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ORIGINAL RESEARCH

Efficacy of oral administration of specific immunoglobulins in preventing neonatal calf diarrhoea in dairy herds

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Abstract

Background: The aim of this study was to evaluate the efficacy of bovine concentrated lactoserum (BCL) containing specific immunoglobulin G against *Escherichia coli*, rotavirus and coronavirus in preventing neonatal calf diarrhoea (NCD).

Methods: A total of 489 newborn calves from 35 herds were orally given either BCL or a placebo before the first feeding of colostrum and clinically supervised by the farmers for the first 14 days of life. The diarrhoea score was defined according to the following criteria: 0 = no diarrhoea; 1 = light diarrhoea without medical treatment; 2 = diarrhoea requiring oral treatment (rehydration and/or antibiotic therapy); and 3 = severe diarrhoea requiring parenteral rehydration or resulting in death.

Results: A total of 138 calves suffered from diarrhoea (28%), and 65 (13%) showed signs of diarrhoea requiring treatment. The odds of getting NCD were reduced (odds ratio = 0.326; $p < 0.001$) in the BCL group. There was a tendency towards a reduction in the duration of NCD in the BCL group (2.25 (±1.7) days vs. 2.88 (±2.7) days in the placebo group) ($p = 0.052$). Furthermore, no calves died in the BCL group, whereas four calves died in the placebo group.

Limitations: Because of the design of the study using animals in practice, the mechanisms explaining the clinical findings remain as hypotheses. Diarrhoea scoring performed by farmers has to be analysed and interpreted with caution.

Conclusions: This study demonstrates that BCL as a single preventive treatment is effective in reducing the incidence of NCD even in a region with good general management of dairy calves and overall good colostrum quality.

INTRODUCTION

Neonatal calf diarrhoea (NCD), defined as diarrhoea in the first month of life, is a health and welfare problem on dairy and beef farms worldwide and is caused primarily by *Escherichia coli*, rotavirus (BRV), coronavirus (BCoV) and *Cryptosporidium parvum*. The risk of diarrhoea for calves is highest in the first week of life, subsequently decreasing with age.[1] NCD has a negative impact on animal welfare and is the main cause of mortality in many cattle-rearing countries.[2–4] For US dairies, the 2007 National Animal Health Monitoring System reported that diarrhoea or other digestive problems accounted for the majority of preweaned heifer deaths (56.5%). Of all preweaned heifers, 23.9% suffered from diarrhoea and 17.9% were treated with antibiotics against diarrhoea.[5]

In order to reduce the use of antibiotics in animal production, disease prevention is becoming increasingly important. A common way to prevent diarrhoea is to vaccinate dams to produce colostrum with higher levels of specific antibodies (AB). In certain situations, alternative methods with fewer constraints can help farmers to achieve similar protection. One option to prevent NCD is to provide specific AB against the main pathogens involved in NCD directly to the calf early after birth. The advantage of direct supplementation of specific AB before colostrum over colostrum alone is that the quantity and quality of specific immunoglobulin G (IgG) received by the calf

is guaranteed, providing immediate local protection. The major risk factor for the development of NCD is the failure of the passive transfer (FPT) of maternal immunity.[6] Lombard et al.[7] proposed a more precise definition of this status as failure of transfer of passive immunity (FTPI), which will be used in our study.

Due to the placental structure in ungulates, maternal immunoglobulin (Ig) cannot cross the placenta. Consequently, calves are born agammaglobulinemic and are entirely dependent on an early administration of high-quality colostrum to ensure adequate passive transfer of Ig. Cessation of macromolecule absorption (closure) occurs at approximately 24 hours postpartum. An IgG concentration of less than 10 mg/mL in a 24–48-hour-old calf indicates an FTPI from the dam to the calf.

The study's primary objective was to evaluate the efficacy of the application of bovine concentrated lactoserum (BCL) given orally to the calves in addition to normal colostrum to prevent NCD under practical conditions. A second objective was to monitor and analyse risk factors and their association with calf health during the first 2 weeks of life. To our knowledge, the BCL formulation used in this study is the only approved product for ruminants containing guaranteed high titres of specific AB against *E. coli*, BRV and BCoV, which allows its testing under practical farming conditions. Our hypothesis was that BCL is efficient in preventing NCD caused by *E. coli*, BCoV and BRV and helps calves reach higher Ig titres than calves in the placebo group.

MATERIALS AND METHODS

This study was carried out between November 2016 and October 2017, and all procedures were conducted in accordance with the guidelines of the Swiss Law on Animal Protection and approved by the responsible Veterinary Office in Brunnen, Switzerland (permit for animal experimentation SZ-28499/16).

Selection and sampling of calves

Calves were selected from 35, mostly small, commercial herds serviced by one veterinary practice in central Switzerland. Only herds without a history of vaccination against NCD were included. Herd size ranged from 14 to 70 dairy cows. The median number of included calves was 14 (range 6−22 calves per herd being part of our study). The sample size calculation was made with the method proposed by Rollin (www.stat.ubc.ca/~rollin/stats/ssize/b2.html). A priori estimations were an expected diarrhoea incidence of 10% in the treated group versus 20% in the control group. This resulted in a group size of 199. In previous analyses (unpublished), we found an intraherd correlation coefficient of about 20%. Therefore, we augmented our sample size by 80 to account for this potential outcome. A total of 489 calves were included: 246 in the BCL group (group A) and 243 in the placebo group (group B). The BCL (Locatim, Biokema) is an oral solution manufactured from the colostrum of cows hyperimmunised against *E. coli* K99/F5, BRV and BCoV. It is a BCL containing high levels of specific IgG to be given orally to the calf during the first hours of life. The placebo consisted of phosphate-buffered saline (PBS). As the PBS buffer is colourless, an industrial food-grade dye was added to obtain the slightly brown colour characteristic of BCL. Both products had comparable viscosities. The treatments were filled in anonymised bottles marked 'A' or 'B' in order to blind the allocation for the farmers and the veterinarians of the practice.

Within each herd, as calves were born, they were alternatingly allocated to receive either 60 mL of BCL or 60 mL of placebo orally before colostrum within the first 6 hours of life. The sequence was fixed within the herd in order to have a similar distribution of treatments during the whole period, as climate and infectious pressure can change with changes in season. The start of the treatment series with A or B was randomly chosen by the flip of a coin.

As soon as possible after birth, every calf was given colostrum from their dam by bottle according to the usual practice of the farm. Simultaneously, a sample of the colostrum was taken by the farmer and stored in the freezer. After 36–48 hours of life, a serum sample was taken from each calf by a veterinarian. These samples were centrifuged as soon as possible and then stored together with the colostrum sample in a freezer until all samples were collected. Meanwhile, the farmer recorded the occurrence of diarrhoea during the first 14 days of life using a clinical scoring form on the calf sheet. The diarrhoea score was defined according to the following criteria: 0 = no diarrhoea; 1 = light diarrhoea without medical treatment; 2 = diarrhoea requiring oral treatment (rehydration and/or antibiotic therapy); and 3 = severe diarrhoea requiring parenteral rehydration or resulting in death. To ensure consistency between farmers in recording these diarrhoea scores, we informed and trained all the participating farmers during an information event. Furthermore, we trained each of them during a visit on the farm and discussed the procedure as well as the importance of the calf sheet, which had to be filled out by the farmers each day during the calves' first 14 days of life.

Furthermore, farmers were advised to take a sample of the faeces of those calves requiring treatment before treating their calves and to bring it to the veterinary practice the same day. There was no faecal sample planned from calves with a diarrhoea score of 1 for practical and economic reasons. Despite that, four samples were taken from calves with a score of 1, and as they were equally distributed between treatment groups, they were included in the analysis. Collection pots (25 mL) were provided and had to be filled directly from the rectum of the calf or collected as calves were passing faeces to avoid contamination and to surely identify the calf. Faecal samples were refrigerated (2°C–8°C) until delivery to the veterinary practice.

Finally, the duration of diarrhoea was recorded in full days. According to the breed of the dam, the calves included in this study were grouped into dairy breeds (Brown Swiss, Holstein and Jersey) and dual-purpose breeds (Brown Swiss crossbreds, Braunvieh, Original Braunvieh, Eringer, Swiss Fleckvieh and crossbreds). All calves were housed in individual pens during the first 14 days and given milk by bottle (first two to three feedings) and from buckets.

Laboratory analysis

Faecal samples from calves with diarrhoea were analysed for the presence of *E. coli* K99/F5, BRV, BCoV and *C. parvum* at the practice's laboratory using the Speed V-Diar 4 test (Bio Veto Test, Virbac). Meanwhile, the total protein (TPROT) in each serum and colostrum sample was measured with an auto-analyser (Cobas-Mira, Hoffmann-La Roche) using a commercial kit (no. AXMJ00067, Axon Lab).

Total IgG was measured in each colostrum and serum sample using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Bovine IgG ELISA Quantitation Set; Bethyl Laboratories), which was specially adjusted for measurements in colostrum and calf serum as previously described.[8] Colostrum samples were thawed at room temperature and serially diluted in ELISA wash buffer (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, adjusted to pH 8.0) to final dilutions of 1:800,000. The interassay coefficient of variation (CV) was 5.7%, and the intra-assay CV was 6.0%. The same procedure was used for serum samples; the final dilution was 1:300,000. The interassay CV was 6.8%, and the intra-assay CV was 5.3%. Colostral and serum IgG concentrations were expressed in mg/mL. IgG levels were categorised according to the recommendation of Lombard et al.[7]: excellent, good, fair and poor, corresponding to serum IgG levels of 25.0 g/L or more, 18.0–24.9 g/L, 10.0–17.9 g/L and less than 10 g/L, respectively. Calves were classified as having FTPI (category 'poor') if their serum IgG was less than 10 mg/mL, which is equivalent to the 'older' definition of FPT.[9]

Specific AB against *E. coli* K99/F5 were determined by means of microagglutination of surface bacterial antigens. Purified K99/F5 antigen (produced by Biokema) was mixed with different dilutions of the serum to be tested in a microagglutination plate and incubated for 18 hours at 37°C. Each plate contained both an antigen control (positive control) and a negative serum control. After incubation, the plates were read using a binocular microscope with a magnification of 10×. The results were expressed in terms of the final dilution of serum, showing the complete agglutination of bacterial antigen. A partial agglutination was considered to be negative.

Specific AB against BRV were determined by means of ELISA. Positive antigen (BRV) and negative antigen (mock infection) were coated directly onto the plate overnight at 5°C. Dilutions of the serum to be tested were then incubated for 1 hour at 37°C. A goat anti-bovine horseradish peroxidase conjugate (Biomol) was used to determine the degree of binding of specific BRV AB. The reaction of horseradish peroxidase with its substrate was stopped after 15 minutes with sulphuric acid (13.4%), and the optical density (OD) at 490 nm was read for each well. The difference in OD between positive and negative antigens was determined for each dilution of the test sample, and the titre of the test sample was determined at the nearest point of $\Delta OD \geq 0.3$. Positive and negative controls were included in each assay to validate the test.

Statistical analysis

Data management was performed with Microsoft Excel. Statistical analysis was carried out with SYSTAT 13 (version 13.00.05; SYSTAT software). To account for the clustering of calves within herds, we performed a hierarchical two-level (HERD/CALF) multivariable logistic regression analysis with the software MLwiN (MLwiN version 3.06, 2022, Centre for Multilevel Modelling).

Diarrhoea scores were considered as categorical data. The score was registered by the farmer every day for the first 14 days of life, and the highest score registered during these 14 days was used in the statistical analysis. Univariate chi-square analysis was performed with diarrhoea scores 0–3 against all explanatory variables (treatment of the calf, sex of the calf, breed of the dam, parity of the dam, parturition, vitality of the calf, suckling behaviour of the calf, additional supply of the calf with, e.g., iron or selenium, season of parturition, timing of the first colostrum feeding, volume of colostrum fed at the first four meals, number of feedings at blood sampling). If the number of observations was smaller than five, some levels of the explanatory variables were regrouped.

In a second step, in order to reduce the potential inaccuracy of farmer observations, diarrhoea scores were coded 0 for scores 0 and 1 (no veterinary intervention) and 1 for scores 2 and 3 (with veterinary intervention) and tested with a chi-square analysis against the regrouped levels of the abovementioned explanatory variables.

Table 1 shows all the explanatory variables that were analysed and offered to the multivariable model. For the multivariable logistic regression analysis, all explanatory variables with a univariate chi-square *p*-value less than 0.15 were included in the starting model. Non-significant variables were successively withdrawn with stepwise backwards elimination, taking the explanatory variable with the highest *p*-value out of the analysis until only variables with a *p*-value less than 0.05 remained in the model.

For the continuous variables TPROT and total IgG, the means of the BCL versus placebo groups were compared with a Student's *t*-test. TPROT and colostrum IgG were also categorised according to the

TABLE 1 Explanatory variables analysed and initially offered to the multilevel (HERD/CALF) multivariable logistic regression model

Variable	Definition	Levels	Explanation of the levels
TREATMENT	Applied treatment (double-blinded)	BCL	Bovine concentrated lactoserum (Locatim)
		Placebo	Placebo (coloured phosphate-buffered saline)
SEX	Sex	Female/male	
BREED	Breed of the cow	Dairy	Brown Swiss, Holstein, Jersey
		Mixed	Braunvieh, Fleckvieh, Eringer and crossbreds
PARITY	Parity of the mother	1/2/3/4/5/6+	
PARTURITION	Parturition process	Normal	Spontaneous birth
		Dystocia	Birth with assistance
		Twins	Twin birth
VITALITY	Vitality of the newborn calf (observation of the farmer)	Good	Gets up and comes spontaneously to feeding
		Reduced	Has to be sought out by the farmer and needs some time to come to him
SUCKLING	Suckling behaviour of the calf (day 1) (observation of the farmer)	Good	Drinks in one gulp
		Medium	Interrupts drinking and needs more time
		Absent	Does not drink spontaneously
SEASON	Season of parturition	Winter/grazing	Detention of the herd in the barn/on pasture
COLO TIME	Time to first colostrum feeding	0–1/2–3/4–6/>6	In hours
NUM FEED	No. of feedings at blood sampling	3/4/5/6+	
TPROT	Total protein in calf serum	Continuous	In g/L
TPROT CAT	TPROT categories	Low/normal/high	<45/46–60/>60 g/L
IGG CALF	IgG in calf serum	Continuous	In g/L
IGG CAT	IGG CALF categories	Excellent/good/fair/poor	≥25.0/18.0−24.9/10.0−17.9/<10
IGG COLO	IgG in colostrum	Continuous	In g/L
IGGCOL CAT	IGG COLO categories	Low/normal/high	<50/51–100/>100 g/L
log K99/F5	Specific AB K99/F5 in calf serum	Titres (microagglutination)	Logarithmic transformation
K99/F5 CAT	K99/F5 categories	Low/normal/high	<2.1/2.2–2.4/>2.4
log ROTA	Specific AB against BRV in calf serum	Titres (ELISA)	Logarithmic transformation
ROTA CAT	ROTA categories	Low/normal/high	<3.4/3.5–3.9/>3.9

Abbreviations: AB, antibodies; BCL, bovine concentrated lactoserum; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.

quartiles of the sample. Both the continuous and the categorised variables were offered to the multivariable logistic regression model in two separate models. Furthermore, serum IgG levels were categorised according to the recommendation of Lombard et al.,[7] mentioned above in the laboratory analysis section. The association of this new variable with the occurrence of diarrhoea was also tested, both in a univariable chi-square test and as part of the multivariable logistic model.

The titres of the specific AB against *E. coli* K99/F5 and BRV were logarithmically transformed, and the means of the BCL versus placebo groups were compared with a Student's *t*-test. As the transformed variables were still slightly skewed, an additional comparison of the median was performed with a non-parametric Mann–Whitney analysis. These two variables were also categorised according to the quartiles of the sample. Both the continuous and the categorised variables were offered to the multivariable logistic regression model in two separate models.

The duration of the diarrhoea episodes was compared between groups with a Student's *t*-test. As this duration was only registered for full days, it can be argued that this variable cannot be considered to be continuous. Therefore, the difference between groups was also evaluated with a non-parametric Mann–Whitney analysis. Meanwhile, the difference in the number of deaths between groups was tested with Fisher's exact test.

RESULTS

Colostrum

Out of a total of 469 analysed colostrum samples, 388 (82.7%) had good quality (IgG ≥ 50 mg/mL). The mean IgG concentration in colostrum was 84.3 mg/mL (SD, 41.7 mg/mL; minimum, 2.1 mg/mL; maximum, 306 mg/mL). In our study, 126 colostrum samples were collected from primiparous cows (25.8%) and 363 were collected from pluriparous cows (74.2%). Farmers indicated that 174 calves (35.7%) were fed their first colostrum within 1 hour, 192 calves (39.3%) within 1–3 hours and 90 calves (18.4%) within 3–6 hours after

TABLE 2 Distribution of cases and controls between treatment groups

	NCD outcome		
	0	1	Total
Treatment			
BCL	225	21	246
Placebo	199	44	243
Total	424	65	489

Note: 0 = no/light NCD (scores 0 and 1). 1 = moderate/severe NCD (scores 2 and 3). Crude odds ratio = 0.422 ($p = 0.002$, Chi-square test).
Abbreviations: BCL, bovine concentrated lactoserum; NCD, neonatal calf diarrhoea.

TABLE 3 Distribution of failure of transfer of passive immunity (FTPI) categories between treatment groups

	FTPI categories[a]				
	Excellent	Good	Fair	Poor	Total
Treatment					
BCL	103 (42.0%)	58 (23.7%)	58 (23.7%)	26 (10.6%)	245
Placebo	104 (42.8%)	58 (23.9%)	51 (21.0%)	30 (12.3%)	243
Total	207	116	109	56	488

Note: Chi-square test (Treatment against FTPI categories) is non-significant ($p = 0.866$).
Abbreviation: BCL, bovine concentrated lactoserum.
[a]According to Lombard et al.[7]

birth. Out of all calves, 32 (6.6%) received colostrum later than 6 hours after birth.

Animals, enteropathogens and explanatory variables

In 28 of 35 herds (80%), at least one calf with diarrhoea was observed. A total of 138 calves (28.2%) suffered from diarrhoea during our study, and 65 (13.3%) showed moderate to severe signs of diarrhoea (scores 2/3) requiring treatment. Four calves died in the first two weeks of life while being observed (0.82% mortality), three of them had severe diarrhoea (score 3), and the fourth had absent suckling activity from the first day of life. All four calves received the placebo. The distribution of NCD cases between treatment groups is shown in Table 2. A total of 488 blood samples were analysed (one blood sample was missing), and 56 of them (11.5%) had FTPI (serum IgG < 10 mg/mL after at least three feedings). The mean IgG concentration of the calf serum samples was 24.9 mg/mL (SD, 13.7 mg/mL; minimum, 0.75 mg/mL; maximum, 86.5 mg/mL). The distribution of FTPI categories between treatment groups is shown in Table 3. For all the calves that had any sign of diarrhoea (scores 1–3), the mean (±SD) duration of diarrhoea episodes was 2.25 (±1.7) days in the BCL group and 2.88 (±2.7) days in the placebo group ($p = 0.052$, Student's t-test). For the non-parametric analysis, the results were comparable: median BCL group = 2 days (interquartile range [IQR] 1–3); median placebo group = 3 days (IQR 1–4) ($p = 0.058$, Mann–Whitney U-test).

During our study, a total of 69 faecal samples were taken and analysed: no enteropathogen was found in

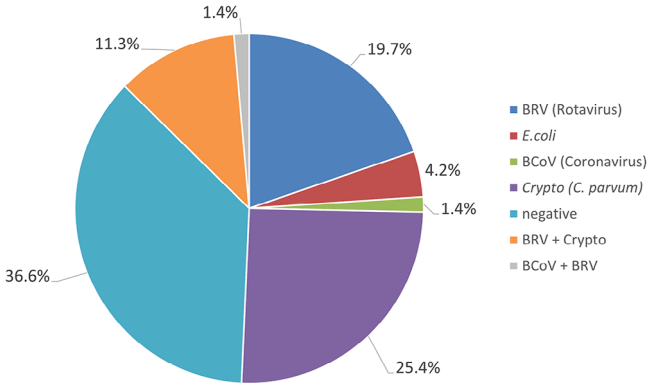

FIGURE 1 Pathogens found in the faeces of the calves with diarrhoea

24 samples, 20 were positive for *C. parvum*, 13 were positive for BRV, eight were positive for *C. parvum* combined with BRV, three were positive for *E. coli* and one was positive for BCoV. There was no statistically significant difference between the treatment groups. Figure 1 shows the distribution of the pathogens found in the faecal samples.

The univariable logistic regression for the relationship between the diarrhoea category and the explanatory variables shown in Table 1 identified a significant association for three variables: treatment, season and breed of the dam. These results are shown in Table 4.

Specific antibodies

Titres of the specific AB for *E. coli* and BRV were analysed in all blood samples. The values are shown in Table 5. No difference was found between groups.

Final model

The results of the multilevel (HERD/CALF) multivariable logistic regression analysis identified the variables treatment (odds ratio [OR] 0.326, $p = 0.001$), breed of the dam (OR 3.900, $p = 0.012$) and season (OR 0.188, $p = 0.033$) to have a significant association with the prevalence of diarrhoea in the calves in our study. Our final model is shown in Table 6. The herd factor was significant ($p = 0.009$), but the intraclass correlation coefficient (ICC) was 19.3%. The ICC of 19.3% indicates that the animals within herds are not fully independent; that is, the risk of getting NCD within herds is different among herds. Furthermore, it demonstrates that correct analysis did require fitting of a multilevel model. The Hosmer–Lemeshow statistic with a very high p-value and the high area under the curve value indicate a high goodness of fit of the model.

Non-significant variables in the final model

The analysis of the data regarding the sex of the calf, parturition, parity of the dam, timing of the first

TABLE 4 Results of the univariable logistic regression for the relationship between the diarrhoea category and the explanatory variables

Variable level	Odds ratio	*p*-Value	95% Confidence interval	
			Lower	Upper
Treatment placebo	1	–	–	–
Treatment BCL	0.422	0.002	0.24	0.73
Season winter	1	–	–	–
Season spring	0.192	0.004	0.05	0.81
Breed mixed	1	–	–	–
Breed dairy	3.030	<0.001	1.59	5.88

Note: Placebo—phosphate-buffered saline. Winter—the period during which the herd was held in the barn. Spring—the period during which the herd was held on pasture. Mixed—breed of the dam = Braunvieh, Eringer, Swiss Fleckvieh and crossbreds. Dairy—breed of the dam = Brown Swiss, Holstein or Jersey.
Abbreviation: BCL, bovine concentrated lactoserum.

TABLE 5 Titres of specific antibodies in blood samples from calves at 36–48 hours old

	log 10 *Escherichia coli* K99/F5[a]	log 10 rotavirus[b]
Mean (±SD) BCL group	2.22 (±0.42)	3.64 (±0.41)
Median (interquartile range) BCL group	2.40 (2.10–2.40)	3.68 (3.42–3.90)
Mean (±SD) placebo group	2.23 (±0.40)	3.65 (±0.41)
Median (interquartile range) placebo group	2.40 (2.10–2.40)	3.68 (3.42–3.91)
p-Value mean (Student's *t*-test)	0.751	0.790
p-Value Kruskal–Wallis	0.609	0.881

Abbreviation: BCL, bovine concentrated lactoserum.
[a]Measured by microagglutination.
[b]Measured by enzyme-linked immunosorbent assay.

colostrum feeding, IgG concentration of colostrum, IgG and TPROT plasma concentrations, four levels of FTPI as proposed by Lombard et al.,[7] and plasma titres of specific AB against *E. coli* and BRV showed no significant association with the presence of diarrhoea in calves in our study.

DISCUSSION

In our study, oral treatment with a concentrate of specific Ig against *E. coli*, BRV and BCoV effectively reduced the occurrence of NCD compared to a placebo group. As no such study regarding BCL has been conducted so far, there are no results to compare. To our knowledge, BCL is the only approved product for ruminants containing guaranteed high titres of specific AB against *E. coli*, BRV and BCoV. There have been no double-blinded studies with other products so far.

Another tool for disease prevention is the vaccination of the dam before calving to increase the level of specific AB in colostrum.[10–12] As the efficacy reports of these vaccines are variable and data on economic benefits are lacking,[4] BCL surely can be considered a valuable complement or enhancement to maternal vaccination. Crouch et al.[10] summarised the improved passive immunity of calves after vaccinating their dams and better overall herd immunity.

Compared to vaccination of the dam, the use of BCL has several potential advantages. First, the treatment is not as labour intensive for the farmer as vaccination. It is quick and simple, and the oral administration of specific AB is safe and guaranteed. Second, it allows the product to be used as a preventive tool in herds where vaccination is not possible or not desired. Finally, it can also help vaccinated herds strategically protect high-value calves or allow targeted use to boost protection for calves with specific risk factors such as dystocia or caesarean section, failure to suckle, cows with insufficient colostrum quantity or quality, etc. With regard to the economic aspect of using this product compared to vaccinating the mother, an evaluation model developed by Biokema demonstrates that when considering factors such as the time commitment involved in coordinating and executing vaccinations, along with scenarios where vaccinations have yielded no significant results (e.g., late abortions, malformations, stillbirths), or proven to be ineffective (FTPI), the return on investment stands as comparable, if not superior, when using BCL.

This study's findings have even more importance considering that diarrhoea or other digestive problems are responsible for 56.5% of preweaned heifer deaths in the United States.[12] As infectious organisms are ubiquitous and holoendemic, being present within the gastrointestinal tract of some, if not most healthy, mature cattle, albeit at low concentrations and without clinical signs of infection,[13] this treatment seems to be an easy and effective way to reduce the impact of NCD. This is especially true when considering the economic impact of NCD due to mortality, poor growth rate and costs caused by diagnosis, treatment and control.[14]

Despite a wide variation, the mean blood IgG concentration in our study was higher (24.9 g/L) than that in a similar study conducted in Switzerland by Reschke et al.,[15] which found a mean serum gamma globulin concentration of 12.3 g/L (SD 6.7 g/L). This can be explained by good colostrum management in these rather small herds.

Calves from dairy cows had greater odds of getting NCD than calves from dual-purpose cows. This association could be explained by the higher IgG concentration in the colostrum of beef cows reported by

TABLE 6 Final model of the two-level (HERD/CALF) multivariable logistic regression analysis

Variable level	Odds ratio estimates			95% confidence interval	
	Odds ratio	Standard error	*p*-Value	Lower	Upper
Treatment placebo	1	–	–	–	–
Treatment BCL	0.326	0.093	<0.001	0.236	0.449
Season winter	1	–	–	–	–
Season spring	0.188	0.088	0.033	0.086	0.411
Breed mixed	1	–	–	–	–
Breed dairy	3.900	1.562	0.012	2.261	6.482

Note: Placebo—phosphate-buffered saline. Winter—the period during which the herd was held in the barn. Spring—the period during which the herd was held on pasture. Mixed—breed of the dam = Braunvieh, Eringer, Swiss Fleckvieh and crossbreds. Dairy—breed of the dam = Brown Swiss, Holstein or Jersey. The multilevel hierarchical model takes into account the clustering of calves within herds. The herd effect was significant ($p = 0.009$), but the intraclass correlation coefficient was 19.3%. Hosmer–Lemeshow statistic = 3.638 ($p = 0.820$); area under the curve of the receiver operating curve = 0.905.
Abbreviation: BCL, bovine concentrated lactoserum.

Guy et al.[16] as well as Muller and Ellinger.[17] Genetics and/or dilution effects can be a reason for this. In contrast, Kessler et al.[18] clarify that colostrum quality of dairy cows is not generally poorer compared to double-purpose cows.

Calves born during the grazing season had reduced odds of getting NCD compared to calves born during the winter months. In the grazing season, we experienced better climatic conditions and less overcrowding, which were responsible for this association. According to Godden et al.,[19] the relationship between the season of calving and colostrum quality or volume remains unclear.

Colostrum

Due to the placental structure, maternal Ig cannot traverse the placenta. Therefore, the calf entirely depends on the supply through colostrum.[20–25] Inadequate intake of colostrum was repeatedly identified as a reason for the development of NCD.[26–28]

A possible cause for the non-significant differences in specific AB concentrations between the groups in our study could be the high quality of colostrum. High-quality colostrum is traditionally defined by IgG concentrations greater than 50 g/L.[19,20] In the present study, more than 80% of colostrum samples (388 of 469) complied with this quality standard. With 17.3% of all colostrum samples below the cutoff of 50 g/L, we found a similar proportion of cows with inadequate colostrum quality as a study published by Kessler et al.,[18] which found 15.3% of colostrum samples with inadequate quality. The same study[18] showed that the colostrum of primiparous cows can also be of sufficient quality, which could be a possible explanation for the non-significant association between the parity of the dam and the presence of diarrhoea in calves in our study. In other studies, the proportion of cows with adequate colostrum quality varies between less than 50%[29] or even 30%[30] and values above 90%.[31,32]

According to different studies, colostrum quality is affected by the time of collection after calving.[33,34] The highest Ig concentrations were observed by Kessler et al.[18] in colostrum milked within 3 hours postpartum, supporting the objective to milk cows as soon as possible after calving to ensure high quality of colostrum. Other studies[32,35,36] found that there was no negative influence of the timing of the first milking on volume and IgG concentration up to 9–12 hours postpartum.

Previous studies found an association between the age of the calf at first colostrum feed (in hours) and the occurrence of FTPI/NCD. However, the results of the present study did not show a significant association between the timing of the feeding of the first colostrum meal or the concentration of IgG in colostrum and the occurrence of NCD. This may be due to the previously mentioned general good colostrum quality and the fact that farmers in our region regularly observe cows during parturition and collect colostrum as soon and as hygienically as possible. Of the calves in our study, 75% received their first colostrum within 3 hours, and about 36% received their first colostrum within 1 hour. This is of elementary relevance as the efficiency of Ig transfer across the gut epithelium is optimal in the first 4 hours postpartum, progressively declining from 6 hours after birth.[37,38] Cessation of macromolecule absorption (closure) occurs at approximately 24 hours postpartum. If the feeding of colostrum is delayed, closure may be extended to 33 hours for IgG.[39] With 11.5% of all calves suffering from FTPI in our study, the percentage of calves with FTPI was lower than in other studies conducted in Switzerland. Reschke et al.[15] reported 43.5%, and Lejeune et al.[40] reported 43% of calves in their studies suffered from FTPI. In the study of Reschke et al.[15] a colostrum IgG concentration of less than 50 g/L for the first feeding was associated with the highest odds for FTPI (OR 10.7; 95% confidence interval [CI] 4.7–24.2; $p < 0.001$). In addition, the timing of the first feeding was significantly associated with risk of FTPI, with calves fed colostrum later than 6 hours after birth at higher risk for insufficient serum IgG concentrations (OR 3.1; 95% CI 1.1–8.6; $p = 0.035$). Lejeune et al.[40] also found that feeding calves their first colostrum more than six hours after birth led to a higher risk of FTPI (OR 7.4; 95% CI 1.4–39.5; $p = 0.02$). The low rate of calves suffering from FTPI in our study may be an effect of high-quality colostrum and management during our study; for example, only

6.6% of all calves received their colostrum more than 6 hours after birth.

Specific antibodies

Despite the clinical difference between treatment groups, specific AB against BRV and *E. coli* were not significantly associated with the treatment applied in our study. One hypothesis is that the clinical difference between the groups was caused by the specific IgG present in the gut before the first contamination to support local immunity in the very early phase. Chase and Kaushik[41] describe a mucosal barrier that consists of mucous and mucins, antimicrobial peptides and IgA. They call this barrier the 'kill zone', which prevents microbial invasion of the epithelium. Crouch et al.[42] mentioned the continuing protective effect of Ig in the gut lumen of the calf. Another possibility is that the high level of the concentration of specific AB is achieved earlier in the BCL group, leading to more efficient protection against early infection through secretion back to the epithelial cells and the 'kill zone'. This would have to be looked at in a study over time with repeated measures. Furthermore, we can speculate that the clear study design and the experience of the farmers concerning the timing of colostrum collection/feeding and nursing of the calves make the differences in specific AB concentrations non-significant.

Limitations

Despite the overall very good quality of colostrum in this study, there was a very wide range of values. This could be explained by the fact that the colostrum samples were taken by the farmers, possibly after the first milking. This variation could explain why colostrum IgG concentration was not statistically significant in the multivariable logistic regression model. However, we also included the calf serum total protein and IgG concentration in the analysis, without significant results. Despite these findings, colostrum management, especially the control of IgG content, remains a main focus of good practices of calf rearing.

Another factor to be considered is that diarrhoea scoring was performed by the farmers. We trained the veterinary staff as precisely as possible, and in turn, the veterinarians trained the farmers at their enrolment. Although the scoring system was designed to be as objective and precise as possible, scoring keeps a touch of subjectivity, and it has to be analysed and interpreted with caution. One measure to reduce potential misclassification, especially in the area of light diarrhoea, was to regroup scores 0–1 and 2–3.

Furthermore, we did not assess the hygienic quality of the colostrum, which is known to have a large impact on IgG resorption and immune protection. However, we are confident that these small herds with their good management did their best to work properly. The relatively low incidence of diarrhoea seems to confirm this impression, but in further studies, the hygienic quality of the colostrum will have to be included in the datasets.

Finally, only 69 faecal samples from 138 calves suffering from diarrhoea could be analysed. No statistically significant difference was found between the treatment groups, but it remains uncertain whether this would have been the case if samples from all the diarrhoeic calves had been analysed. However, we tend to think of NCD as a syndrome rather than a disease caused solely by enteropathogens. There was a clinical benefit to receiving BCL despite the fact that some of the samples revealed pathogens other than the target ones or no pathogen at all.

CONCLUSION

The results of this study show that the administration of BCL containing specific IgGs against *E. coli*, BRV and BCoV is effective in preventing NCD. However, the mechanisms of action through a local effect on the intestinal immune system remain as hypotheses, as it was not possible to verify this in our setting using commercial herds. The longer-term impact of the application of a BCL on the gut microbiome and overall calf health must be addressed in future studies.

AUTHOR CONTRIBUTIONS

Study design, data collection, data analysis and preparation and correction of the manuscript: Olivier Nussbaum. *Laboratory analysis, supervision of the study and review of the manuscript*: Josef J. Gross and Rupert M. Bruckmaier. *Study design, data analysis, statistical analysis and review of the manuscript*: Richard Eicher.

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CONFLICT OF INTEREST STATEMENT

The research being reported in this paper was supported by Biokema, a company with financial interest in the subject matter discussed in this manuscript. The last author (Richard Eicher) is a part-time employee of Biokema, which produces the bovine concentrated lactoserum used in this study.

DATA AVAILABILITY STATEMENT

Study data are available through the corresponding author (subject to written confidentiality agreement).

ETHICS STATEMENT

All procedures in this study were conducted in accordance with the guidelines of the Swiss Law on Animal Protection and were approved by the responsible Veterinary Office in Brunnen, Switzerland (permit for animal experimentation SZ-28499/16).

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