).

The practice of prolonged CCC is diverse and is done in a variety of ways. In a system with prolonged CCC, the calf stays with the cow for more than the standard period, which can be anything from several months to as little as 3 days, either with the dam or a nurse cow. CCC can be full contact or half contact, depending on the possibilities on the individual farm. This diversity is perhaps one reason for the lack of scientific studies on prolonged CCC. In this study alone, four different examples of prolonged CCC are represented (Table 1).

Studies have been conducted on the influence of prolonged CCC regarding the psychological welfare of the calf and cow, both during the contact and after they are separated. They found that prolonged CCC can promote normal social behaviour and decrease abnormal oral behaviour of calves (Wagenaar et al., 2007; Meagher et al., 2019). Meanwhile, the distress seen in calves after rearing depends on the type of prolonged CCC, with full contact increasing the risk of distress as opposed to partial contact (Wenker and Reenen, 2022). Contrary Roadknight et al. (2022) found that in systems with half day contact opposed to full day contact, cows showed more restlessness when apart, and discomfort and unease when reunited with a hungry calf.

The positive and negative physiological impact of prolonged CCC on calf health has been the focus of a few studies, why the following thesis is pertinent for potentially filling the knowledge gap.

One dilemma with prolonged weaning is the fact that the farmer breeds cows to produce milk, and in some sense, the calf is a by-product of this practice (Wagenaar et al., 2007). The calf is either used to sustain the size of the herd or sold for beef production.

A calf would normally suckle the cow in the first months of life, and the farmer makes a living selling the milk from the cows, thereby those two needs clash. Therefore, many farmers use milk substitutes, leftover milk that cannot be sold, or some combination of the two to feed the calves.

Holstein is the most common breed used for milk production in Denmark (Åbent Landbrug), and Holstein calves should be offered at least 8 litres of milk per day (NASEM, 2021) while the average Holstein cow in Denmark produces 32 litres of milk per day (Åbent Landbrug).

There are many arguments against prolonged CCC and many arguments for early separation. Many argue that there is an economic loss when the calf is left to suckle the cow, but this depends on the practices of the individual farmer and the price of the alternative. Feeding calves artificially is thought to increase the control of immunisation, nutrition, and disease. Furthermore, it is thought that the separation from the calf facilitates milk let-down, and early separation is thought to decrease stress (Meagher et al., 2019).
Besides that most stables in Denmark are not built for a system where cows and calves are housed together, the practice of prolonged CCC requires more space and a different type of bedding, like hay, to fulfil the needs of the calf (BEK 1743 §43). To shift from one practice to another can be a considerable change for some farmers and this risk may stop them from trying. This proves a need for scientific evidence to determine if the practice of prolonged CCC is worth it.

1.2 Immunisation of calves

The placenta of a cow is syndesmochorial, which means that there is a separation between the blood supply of the mother and foetus in utero. Therefore, the calf is born without immunoglobulins (Ig) from the cow meaning neonate calves are agammaglobulinemic at birth (Morrill et al., 2015; Bush and Staley, 1980; Pakkanen et al., 1997). To protect the calf against disease, it must successfully have a transfer of passive immunity (TPI) by ingesting adequate colostrum in the first 24 hours to avoid a failure of transfer of passive immunity (FTPI). FTPI predisposes to disease and is associated with increased morbidity and neonatal mortality (Weaver et al., 2000; Sutter et al., 2023; Lora et al., 2018). Via colostrum, the calf gains maternal Ig, especially IgG (Pakkanen et al., 1997). The neonate calf absorbs the Ig by pinocytosis via enterocytes in the small intestine within the first 24-36 hours. By exocytosis, the Ig is transported into the lymphatic system and via the thoracic duct to the circulatory system (Pakkanen et al., 1997; Weaver et al., 2000). The absorption in the intestine is greatest in the first 4 hours and is shown to decline at 12 hours as reviewed by Weaver et al. (2000).

Studies have shown that an increased percentage of FTPI has been associated with calves who are left to suckle the cow, compared with feeding through a nipple bottle or oesophageal tube, the latter resulting in the least calves with FTPI (Weaver et al., 2000; Besser et al., 1991). The risk of suckling is due to inadequate intake and quality of colostrum (Weaver et al., 2000). Contrary Selman and McEwan (1971) found that the concentration of absorbed immunoglobulins was higher when calves were housed in the proximity of the cow compared with calves separated from the cow. No difference in volume intake was found, but the calves left with the cow had a significantly higher IgG concentration on average at 48 hours postpartum.

Health benefits of prolonged CCC have been documented, e.g. decreased mortality rates for calves (Alvarez et al., 1980), reduced risk of calves getting severe scours as well as reduced duration (Nocek et al., 1984; Rajala and Castrén, 1995; Wagenaar et al., 2007), a positive effect on calf growth (Wenker and Verwer, 2022; Meagher et al., 2019) and others found a reduction in the risk of cows getting mastitis (González-Sedano et al., 2010; Beaver et al., 2019).

1.3 Methods of measuring IgG

The serum concentration of IgG can be tested in several ways to evaluate the level of passive immunity (Lombard et al., 2020).

The only tests that directly measure the serum IgG concentration are enzyme-linked immunosorbent assay (ELISA) and radial immunodiffusion, which are considered "golden standard". Other tests as a refractometer or Brix (digital refractometer) estimate IgG concentrations based on the concentrations of other proteins that are associated with IgG e.g., the concentration of total serum protein or total globulins as reviewed by Weaver et al. (2000). For on-farm use, a Brix refractometer is quick and easy to handle when assessing passive transfer in a herd.

To measure the transfer of passive immunity a blood sample is taken from a calf between 1 and 7 days of age. When measuring TPI in this timeframe, the concentrations of IgG or STP provide reliable estimates of TPI at 24 hours, which is when the concentration peaks (Lombard et al., 2020; Hancock et al., 1985; Wilm et al., 2018). To ensure a correct measurement the calf must be clinically assessed as if they are dehydrated or sick STP and IgG can be falsely elevated as reviewed by Buczinski et al. (2018).

The standards for measuring TPI as categorized by Lombard et al. (2020) are excellent (≥25.0 g/L IgG), good (18.0-24.9 g/L IgG), fair (10.0-17.9 g/L IgG), and poor (<10.0 g/L IgG). As for Brix% (%), the value for each category is ≥9.4, 8.9-9.3, 8.1-8.8, and <8.1 respectively. For total serum protein (g/dL) the corresponding values are ≥6.2, 5.8-6.1, 5.1-5.7, and <5.1. These are the values used in this study as standard measurements for evaluating TPI. On a herd level, the recommended percental distribution is excellent >40%, good ~30%, fair ~20%, and poor <10%. Poor (<10 g/L) was in this study equated to FTPI (Tyler et al., 1996; Godden et al., 2019; Lombard et al., 2020).

1.4 Colostrum

The production of colostrum begins several weeks before calving, by the influence of lactogenic hormones e.g., prolactin, and the production ceases at parturition (Godden et al., 2019).
Colostrum consists of nutrients such as immunoglobulins, fat, casein, vitamins, and minerals. Non-nutritive factors consist of hormones, leukocytes, cytokines, growth factors and trypsin. These components are great in numbers (Godden et al., 2019; Pakkanen et al., 1997).E.g., Trypsin levels are 100% higher in colostrum than in regular milk, and Trypsin helps protect IgG and proteins in the intestine. Fat and lactose levels are also increased in colostrum which provides energy that is critical for body temperature regulation and thermogenesis. The content of leukocytes has proven to be critical for displaying a functional immune response within 1 week of life opposed to 3 weeks when given colostrum without leukocytes. All these components are greatest at first milking and decline with each milking (Godden et al., 2019).
The colostrum consists of immunoglobulins which are a combination of IgG, IgM and IgA respectively distributed as about 85-90%, 7% and 5%, in total IgG1 constitutes 80-90%. This distribution varies between cows (Godden et al., 2019). The concentration of IgG in colostrum has been shown to differentiate between breeds, with the Jersey cow producing higher quality than Holstein as reviewed by Weaver et al. (2000).

In the field, a Brix refractometer is easily used to determine the quality (Buczinski et al., 2016; Quigley et al., 2013; Morrill et al., 2015). A Brix% ≥22% is used to determine if colostrum is of good quality (≥ 50 g/L IgG) with a probability of 94.3% of it being true. When discarding colostrum with <22% up to 50% of these samples will have a quality of ≥ 50g/L IgG. A more precise cut-off when determining if colostrum is of poor quality is <18%, where only 22.7% will have a quality of ≥ 50g/L IgG. Buczinski et al. (2016) propose that ≥ 22% is used for colostrum feeding, <18% is discarded as poor quality and colostrum replacer or frozen colostrum is used instead. Everything in between those cut-offs is recommended to be used in combination with a colostrum replacer or supplement (Godden et al., 2019; Buczinski et al., 2016).

Other than the concentration of IgG, bacterial contamination, the amount of growth factors and leukocytes etc. are important to consider when evaluating colostrum quality (Buczinski et al., 2016; Godden et al., 2019).

1.5 Colostrum management

The calf should be fed colostrum as soon as possible (Selman et al., 1970; Lora and Barberio et al., 2018). Feeding after 6 to 12 hours decreases the transfer of passive immunity (Fischer et al., 2018) and by Danish law, the calf must be fed colostrum within the first 6 hours after birth (BEK 1743 §48 stk. 2). To avoid FTPI, calves must ingest the adequate amount and quality of colostrum at the right time (Lora and Barberio et al., 2018; Selman et al., 1970), and by giving a second feeding of colostrum the risk of FTPI is lowered (Chigerwe et al., 2009; Hare et al., 2020; Abuelo et al., 2021; Morin et al., 1997). A calf should be fed 10-12% of its body weight and for a Holstein calf, this is equivalent to 3-4 L of colostrum (Godden et al., 2019).

Many herds have a colostrum bank containing tapped colostrum with a known Brix% (Moore et al., 2005), typically stored in a freezer. This is helpful if a calf is born from a cow with low-quality colostrum or if there is a general problem in the herd with colostrum feeding and calf health. Under Danish law (BEK 1743 §52) calves should not be fed colostrum with a feeding tube, only when used as a treatment for a disease, therefore staff needs to ensure that calves suckle from the cow in the first 24 hours to ingest colostrum or use other means of artificial feeding.

For optimal quality, the colostrum must be collected as soon as possible as the quality decreases over time (Morin et al., 2010; Quigley et al., 2013; Sasaki et al., 1976; Moore et al., 2005) and decreases by the amount of milking (Stott et al., 1981).

1.6 Aims and Objectives

Considering the limited knowledge of the physiological health benefits of prolonged CCC, this study had three objectives. The first objective was to describe the health and immunisation of the calves in the sampled herds with prolonged cow-calf contact. The second objective was to answer if there was a significant difference in TPI between conventional herds and herds with prolonged

CCC. The last objective was to determine if the colostrum quality of the cow measured by Brix% is correlated with the calf's serum Brix%.

The necessary data was collected from clinical assessment of calves including blood samples, and colostrum samples from the cows, and questionaries regarding farmers' colostrum management as well as calving and housing arrangements.

2. MATERIALS AND METHODS

2.1 Literature search

The databases primarily used in the literary search, were Ovid MEDLINE(R) ALL and Web of Science. The keywords used were variations of cow-calf contact, cow, bovine, calf, colostrum, immunisation, immunoglobulin, transfer of passive immunity, suckling, and Brix.
The keywords were combined using OR or AND. The languages were limited to Danish and English. Supplementary the snowballing method was used using Medline, Web of Science and Scopus.

2.2 Ethical approval

The laboratory animal permit (2023-15-0201-01520) was issued on 02.10.2023. The local ethical approval (2023-012) was issued on 04.09.2023.

2.3 Data

In the fall of 2023, the writer of this thesis collected blood samples, clinical assessments, and milk samples once a week for all four herds. All collections and processing of data were performed by this writer, excluding one round of samples which were processed by supervisor Kirstin Dahl-Pedersen.
The four herds were chosen through the project 'Sundhed og sygdom i ko-kalv samværssystemer'. They showed interest in participating, had a considerable size and were geographically located in the same area of mid-Jutland, except for herd 4 which was located on Zealand. The herds were chosen based on being a considerable size assuring a fair number of births in the short data collection time. Due to the small number of herds with prolonged CCC and the practical circumstances of proximity between the herds, the number of farms was limited.
All herds were organic herds that had switched to a system of prolonged cow and calf contact within the last 6 years.
Upon the first visit, each farm was questioned using Appendix 1 (attached questionnaire) to depict each herd's routines in calving and colostrum management. The data will be called Cowcalf when compared with other studies in further analysis and discussion.

2.3.1 Data collection

Each calf was given a clinical assessment, including rectal temperature, assessment of umbilical cord, respiratory score, gastric score, hydration score and general attitude following Appendix 2, which was inspired by O'Reilly and Kirkegaard (2023, Appendix 2).

Rectal temperature was missed for one calf sample, no. 40.

There were 6 parameters in the clinical assessment, all 6 were included in the statistical analysis.

2.3.2 Colostrum sampling

Colostrum samples were collected from 77 cows.

The aim was to get a colostrum sample from each cow. This proved to be difficult when herds had varying methods of handling cows versus heifers, and one farm had difficulty implementing a new routine.

Farm 4 differed from the following as the Brix percentage of colostrum was measured on the farm by the farmer, this included 10 samples.

The colostrum samples were kept at freezing temperature (-20°C) after collection. Before analysis, the samples were kept in a cooling box with a freezing element for up to 4 hours.

Before each round of samples, the Brix refractometer was tested using 3 drops of ionized water (0.0%) and was then calibrated.

The samples were slowly inverted before 3 drops were transferred to a Brix refractometer 'KRUUSE Digitalt refraktometer' with a single-use pipette. The Brix refractometer was then wiped clean with a paper towel before another 3 drops from the same sample were analysed.

Between analysis of each sample, the refractometer was wiped clean with a towel, and if the milk was very thick, ionized water was used as well.

2.3.3 Blood sampling

Blood samples were collected from vena jugularis from 126 calves. Before sampling the area was sanitized with 70% alcohol. For each calf, 1 blood sample was taken and filled in 2 serum tubes (each 8.5 ml) with stable gel (BD Vacutainer SST).

All blood samples were kept at a maximum of 5°C and above freezing temperatures either by refrigerator and cooling box or exclusively by cooling box. Samples no. 90-93 were stuck in the centrifuge at room temperature for 3-4 hours due to a technological error. All samples were analysed within 36 hours of collection.

Each blood sample was centrifuged at 1800 G (4327 RPM) for 10 minutes on 'Hettic EBA 20'.
The Brix refractometer and classic refractometer were tested using 3 drops of ionized water for correct calibration before each round of samples.
The samples were then examined once by classic refractometry 'ATAGO SPR-T2' (serum protein) and twice by Brix refractometry 'KRUUSE Digitalt refraktometer'. Between each analysis, the refractometers were cleaned using either a paper towel or ionized water as per the instruction manual. For both classic refractometry and Brix refractometry, 3 drops of serum were applied using a single-use pipette, this procedure was repeated for the Brix refractometer.
After each round of samples (each week) the refractometer was reset.

2.4 Statistical analysis

All data was transcribed in Microsoft Excel (version 16.77). The data were then analysed using Microsoft Excel and the statistical program R Core Team (2023). Descriptive statistics as clinical scoring was evaluated by prevalence, while the questionnaire was used as a reference in the discussion of results.
To compare the distribution in the 4 groups (Lombard et al., 2020) for herds 1 and 2, a Chi-squared test was used. The same test was used to analyse the distribution in the 4 groups for Cowcalf and Conventional respectively compared with the recommendations from Lombard et al. (2020), as well as compare the distribution in the 4 groups between Cowcalf and Conventional.
A T-test was used to compare the mean Serumbrix% of Cowcalf and Conventional, along with the mean Serumbrix% of calves categorised as sick versus healthy.
A proportion test was used to compare the proportions of calves with FTPI in Cowcalf and Conventional.
A significance level of 0.05 was used in each of these analyses.
The calves with a total health score (THS) ≥ 2 across the 6 categories (respiratory, gastric, welfare, umbilicus, palpation, and hydration) were categorised as sick, while calves with THS < 2 were categorised as healthy.

2.5 Data from conventional herds

Data on serum Brix% from a large Danish research project called "Robuste kalve", were used for comparison to the calves within this study. 572 calves aged 3-7 days were blood sampled from 83 Danish, intensive, commercial, dairy herds, and Brix% was measured on serum. The details are presented in Goecke et al. (2021), but in summary, they were sampled in the winter period of 2018-2019. The calves were primarily kept in single pens, and feeding regimes differed according to management, but calves were typically fed milk for 8-12 weeks. This study will be called Conventional in further analysis and discussion.

3. RESULTS

3.1 Herds

Herd	1	2	3	4
Number of cows	750	380	240	120
Race	Holstein Beefmix for sale	Holstein with a Dutch breed Beefmix for sale	Holstein core with Jersey, Red dairy breed and Beefmix for sale	Jersey Beefmix for sale
Calving conditions	Joint box	Single box	Joint box	Joint box and single box
Colostrum routines				
First feeding	After 2 hours	As quickly as possible	As quickly as possible	None
Amount (L)	3 L	3,5-4 L	4 L if possible	None
Number of feedings	1	1	1	None
Quality tested (Brix%)	Yes	No	Yes	No
Feeding tube or suckle	Feeding tube	Feeding tube	Feeding tube	None
Housing				
CCC exclusively with dam	72 hours in single box	Min. 24 hours in single box	Min. 24 hours in single box	72 hours in single box
Hours per day	All day except milking	~19 hours (- 5 when cow was in the field)	All day except milking	All day
CCC in total	3 months	4-5 months	4-5 months	Min. 3 months
Housing (majority)*	Joint box	Joint box	Joint box	Field (joint)
Comment	Joint box with the dam for 1 week, the remainder with nurse cow	Joint box with the dam for 3 weeks in total, the remainder with nurse cow	Joint box with nurse cow for the remainder of the period	Joint with the dam for the entire period

*Table 1: Overview of farmers' questionnaire depicting the general routines and conditions of each farm for housing of calves and cows, as well as colostrum management. *The duration in each section varied depending on the number of births.*

Of the 4 herds, 3 herds had primarily Holstein cows while the fourth had Jersey cows. For half of the herds, the cows calved in a joint box, while the others calved in a single box. Only herd 4 had no colostrum management and it was also the only herd which had 24-hour contact through the whole period of CCC. The herds which had colostrum routines fed from 3 to 4 litres of colostrum by oesophageal tube as soon as possible, and of those, herd 2 didn't check the quality of the colostrum.

Herd 3 housed the calf with the dam for the shortest period before the calf was housed with a nurse cow, while herd 4 had the longest period of contact with the dam. All herds practised CCC for over 3 months (Table 1).

3.2 Animals

After consideration, it was decided that only the calves aged 3-7 days would be used to describe the health of the population (n = 83) to realistically evaluate prolonged CCC. This excludes calves aged 1-2 days and two calves aged 8 days. For herd 1 this resulted in n = 43, herd 2 n = 24, herd 3 n = 11, and for herd 4 n = 5. For herd 4 the herd effect is harder to describe and has less strength when describing significance. When excluding the calves aged 1-2 days the according colostrum sample was also excluded, leaving 50 samples for analysis.

There was a significant correlation between the two Brix% measurements on serum with $R = 0.94$ and $p < 2.2 \cdot 10^{-16}$ (Figure 1). By performing an Intraclass correlation (ICC) calculation it was possible to further defend the use of the mean between the Brix% measurements on serum. The ICC value was 0.964, $p = 8.49 \cdot 10^{-50}$, and the 95% confidence interval was $[0.945 ; 0.977]$, all suggesting a strong agreement. The mean Brix% of serum was used onwards and was called Serumbrix%.

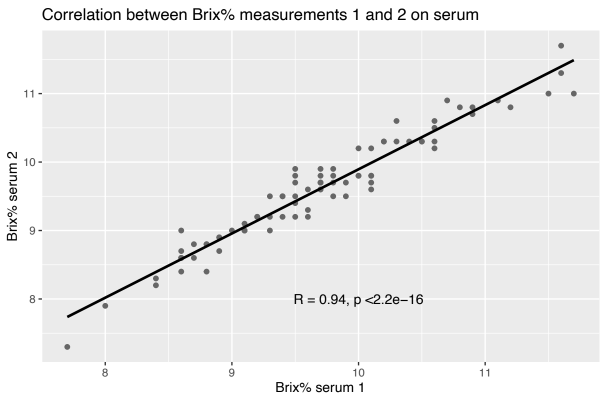


Figure 1: Correlation between brix measurements 1 and 2 on serum, Significant correlation with R = 0.94, p $< 2.2 \cdot 10^{-16}$

There was a significant correlation between the two Brix% measurements on colostrum with $R = 0.975$ and $p < 2.2 \cdot 10^{-16}$ (Figure 2). For the two measurements on colostrum, the same test (ICC) was performed to defend the use of the mean. The ICC value was 0.985, $p = 8.63 \cdot 10^{-40}$, and the confidence interval was $[0.973; 0.991]$ and suggested a very high agreement between the two measurements defending the use of the mean. The mean Brix% of colostrum was used onwards and was called Colostrumbrix%.

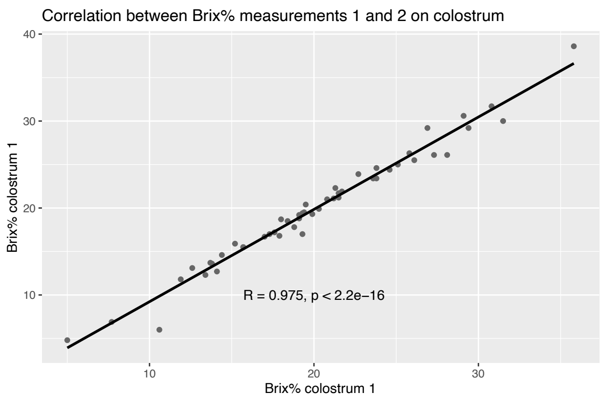


Figure 2: Correlation between brix measurements 1 and 2 on colostrum. Significant correlation with R = 0.975 and $p < 2.2 \cdot 10^{-16}$

For analysis of both Brix% on serum and colostrum, it was advantageous to use an average between the two measurements for both serum and colostrum.

A significant correlation was seen between the Brix% measurement on serum and the corresponding total serum protein by refractometer, with $R = 0.93$ and $p < 2.2 \cdot 10^{-16}$ (Figure 3).

Figure 3: Correlation between Serumbrix% and Total Serum Protein. Significant correlation with R = 0.93 and $p < 2.2 \cdot 10^{-16}$.

The mean Colostrumbrix% was 20.07%, the lowest Colostrumbrix% was 4.90% while the highest value was 37.20%. The mean Serumbrix% was 9.63% for all herds, the lowest Serumbrix% being 7.5% and the highest 11.65% (Table 2).

Parameter	Mean (SD)	Median (IQR)	Min	Max
Brix% serum (%)	9.63	9.65	7.5	11.65
Total serum protein (g/dl	6.28	6.20	4.00	8.80
Brix% colostrum (%)	20.07	19.52	4.90	37.20

Table 2: Total serum protein and Brix% for serum and colostrum with continual values.

No significant correlation was seen between Serumbrix% and the corresponding colostrumbrix% with $R = 0.00018$ and $p = 0.93$ (Figure 4).

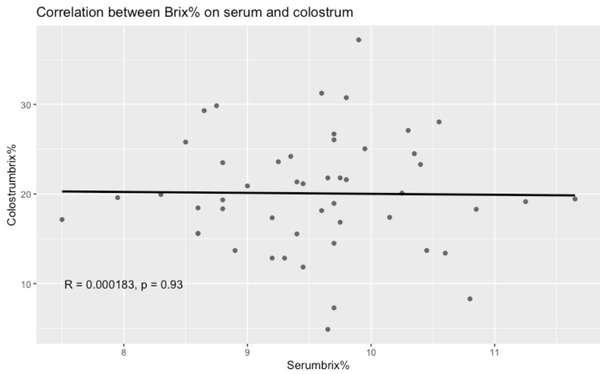
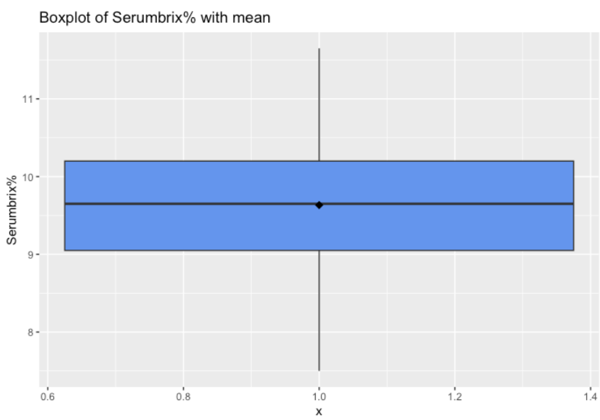


Figure 4: Correlation between Colostrumbrix% and Serumbrix%. No significant correlation with R = 0.00018 and p = 0.93.

3.2.1 Immunisation



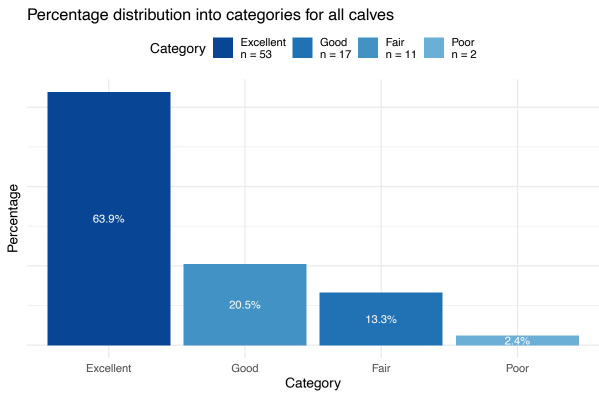


Figure 5: Boxplot for all calves (1-4) of Serumbrix% with mean.

Figure 6: Percentage distribution in the four categories by Lombard et al. (2020) for all four herds.

When grouping the Serumbrix% levels of the four herds according to the categories proposed by Lombard et al. (2020) the following results were attained. For all sampled calves aged 3-7 days, 63.9% were in the excellent category, 20.5% were good, 13.3% were fair and 2.4% were poor (Figure 6). All results are within the recommended intervals (excellent >40%, good ~30%, fair ~20%, and poor <10%). This distribution in the categories was found to be significantly different from the expected (Lombard et al., 2020) distribution with $p = 0.000106$. When looking at the data it is obvious that the difference is positive, with a left shift towards the better categories.

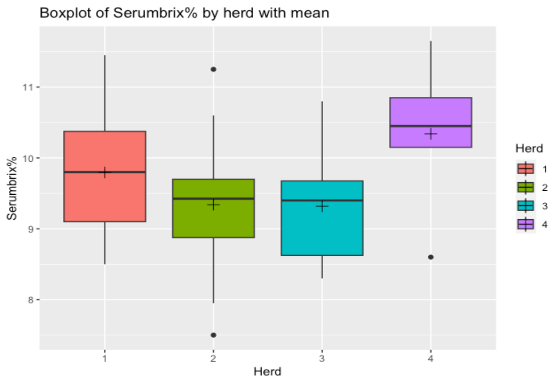


Figure 7: Boxplot for all calves divided by herd (1-4) of Serumbrix% with mean.

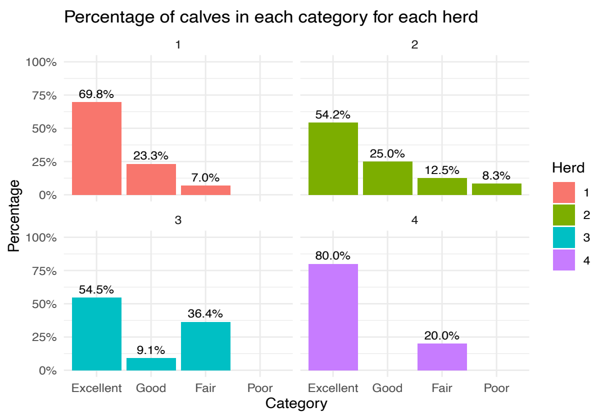


Figure 8: Percentage distribution in the four categories by Lombard et al. (2020) for all calves divided by herd (1-4).

Predictor	**Brix% category**				**Brix% mean**
Herd	*Excellent (>9.4%)*	*Good (8.9-9.3%)*	*Fair (8.1-8.8%)*	*Poor (<8.1%)*	
1 (*n* = 43)	69.8% (30)	23.3% (10)	7.0% (3)	0% (0)	9.80
2 (*n* = 24)	54.2% (13)	25.0% (6)	12.5% (3)	8.3% (2)	9.34
3 (*n* = 11)	54.5% (6)	9.1% (1)	36.4% (4)	0% (0)	9.32
4 (*n* = 5)	80.0% (4)	0% (0)	20.0% (1)	0% (0)	10.34
Combined (*n* = 83)	63.9% (53)	20.5% (17)	13.3% (11)	2.4% (2)	9.63

Table 3: Each herd and their distributions in Brix% categories and their mean Serumbrix% (Figures 6-8).

All 4 herds had the largest proportion of calves in the excellent category and the smallest if any in the poor category. Only 2 calves were categorized as having FTPI (<10 g/L), both calves were from herd 2. Herds 1, 3 and 4 had 0 calves in the poor category but herds 3 and 4 had more calves in the fair category than the good category, and only herd 3 had a higher proportion in the fair category than recommended. Herds 3 and 4 were also the smallest, both in herd size and sample size. When applying the Chi-squared test to the distribution across the 4 categories for herds 1 and 2, there was no significant difference with $p = 0.19$. The test wasn't applied to the other herds. The mean Serumbrix% was 9.63% for all herds and ranged from 9.32% to 10.34% (Table 3).

3.2.2 Clinical examination

Parameter	Score results (*n*)	Prevalence (%)	Mean (SD)	Median (IQR)	Min	Max
Respiratory	0: 82 1: 1 2: 0	0: 98.80% 1: 1.20% 2: 0				
Gastric	0: 68 1: 15 2: 0	0: 81.93% 1: 18.07% 2: 0				
Welfare	0: 82 1: 1 2: 0	0: 98.80% 1: 1.20% 2: 0				
Umbilicus	0: 63 1: 19 2: 1	0: 75.90% 1: 22.89% 2: 1.20%				
Palpation	0: 83 1: 0 2: 0	0: 100% 1: 0 2: 0				
Hydration	0: 82 1: 1 2: 0	0: 98.80% 1: 1.20% 2: 0				
Temperature (C)			39.22	39.30	37.50	40.10
Age (days)			4.64	4.00	3.00	7.00

Table 4: Clinical scores with categorial values. Age and temperature with continual values. Per Appendix 2.

Out of all 83 calves, 84.34% (*n* = 70) of the calves had a temperature between 39.0°C and 40.5°C, whereas only two calves out of the 70 calves had a temperature over 40.0°C, and 15.66% (*n* = 13) out of all the calves had a temperature below 39.0°C.

The calves were assessed per Appendix 2. There were 1.20% of the calves that showed respiratory symptoms with a score of 1, consistent with nasal discharge (serous) or slight coughing (*n* = 1), while 18.07% (*n* = 15) of the calves showed gastric symptoms consistent with loose manure and soiling of the back legs. Of all 83 calves, 22.89% (*n* = 19) of the calves had either a swollen or dolent umbilicus, while 1.20% (*n* = 1) scored 2 when assessing the umbilicus, implying it was swollen and dolent.

When assessing the general attitude only 1.20% (*n* = 1) of the calves were assessed as slightly depressed, being less interested in their surroundings and less reactive when approached and handled. Light signs of dehydration (skinfold standing 1-3 sec) were assessed in 1.20% (*n* = 1) of the calves.

In neither of the categories did over half of the calves score 1 or 2, within every category over 50% of the calves scored 0, and in total 60.24% (*n* = 50) of the calves scored 0 in all 6 categories. Only one calf scored 2 in any category (Umbilicus), 32.53% of the calves (*n* = 27) scored 1 overall and 7.23% (*n* = 6) scored 2 overall.

The calves with a total health score (THS) of 2 across the 6 categories were categorised as sick. T-test was applied to the calves categorized as 'healthy' (THS < 2) and 'sick' (THS = 2) compared with the Serumbrix%, the T-test showed no significant difference with a t-value of -0.08 and $p = 0.94$.

3.3 Cowcalf compared with Conventional

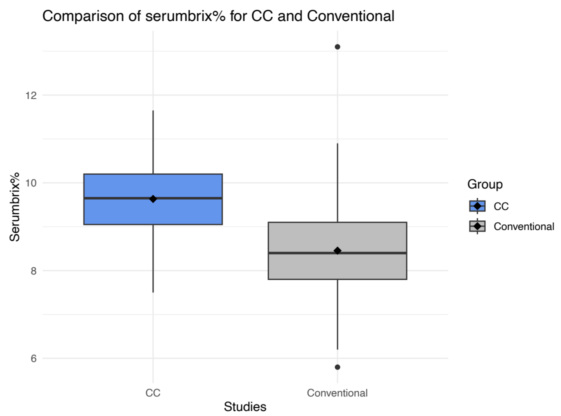


Figure 9: Boxplot of the datasets Cowcalf (CC) and Conventional of Serumbrix% with mean.

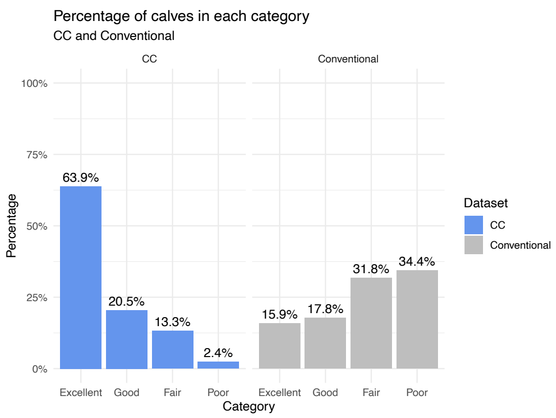


Figure 10: Percentage distribution in the four categories by Lombard et al. (2020) for the two datasets Cowcalf (CC) and conventional respectively.

There were 83 calves in Cowcalf (CC) and 572 in Conventional when sorted by age, 3-7 days.

Serumbrix%	**Cowcalf** (*n* = 83)	**Conventional** (*n* = 572)
Mean	9.63	8.46
Q1	9.05	7.8
Median	9.65	8.4
Q3	10.2	9.1

Table 5: Serumbrix% for datasets CC and Conventional with continual values (Figure 7).

The mean Serumbrix% for Cowcalf was 9.63% while it was 8.46% for Conventional. Using a T-test, the difference in mean was assessed as significantly different with $p < 2.2 \cdot 10^{-16}$.

When evaluating the distribution of calves across categories for Conventional compared with the expected (recommended) proportions there was a significant difference with $p < 2.2 \cdot 10^{-16}$. The distribution of Conventional was worse than recommended and right shifted as opposed to Cowcalf. The largest proportion of calves from Cowcalf were in the excellent category while the largest proportion of Conventional was in the poor category. There was a significant difference between the two studies with $p < 2.2 \cdot 10^{-16}$ when applying the Chi-squared test.
There was a significant difference in the proportions between calves with FTPI and those who were satisfactorily immunised between the two studies with 2.4% having FTPI in Cowcalf and 34.4% in Conventional with $p = 6.561 \cdot 10^{-9}$.

4. DISCUSSION

One objective was to describe the immunisation and health of the calves in the four sampled herds by interviewing the farmers, performing clinical examinations, and collecting serum samples from the calves. The calves were found to be healthy, with 7.23% of calves presenting with signs of disease, whereas the majority presented with gastric symptoms (Table 4). 60.24% of the calves had a THS of 0. This was substantiated by the mean Serumbrix% which was 9.63%, where only 2.4% of the sampled calves had FTPI (Table 3).
The transfer of passive immunity for calves with prolonged CCC was compared with calves with normal CCC (12-24 hours), and the difference was found to be significant. The Colostrumbrix% for the cows was compared with the Serumbrix% of the calves and there were found no significant correlation.

4.1 The health of the calves

The results indicate that the sampled calves were in good health.
In total, 60.24% of the sampled calves had a THS of 0 while only 7.23% (n = 6) were categorised as sick as their THS was 2, and no calf had a THS higher than 2. Of all calves, 18.07% showed symptoms of gastric disease, which was the highest frequency across the 6 categories (Table 4).
A T-test was used to compare the calves with THS = 2 and < 2, the test was insignificant meaning the mean Serumbrix% for the two groups was not significantly different. The lack of significance could be explained by Buczinski et al. (2018) who stated that the IgG concentration can be falsely elevated when the calves are sick or dehydrated. Still, only one calf had a health score of 2 in any single category meaning most calves with a THS > 0 only showed light symptoms.
The category in which most calves scored was gastric, as opposed to respiratory where only 1 calf scored (Table 4). This is to be expected as calves in this age group typically suffer from enteric disease, whereas respiratory disease is more common in calves older than 30 days (McGuirk et al., 2008).

The Serumbrix% values were high, and the majority of samples were categorised as excellently immunised. The mean Serumbrix% for all calves combined was 9.63% and ranged from 9.32% to 10.34% (Table 3).

There was a significant difference, when the distribution of all sampled calves across the 4 categories was compared with the recommended proportions (Lombard et al., 2020) with $p = 0.000106$. It was clear from the percentages in Figure 6, that Cowcalf was distributed better than the recommendations with 63.9% of the calves having a Serumbrix% higher than 9.4% and only 2.4% with Serumbrix% lower than 8.1% (FTPI).
All calves except those from herd 4, were fed colostrum by oesophageal tube soon after birth, this was likely one of the reasons the herds were so well immunised. Assuming all calves from herds 1, 2 and 3 suckled the cow before and/or after this artificial feeding they received at least two feedings of colostrum, one of which was quality checked (except for herd 2) (Table 1). All calves were kept in a separate box with the cow for at least the first 24 hours, as this decreases the infection pressure and allows the cow and calf to bond.
Herd 4 differed the most in management, being that they did not feed colostrum artificially and the calves were with the cows 24 hours of the day (Table 1). It would have been interesting if the sample size had been bigger so they could have been compared with the other herds.

There was made no statistical comparison between all four herds as the sample size for herd 4 (n = 5) was deemed too small, to make strong and reliable conclusions. The strength of the sample was deemed to be strongest when they were assessed together.
Herd 1 and 2 were very similar, both in sample size and their distribution in the categories. When applying the Chi-squared test there was found no significant difference across the 4 categories between the two herds with $p = 0.19$. In terms of the calving management, they differed when comparing contact hours during the day, and for colostrum management herd 2 did not check the quality of the colostrum fed by oesophageal tube, and the amount in that feeding differed slightly between the two. Still, out of the four herds they were most alike when comparing management (Table 1). The difference in contact hours may not be of great influence because of the ecology of the species where cows commonly leave the calves while grazing (Vitale et al., 1986).

It is difficult to purely assess the effects of prolonged CCC, both positive and negative when many variables differ and may affect the assessment. As with herd 4, it would have been interesting to explore what effect prolonged CCC had on the transfer of passive immunity without the intervention of oesophageal tube feeding. CCC was found to have a positive effect on immunisation by Selman and McEwan (1971). Contrary other studies have found that suckling the cow increases the risk of FTPI (Weaver et al., 2000; Besser et al., 1991). This is thought to be because of inadequate volume and quality, and the artificial feeding decreases the risk of this.

4.2 Cowcalf compared with Conventional

A significant difference was found when comparing Cowcalf to Conventional with $p < 2.2 \cdot 10^{-16}$. Figure 10 shows the distribution of calves in the four categories for both datasets. The trend seems reversed for Cowcalf and Conventional. When comparing Conventional with the four herds in Cowcalf there was a significant difference between the immunisation of the two groups, with Cowcalf herds being significantly better immunised and all within the recommended distributions by Lombard et al. (2020), while Conventional was not. The largest proportion of calves in Cowcalf (63.9%) was in the excellent category and the largest proportion for Conventional (34.4%) was in the poor category. The difference in proportions of calves with FTPI in the two datasets was found to be significant.

The mean Serumbrix% for Cowcalf was 9.63% while it was 8.46% for Conventional (Table 5) and this difference was found to be significant when the T-test was applied. Other studies have shown contradicting results with cow-reared calves having worse health than calves separated shortly after birth (Wenker and Verwer, 2022). It is important to consider that the environment is still essential when evaluating health. Full contact calves in the study by Wenker and Verwer (2022) were housed 3 days in the maternity pen before they were moved to a separate box with the cow. The environment makes for great infection risk and conditions such as draft, bedding etc. increase morbidity.

Failure of transfer of passive immunity is not a disease but increases the risk of disease and mortality. An adequate or even excellent passive transfer is not equal to disease-free calves, and likewise, a calf with inadequate passive transfer placed in a clean environment is not guaranteed to become sick. The environment, management and level of virulent organisms are as influential if not more (Weaver et al., 2000; Wenker and Verwer, 2022).

Both datasets were limited to calves aged 3-7 days to make the samples equal. The calves in Conventional came from Danish big-scale conventional farms and the herds in Cowcalf were all organic Danish farms but differed considerably in herd size from 120 to 750 cows. It is unknown to this author how long the calves from Conventional were left with the cows, but as they were all categorised as conventional farms the standard by law is 12 hours. All calves from Cowcalf were left at least 24 hours with the cow and for herds 1, 2 and 4 they were left with the mother for more than 72 hours. The remainder was then spent either with the mother or a nurse cow.

There was a significant difference in sample size with Conventional having an almost 7 times bigger sample size than Cowcalf. The number of calves categorised as having FTPI was compared across the two datasets and a significant difference was found. This implies that prolonged CCC equals better immunisation and lesser risk of FTPI than standard CCC. It is worth considering that the calves in Cowcalf, except 5 calves, were fed colostrum with an oesophageal tube within the first hours of life, it is unknown if the same applies to Conventional. This may influence the results of Cowcalf if the same or similar practice was not implemented in the herds included in Conventional and lessen the significance of the effect of prolonged CCC on immunisation (Weaver et al., 2000; Besser et al., 1991).

4.3 Colostrumbrix% and Serumbrix%

The study found no linear correlation between the Colostrumbrix% and Serumbrix% with $R = 0.00018$ and $p = 0.93$ (Figure 4). Colostrum quality decreases significantly within the first hours as well as with each milking (Morin et al., 2010; Quigley et al., 2013; Sasaki et al., 1976; Stott et al., 1981), making the source of error great when collecting these samples and assuming that they are equal. Many calves are born at night and so is the case for this study, where the exact time of birth was not noted. Some farmers only took the sample at first milking, thereby decreasing the validity when equating the sample to the actual colostrum quality when the calf feeds. This makes it unreliable to conclude anything on the Colostrumbrix% correlated with Serumbrix%. Colostrumbrix% can be used to determine the quality at collection, which could be lower than the initial quality, but this cannot be said with certainty. The mean Colostrumbrix% was 20.07% which is lower than the typical cut-off point of 22%. The samples ranged from 4.90% to 37.20% (Table 2). Other studies prove that the quality as well as the amount of colostrum fed in the first 24 hours is key to avoiding FTPI and achieving a great immunisation (Renaud et al., 2020; Chigerwe et al., 2009), even though it was not possible to show in this study.

4.4 Limitations

For some of the herds, the sample size was relatively small, and this made the intercomparison less strong. This could have been avoided with bigger herds or a longer sampling period.

When analysing the data, it was decided only to use the samples from calves aged 3-7 days, this equated to 83 calves out of the total 126 calves sampled. This made the samples from the calves aged 1-2 days irrelevant, and this should have been considered before conducting the sampling.

The health scoring is only a depiction of the symptoms seen at the time of sampling, as the ages of the calves ranged from 3 to 7 days, some calves may have shown symptoms if they were examined later due to incubation. If more time had been available a follow-up examination of the calves could have been valuable. To e.g., determine if the Serumbrix% would later influence the THS of the calves.

There were great sources of error with colostrum sampling, as the exact time of sampling was not noted. This was first known when the literature was gathered, as the quality of colostrum is known to decrease over time and with each milking (Godden et al., 2008; Quigley et al., 2013; Sasaki et al., 1976; Moore et al., 2005; Stott et al., 1981), the time of sampling is paramount when assessing the quality regarding the purpose of this study. The aim was to determine the quality of the colostrum ingested by the calves, but by the colostrum quality decreasing with each hour, it was not possible to regard the samples as equals, the samples were likely assessed as worse quality than they were initially. This could have been avoided by instructing the farmers in noting the exact time of sampling and more importantly, having them sample the cows as soon as possible.

5. CONCLUSION

The 83 calves aged 3 to 7 days in the study were healthy and excellently immunised with a low prevalence of disease at 7.23%, most frequently with signs of gastric disease, and a high mean Serumbrix% of 9.63%. Only 2.4% of the calves had FTPI.
They differed significantly from the herds in Conventional, being better immunised with Conventional having a mean Serumbrix% of 8.46% as opposed to 9.63%. The largest proportion of calves in Cowcalf (63.9%) were excellently immunised while the largest proportion of Conventional (34.4%) had FTPI. The lack of management knowledge of Conventional makes it hard to distinguish where the exact differences lie and therefore difficult to make strong conclusions. The extra artificial feeding of the calves may influence the data more than the actual prolonged contact. It is known that there is great risk in letting the calf suckle the cow, but for the four herds in this study transfer of passive immunity was accomplished despite this. The key to great immunisation is in the management and human intervention may be essential to ensure the transfer of passive immunity (Lora et al., 2019). It can be concluded that prolonged CCC in this study did not increase the risk of FTPI.
The Colostrumbrix% were not linearly correlated with the Serumbrix% of the calves. This is contrary to what is found in the literature where the quality of colostrum is important to ensure adequate transfer of passive immunity (Lora and Barberio et al., 2018). The sources of error were too great when evaluating the colostrum samples making it difficult to make strong conclusions. The source of error is thought to be the reason for the lack of significant correlation between Colostrumbrix% and Serumbrix% found in this study.

The building of the immune system starts in the first 36 hours, making these critical hours in the calf's life. As the calf is born agammaglobulinemic, it needs to be fed colostrum while the small intestine is penetrable. It is important that calves that are left with the cow suckle within the first 6 hours (Selman et al., 1970; Lora and Barberio et al., 2018; Fischer et al., 2018). Calves should be fed good quality colostrum, whilst also receiving an adequate amount and at the right time, even twice if possible. It is easy to measure the quality of colostrum and with a careful guide on how to interpret the results it can become an easy routine.
In conclusion, the transfer of passive immunity can be achieved from prolonged CCC. Considering the literature, calf and colostrum management must be a primary focus as the risk is great when there is no intervention.

6. PERSPECTIVES

This study shows that prolonged CCC is possible, while still achieving a high percentage of transfer of passive immunity. The knowledge is limited, and further studies should be carried out to further investigate the possibilities of prolonged CCC in modern dairy farming. The diverse practice of prolonged CCC is likely a reason for the lack of studies and impacts their relevance.

Most farms in Denmark are not built for a system with prolonged CCC, and this may slow some farmers in making the changes. It is both a question of financial limits, the willingness to change routines and the potential risk that follows.

If prolonged CCC should work in practice the housing arrangements need to be carefully evaluated, as they must ensure that the calf is warm and dry and minimise the exposure to infections. Farm staff needs to have a keen eye on the calf in the first 24 hours, ensuring a successful feeding of colostrum within the first 6 hours, possibly feeding the calf with good quality complementary colostrum.

When considering the health benefits and issues of prolonged CCC it is also important to consider the effects on the cow, both the physical of the udder and the stress when they are separated (Roadknight et al., 2022). The field needs more knowledge of the physiological effects of prolonged CCC but in the end, it is both the physiological and psychological impact that needs to be considered when evaluating this practice.

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8. APPENDIX

8.1 Appendix 1

Besætningsoplysninger

Antal køer:

Race:

Kælvningsforhold (enkelt/fællesboks):

Råmælksrutiner:

1. **fodring:**

   Mængde:

   Kvalitet:

   Får de flere gange?:

   Sonde eller sut?

Hvor længe går koen med kalven:

Hvor mange timer om dagen:

Opstaldning af ko og kalv (fælles/enkelt):

8.2 Appendix 2

Clinical assessment guide

(inspired by O'Reilly and Kirkegaard (2023, Appendix 2))

Category	***Criteria***
General condition - *Based on Wisconsin-Madison Calf Health Scoring Criteria.*	**0**: Interested in its surroundings and reacts when approached and handled, good appetite. **1**: Slightly depressed, unilateral eardrop, less interested in its surroundings and less reactive when approached and handled. **2**: Quiet and lying down. Sunken eyes, drooping ears, inappetence, dull fur and possibly comatose.
Hydration	**0**: Skinfold stands max. 1 sec **1**: Skinfold stands 1-3 sec **2**: Skinfold stands >3 sec
Umbilical cord	**0**: Normal **1**: Lightly swollen or dolent **2**: Swollen and dolent
Gastric	**0**: Normal faeces **1**: Loose faeces and soiling on the hindlimbs **2**: Watery or bloody faeces (treatment required)
Respiratory - *Based on Wisconsin-Madison Calf Health Scoring Criteria.*	**0**: Normal respiration **1**: Nasal discharge (serous/seromucous) or slight cough **2**: Nasal discharge (purulent), cough and forced breathing (changes when auscultation)
Palpation of limb joints (carpal and fetlock)	**0**: Normal palpation **1**: Slightly swollen or warm over carpal or fetlock joint **2**: Swollen and warm over carpal or fetlock joint