A novel vaccine demonstrating prevention of neonatal calf diarrhoea

MATT YARNALL[1]*, EDMOND JOLIVET[2], MATHIEU CHEVALIER[2], BENJAMIN HATAT[2], MARIE-PASCALE TIBERGHIEN[3], BARBORA MALYSKOVA[4], JURAJ KUCERAK[4]

[1] Boehringer Ingelheim Vetmedica GmbH, Binger Strasse 173, 55216 Ingelheim, Germany
[2] Boehringer Ingelheim, 29 avenue Tony Garnier, 69007 Lyon, France
[3] Consulting, 70 impasse de Floretille, 71250 Cortambert, France
[4] Bioveta, Komenského 212/12, 683 23 Ivanovice na Hané, Czech Republic

SUMMARY

A new maternal vaccine has been developed against neonatal calf diarrhoea associated with bovine rotavirus (BRV), bovine coronavirus (BCV) and Enterotoxigenic *E. coli* F5 (K99) (ETEC). This paper describes the results of 5 efficacy studies in target animals, conducted to gain Marketing Authorisation. Four studies were laboratory oral challenges (ETEC at 12 hours, BRV at 7 days, BCV at 7 and 14 days of age) in calves fed colostrum and milk for 7 days, from dams either vaccinated with a minimum potency vaccine, or placebo-injected, 11-12 weeks prior to expected parturition. One study was a field safety and efficacy study where pregnant cattle on a total of 3 farms were either vaccinated with a standard potency vaccine, 12 to 3 weeks prior to expected parturition, or left untreated. All animals were monitored - as well as their offspring, which received colostrum and milk from their dams - from 2 hours of age until 2 weeks post calving. The results of the challenge tests demonstrated that the vaccine successfully induced a significant and sustained antibody increase in cows and heifers to each vaccine component. Specific antibodies in colostrum and milk from the vaccinated dams transferred to calves, completely prevented clinical signs of diarrhoea after challenge from ETEC, BRV and BCV at 7 days, and significantly reduced their faecal virus shedding, compared with calves fed colostrum from placebo dams. In the field study, vaccinated animals showed a modest temperature increase (+0.69°C on average) for a limited period (1 day on average), compared with untreated cows. No adverse impact was observed on pregnancy outcomes. Calves born to vaccinated cows showed a significant increase of all specific antibodies, compared with calves from untreated controls. The novel, non-oil adjuvanted vaccine achieved very high levels of efficacy and safety in rigorous studies and provides vets and farmers with a new tool against neonatal calf diarrhoea.

KEY WORDS

Calf, scour, diarrhoea, prevention, Fencovis.

INTRODUCTION

Neonatal calf diarrhoea (NCD) is a multifactorial disease occurring in both dairy and beef breeding herds, with an estimated prevalence of 19-35% (1) incurring high costs to farmers through direct costs of deaths and care of sick animals, and indirect losses associated with delayed growth (2), higher age at first calving (3) and genetic losses. Risk factors for NCD include pathogen pressure in calf housing, management aspects and host immunity. Enterotoxigenic *E. coli* F5 (K99) (ETEC), Bovine rotavirus (BRV), Bovine coronavirus (BCV), Cryptosporidia and *Salmonella spp.* are recognised as the major pathogens associated with diarrhoea in neonatal calves. Disease occurs mostly within a few hours (ETEC) to a few days or weeks after birth (BRV and BCV) (3)(4).

Corresponding Author:
Matt Yarnall (matt.yarnall@boehringer.com)

While disease is often mild, impact on calf well-being is marked, as is the emotional impact on staff of treating sick calves. Treatment of NCD is often symptomatic and dependent on severity, through provision of oral or intravenous fluids and often antimicrobials. The justification for the use of antimicrobials in the treatment of diarrhoea in calves is not always clear. Many cases are viral in origin, however primary ETEC, *Salmonella spp.* or protozoal causes would justify antimicrobial treatment. As would calves affected with viral diarrhoea suffering overgrowth of *E. coli* which may often become systemic, showing systemic illness or severe gastrointestinal damage by the presence of diphtheritic membranes, mucus or blood in the diarrhoeic faeces (5).

The livestock industry is working to reduce the use of antimicrobials on farms owing to the possibility of increasing the antibiotic resistance of bacteria which infect both humans and animals. It also impacts seriously on the animal's gut microbiota (6).

Therefore prevention of disease should be the primary goal of

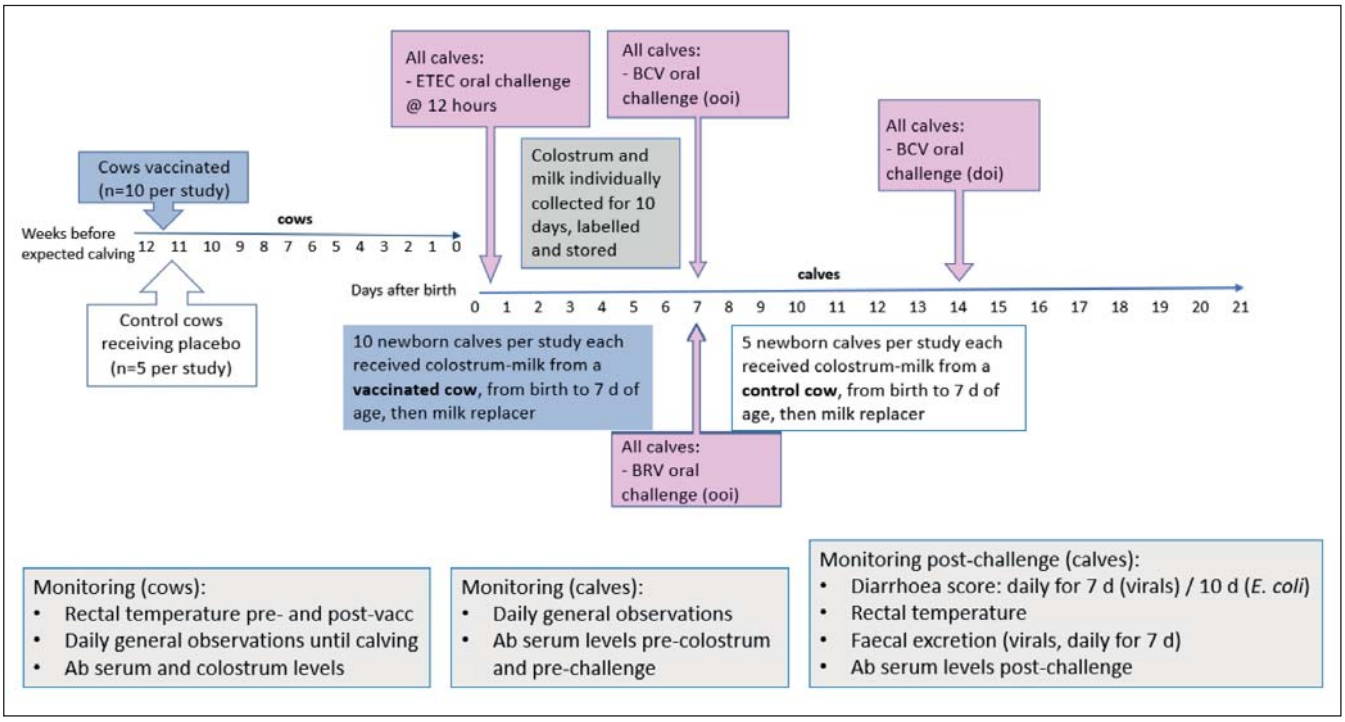


Figure 1 - Study design of the four cow vaccination-calf challenge studies.

calf management, through priming of the calf's immune system. There are however challenges due to the immature nature of the immune system and the early onset of disease in the calf's life. This means passive immunity is key to protection. However, due to the synepitheliochorial placentation in cattle (7), calves are born without the benefit of maternal antibodies and must acquire them from colostrum absorption.

The early onset of neonatal diarrhoea and the need for colostrum intake by calves have led to the development of maternal vaccines, administered to dams for the benefit of offspring after colostrum suckling.

This project sought to develop a new non-oil adjuvanted maternal vaccine to provide a higher level of protection for calves against BRV, BCV and ETEC than currently available solutions.

MATERIALS AND METHODS

Vaccination-Challenge Studies

The studies were carried out in accordance with the Act on An-

		Field safety and Efficacy (inactivated trivalent vaccine of standard potency)	Cow vaccination + calf challenge studies (inactivated trivalent vaccine formulated at minimal potency*)	Challenge strain/dose
Vaccine	**BRV**	BRV strain TM-91, RP ≥ 1 **	Strain TM-91, [G6P1] $10^{4.7}$ $TCID_{50}$/ ml	CAMP V-422 [G6P1], Vet. Res.Institute, Brno 10 ml orally of a solution containing $10^{5.8}$ $TCID_{50}$/ml
	BCV	BCV, strain C-197 RP ≥ 1 **	Strain C-197 $10^{5.0}$ $TCID_{50}$/ ml	Strain S379 Riems, Friedrich Loeffler Institute, Germany 10 ml orally of a solution containing $10^{6.8}$ TCID50/ml
	E coli	Escherichia coli adhesin (F5), RP ≥ 1 **	Enteropathogenic *E. coli* [O8:K99] 3.3 109 CFU/ ml	Enteropathogenic *E. coli* challenge strain, EC 8044 [O101:K99, F41], Vet. Res.Institute, Brno 10 ml orally of a solution containing 10^{10} CFU/ml
	Adjuvant	Aluminium hydroxide & Quil A		
	Preservative	Thiomersal		
Placebo		None used: control animals were left untreated	Phosphate Buffered Saline (PBS) + Aluminium hydroxide, Quil A and thiomersal	
* Minimal potency = the lowest possible dose of a culture at the highest passage level				
** RP = Relative potency (RP) is determined by serological method (ELISA); It is determined by comparing the level of antibodies in the serum of guinea-pigs vaccinated with the test vaccine with the level of antibodies in the serum of guinea-pigs vaccinated with the vaccine compliant with the challenge test on target animals.				

Table 1 - Vaccines and challenge strains used.

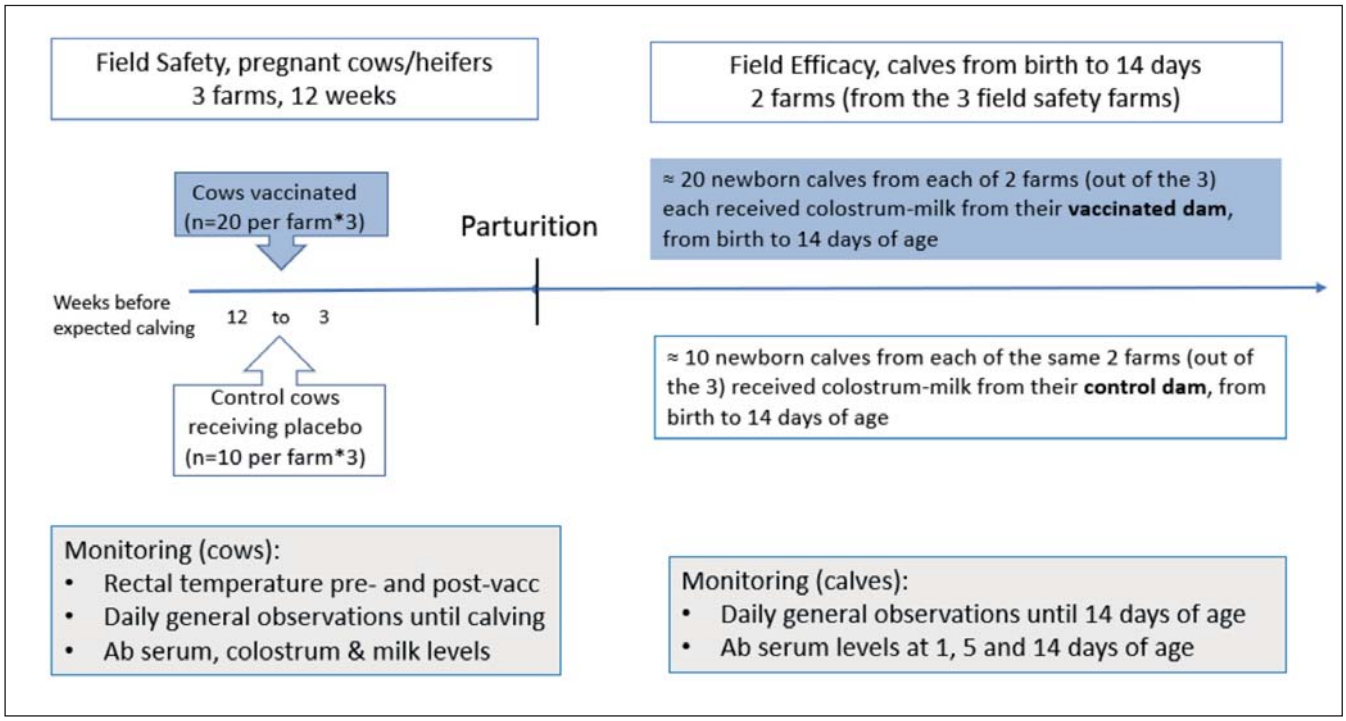


Figure 2 - Study design of field safety-efficacy study.

imal Health and Animal Welfare of The Czech Republic. The test sites used for purchase and challenge of the calves had accreditation allowing biological testing on animals. Any study procedure was carried out under this licence. All personnel involved in the in-life phase of the study were fully trained and provided with full details of the study, necessary instructions and support to ensure that the care and welfare of the animals were not compromised during the study period.

The clinical trial of the safety and efficacy of the test vaccine was approved by the Institute for State Control of Veterinary Biologicals and Medicines (ÚSKVBL) in Brno, to the extent specified in Clinical Trial Permit No. 003/2016/CT. During the study, the clinical trial was checked by the ÚSKVBL Brno on one of the farms and it was stated that the ongoing field trial was in accordance with the approved clinical trial protocol. During the study, the clinical trial was controlled by the clinical trial monitor and auditor of the sponsor Bioveta. a.s. All participating farmers had signed an informed consent form.

Four vaccination-challenge studies were conducted with a novel vaccine for ETEC, BRV and BCV (Fencovis/Biobos RCC, Bioveta, Ivanovice na Hané, Czech Republic) in cattle. The overall study design is shown in Figure 1.

For each study 15 pregnant cows and heifers were selected based on the number of animals required according to European Pharmacopeia monographs (01/2017:1954, 01/2017:0961, 01/2017:1953). The target animal are those most sensitive to the antigens, which are those that are seronegative or with low seropositivity. For this reason there was not an even balance of heifers and cows between studies, with no heifers in either of the BRV or BCV OOI studies, 2 hiefers in the ETEC study and 8 heifers in the BCV DOI study. Animals were housed in the same airspace on straw-covered concrete, previously unvaccinated, with no recent history of neonatal disease in the herd and with no significant difference in levels of initial antibodies to the components tested (BioX Diagnostics, Rochefort, Belgium. BIO K 126 for antibodies against BRV and BIO K 392 for antibodies against BCV). Animals received a single injection of vaccine (n=10) or placebo (n=5) at 12-11 weeks prior to expected delivery. Following delivery colostrum and milk were collected daily from each animal using a milking machine and vacuum pump into a stainless steel collection vessel and stored in 1–3l plastic bottles at -20 °C.

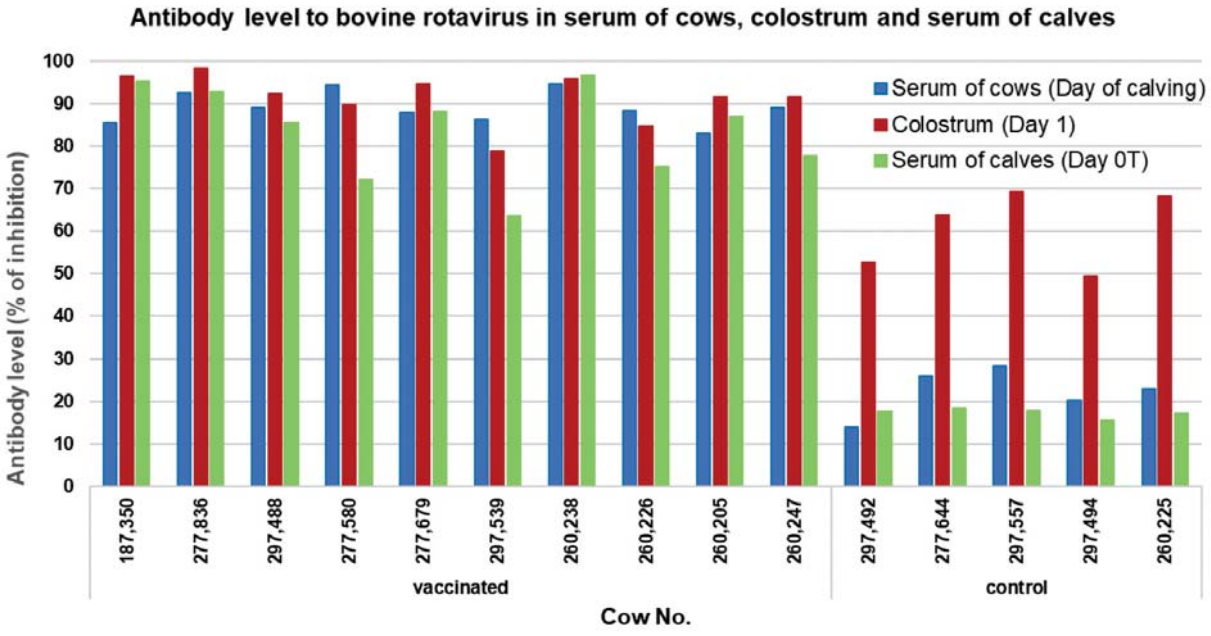
Fifteen newborn calves born weeks later to different dams were colostrum-deprived until they received their 1st colostrum meal (1.6l +/- 0.1l) from vaccinated or placebo cows within 6 hours of birth. These calves were selected by being of normal physical condition and without clinical symptoms of any disease. They were housed on straw in individual pens within the same building, and allocated to receive vaccine (n=10) or placebo (n=5) colostrum and subsequently transition milk for 7 days. Each calf received 3 daily feeds (4.5 - 9l/day) out of the previously collected colostrum or transition milk from a single dam for each calf. All calves - except those to be challenged with ETEC- also received a daily injection of amoxycillin + clavulanic acid from birth to 5 days of age, to protect against bacterial infection in these susceptible animals.

Three of the studies tested the onset of immunity (ooi) of each vaccine antigen component by oral administration to calves of a virulent suspension challenge, although by definition immunity is provided from the first colostrum feed. This was performed for BRV and BCV at 7 days of age and for ETEC at 12 hours of age, and the fourth study evaluated the duration of immunity (doi) of BCV by oral challenge in calves aged 14 days. Vaccine of minimal potency and placebo as well as challenge strains used in the studies are described in Table 1. Rectal temperature was monitored in pregnant females before vaccination and then 4 hours and once a day for 4 days thereafter. Antibody levels to the specific study pathogen (BRV, BCV and ETEC) were individually monitored by indirect competitive ELISA (BioX Diagnostics, Rochefort, Belgium. BIO K 126 for antibodies against BRV and BIO K 392 for antibodies against BCV) or non-competitive ELISA (ETEC, F5 antigen of en-

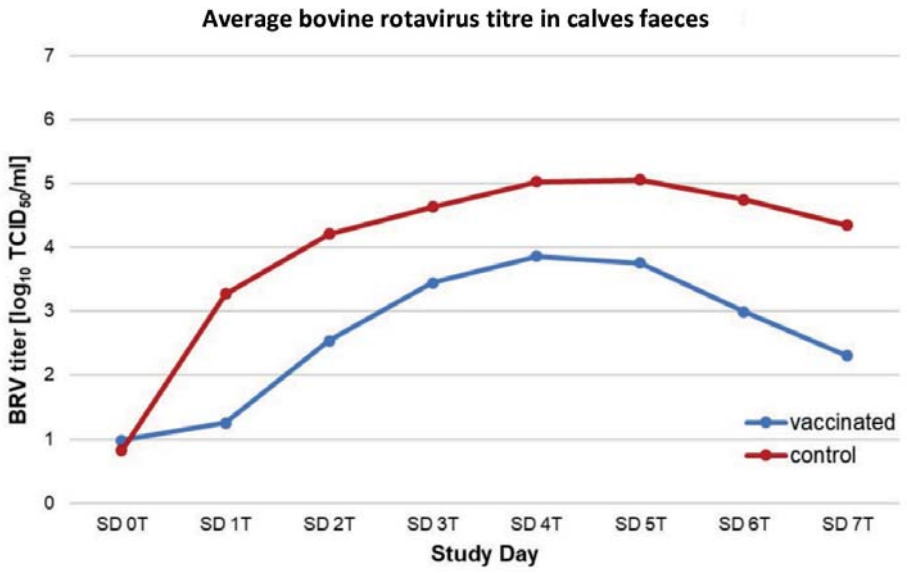
Figure 3 - Overview of calves diarrhoea symptoms in the four cow vaccination-calf challenge studies

teropathogenic *E. coli* in laboratory of Bioveta a.s. using indirect ELISA method) in dams' serum before and 3 weeks after vaccination and on the day of calving, as well as in colostrum and in milk from the day of calving until 10 days thereafter. Antibodies were also measured in calf serum immediately before challenge and at the end of the post-challenge observation period. After challenge the calves were monitored clinically for 7 days (viral studies) or 10 days (ETEC study), notably for signs of diarrhoea, using an adapted scoring system (8,9). In the BRV and BCV studies, individual rectal swab samples were collected from all calves to monitor viral shedding in faeces. Samples were taken directly from the rectum of each calf using cotton buds, then collected into sterile tubes prior to the challenge strain administration on Study Day 0T (7 days after birth of each calf) and then daily until Study Day 7T (7 days after challenge). Immediately after collection, 10% suspension of faeces in saline was prepared for the analysis. Each sample was labelled with the study number, sample identification and date of sampling. All samples were stored in a freezer at -80 °C prior to analysis. BRV and BCV were detected using an in-house laboratory using the reverse transcription method - quantitative real-time PCR (RT-qPCR).

Bias reducing methods were used: The Study Director (SD) was unblinded. Laboratory staff, the examining veterinary surgeon and all other study personnel performing observations were blinded. The SD stored the unblinded random treatment plan



Serum and colostrum BRV antibody concentrations in vaccinated cows at calving significantly higher ($p<0.01$) than in control animals. Significantly higher ($p<0.01$) BRV antibody levels on day of challenge in serum of calves fed from vaccinates compared with control calves.



Faecal excretion, as measured by Area Under the Curve (AUC) post challenge significantly higher in control calves compared with calves suckled from vaccinated cows ($p=0.014$)

Figure 4 - BRV onset of immunity (ooi) - Antibody levels in dams and calves; Average faecal excretion.

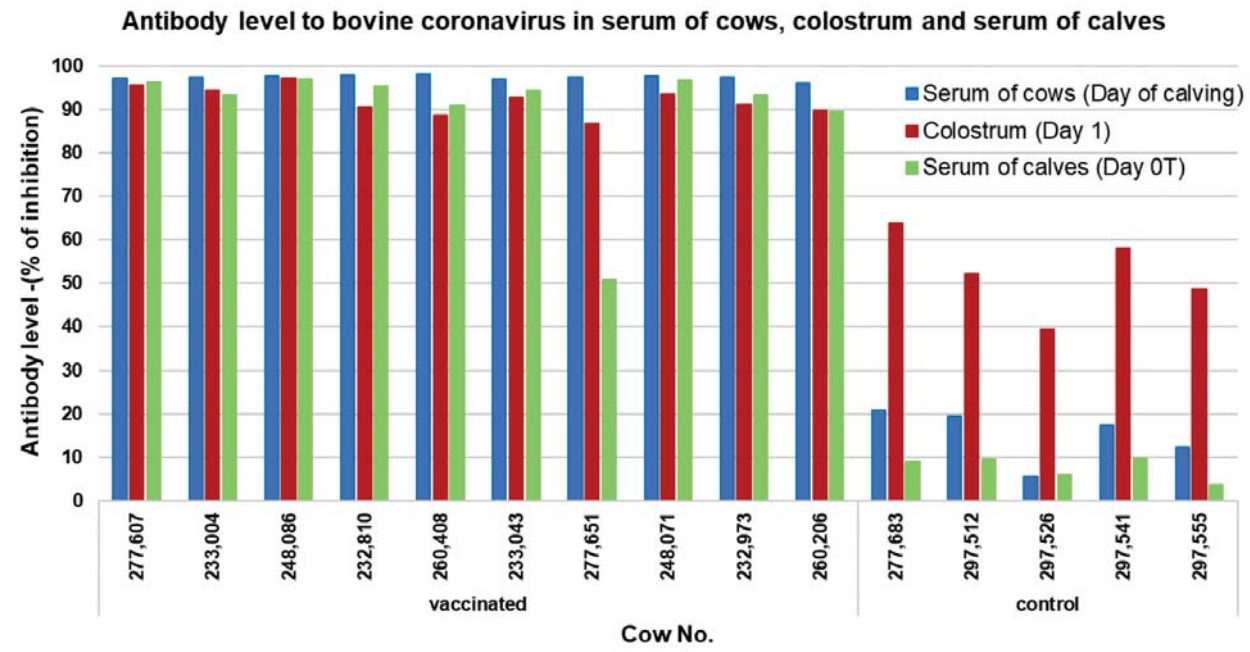
and the animal dosing forms. On the day of vaccination, the SD prepared the vaccine and placebo according to the unblinded plan and drew the appropriate amount into a syringe. Vaccine and placebo were administered by a suitably qualified person unaware of treatment group. The examining veterinary surgeon and other personnel were unaware of the allocation to treatment groups for the duration of the study.

Field efficacy and safety study

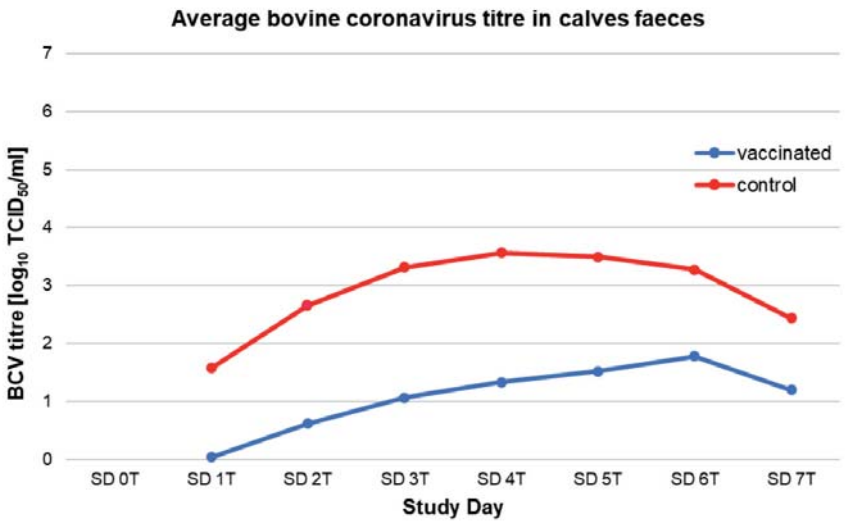
In addition, a field study was implemented in 3 farms to provide confirmation of both the safety of vaccination in pregnant cows and heifers when administered 12 to 3 weeks prior to expected parturition, and the efficacy (on 2 sites only for logistical reasons of fresh sample transportation) of antibody transmission to their offspring. The design of the field study is shown in Figure 2.

Three farm sites were recruited, which conducted no routine vaccination against the 3 vaccine pathogens. On each farm site, 30 healthy cows and heifers, due to calve 3-12 weeks later were included in the study; 20 animals received a single vaccine injection, and 10 animals were untreated controls.

The vaccine for ETEC, BRV and BCV (Fencovis/Biobos RCC, Bioveta, Ivanovice na Hané, Czech Republic) used for the clinical trial was a standard production batch of the test product; it was manufactured by Bioveta according to the approved manufacturing procedure.



Serum and colostrum BCV antibody concentrations in vaccinated cows at calving significantly higher ($p<0.01$) than in control animals. Significantly higher ($p<0.01$) BCV antibody levels on the day of challenge in serum of calves fed from vaccinates compared with control calves.



Faecal excretion, as measured by Area Under the Curve (AUC) post challenge significantly higher in control calves compared with calves suckled from vaccinated cows ($p=0.003$).

Figure 5 - BCV ooi - Antibody levels in dams and calves; Average faecal excretion.

Safety was evaluated by monitoring rectal temperature, injection site reactions, and general health until calving in all dams. Efficacy was evaluated in 60 animals in total between groups in terms of serum, colostrum and milk antibody kinetics to the 3 vaccine antigen components in the cows/heifers. Blood samples (5 ml) were collected from the *vena coccygea* (cows) or *vena jugularis* (calves), using sterile Hemos sampling tubes, labelled with animal identification number and date of collection. The samples were processed at Bioveta within 24 hours of collection, using centrifugation at 3000 rpm for 10 minutes before serum removal. Serum samples were stored at or below -15°C until analysis. Colostrum/milk samples (5 ml) were collected into 10 ml tubes from each cow and heifer, in preclinical studies daily from the day of calving for the 10 days and in the field study daily from the day after calving until 14 days later. Samples were stored at or below -15°C until determination of antibody concentrations. Serum antibody kinetics to the 3 vaccine antigen components in the calves born to the cows/heifers included in the study were also measured using. These calves had received colostrum and milk from their dams from the day of birth, within 2 hours, until 14 days later, fed to the calf via a teat-bucket (farm 1) or an oesophageal feeder (farm 2).
Bias reducing methods were used: the monitor was aware of the animal allocation to groups; the investigators performed pre-allocation blood sampling and health check, and then conducted vaccination on each site on Day 0, under supervision

from the monitor. Farm personnel performing observation of animals and laboratory personnel involved in sample analysis were blinded to treatment; study animals (vaccinates and controls) remained together in the same housing section.

Statistical Analysis

Clinical diarrhoea scores in calves post challenge were calculated as the sum of daily scores (details shown in Figure 3). Total individual scores were compared between groups using non-parametric Mann-Whitney one-sided tests for all challenge studies. Specific antibody concentrations were compared in cows (vaccinated vs placebo) and then in calves (vaccinates-suckled vs placebo-suckled) using analysis of variance (ANOVA) methods (QC Expert v. 3.2, TriloByte). A two-way ANOVA was used (factor 1: treatment group, factor 2: sampling point). When significant differences were detected, Scheffé's method for correcting for multiple comparisons was implemented. The level of significance was set at $p<0.05$.

Faecal excretion of virus in each calf after challenge was quantified by calculating the AUC (area under the curve) of virus shedding during the observation period. AUC values were compared between groups using Mann-Whitney one-sided tests (STATISTICA v. 9CZ, StatSoft).

RESULTS

Vaccination-challenge studies

The results are illustrated in Figures 3 to 7. Figure 3 presents an overview of individual calf diarrhoea scores post challenge per group for all 4 studies. Figures 4 to 7 show for each study: antibody levels in dams (serum and colostrum) and in calves, and average faecal excretion per group for the viral challenge studies. Each figure includes self-standing comments and p values of comparisons between groups are specified.

In the 3 ooi studies, the control calves (5 per study) each showed clear signs of diarrhoea; 2 control calves in the BRV study and 1 control calf in the ETEC study died from severe diarrhoea. Meanwhile, all 10 calves per study which had received colostrum and milk from vaccinated cows and heifers, remained free from diarrhoea symptoms, the difference was significant ($p<0.05$).

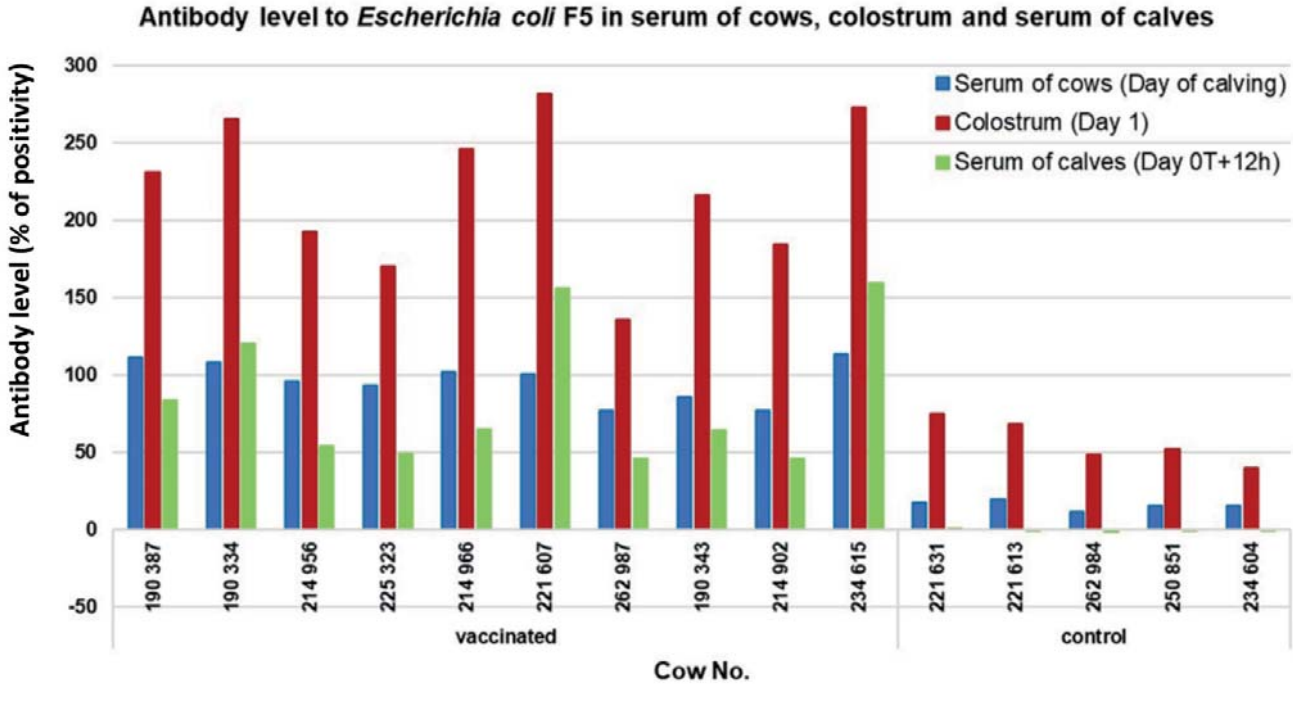
In the BCV doi study, all 5 control calves exhibited signs of diarrhoea lasting 3 to 7 days, while 8/10 calves which had received colostrum and milk from vaccinated cows and heifers remained free from diarrhoea symptoms, and the last 2 showed mild and transient diarrhoea, lasting 1 to 2 days, the difference still being significant ($p<0.05$).

For each study, there were significant differences between serum and colostrum antibody levels in vaccinated vs control cows on day of calving (higher in vaccinates) although not different before vaccination, and serum antibody level in calves suckled with colostrum and milk from vaccinated vs control cows (higher in vaccinates). There was no difference in quantities or times from birth to first colostrum feed between each group. Viral faecal excretion was significantly reduced in calves which had received colostrum and milk from vaccinated cows, compared with control calves, for all viral studies.

Field efficacy and safety study

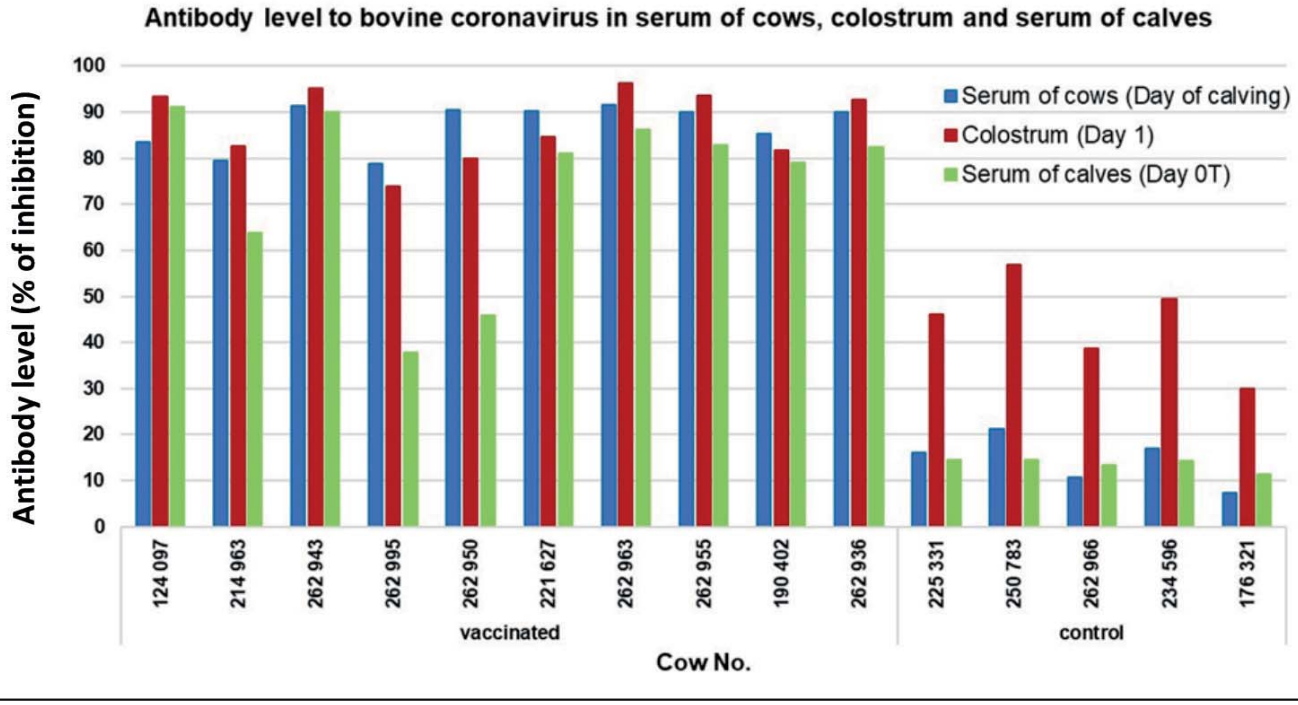
The results are illustrated in Figures 8 and 9.

For all 3 vaccine components on both sites: the calves having received colostrum and milk from vaccinates, showed significantly higher antibody concentrations than calves fed with colostrum and milk from control dams at all timepoints ($p<0.001$). Calves from vaccinates showed the highest con-

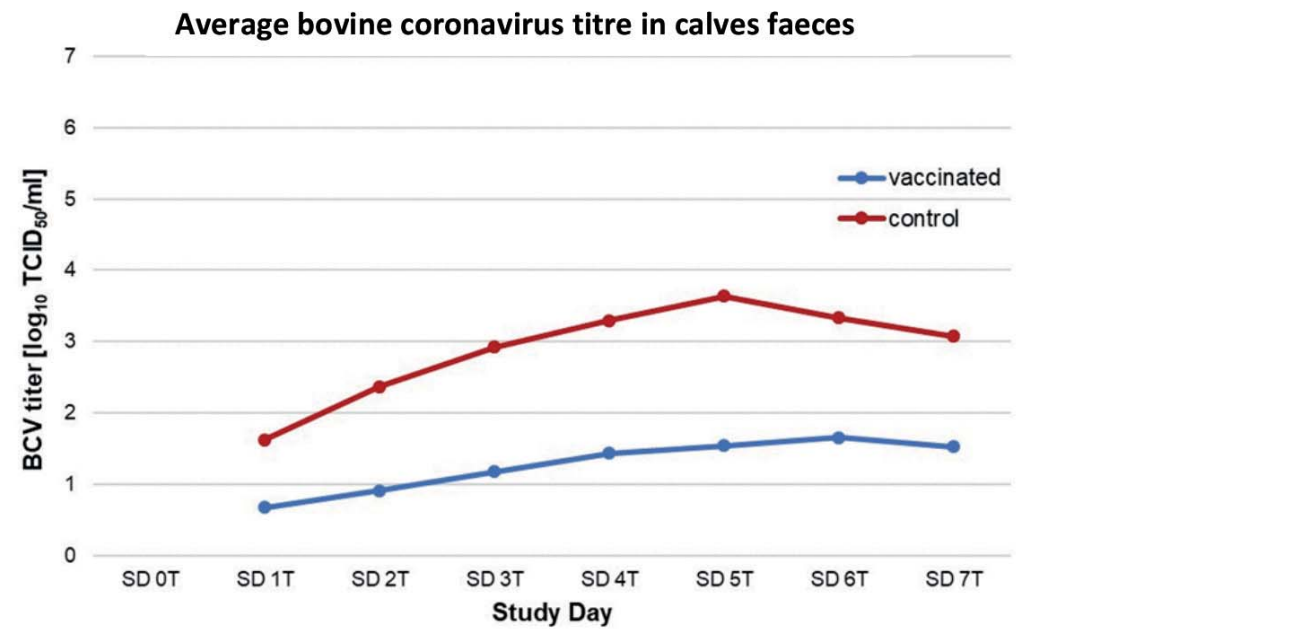


Serum and colostrum *E. coli* F5 antibody concentrations in vaccinated cows significantly higher ($p<0.05$) than in control animals.

Figure 6 - *E. coli* ooi - Antibody levels in dams and calves and diarrhoea scores.



Serum and colostrum BCV antibody concentrations in vaccinated cows at calving significantly higher ($p<0.05$) than in control animals. Significantly higher ($p<0.05$) BCV antibody levels on the day of challenge in serum of calves fed from vaccinates compared with control calves.



Faecal excretion, as measured by Area Under the Curve (AUC) post challenge significantly higher in control calves compared with calves suckled from vaccinated cows ($p=0.003$)

Figure 7 - BCV duration of immunity - Antibody levels in dams and calves; Average fecal excretion.

centrations on Day 1, declining slowly until Day 14. Conversely for calves from controls, the concentrations remained low and relatively stable between Day 1 and Day 14. No wild strain challenge occurred on any of the farms during the study period. Mean rectal temperature over time in vaccinates *vs* control animals indicated that vaccination led to a modest increase in rectal temperature- on average 0.69°C- which occurred between 4 hours and 1 day post vaccination (depending on the site), returning rapidly to normal, by day 2 post vaccination. There were farm differences, with animals in farm 1 showing a much flatter increase, 4 to 6 hours post vaccination, whereas the increase was later and more pronounced on farm 2 and farm 3. One animal (out of 60 vaccinates), from farm 3, showed an increase of body temperature of 2.05°C which lasted for 1 day. No ad-

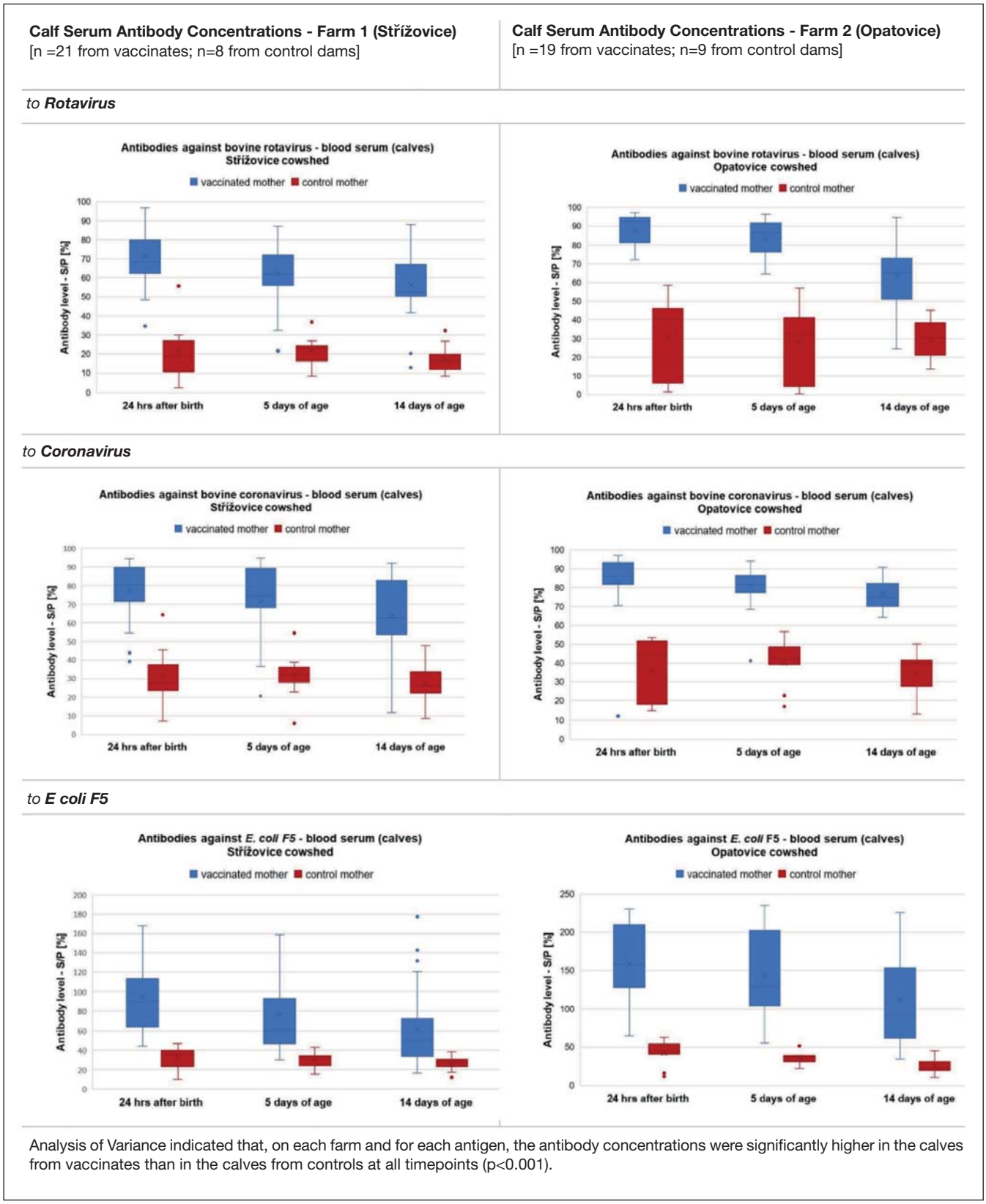


Figure 8 - Field Study: Antibody levels (to BRV, BCV and ETEC) in calves per treatment group at D1, D5 and D14 of age on each of the 2 study sites, shown as boxplots.

verse effect occurred, on the animals' general demeanour or on the pregnancy outcome.

DISCUSSION

All challenge strains used in the studies were heterologous to the vaccine strain being evaluated.

The BRV challenge strain, although its nucleotide sequence differed in both the G6 and P1 genes, was of the same serotype [G6P1] as the vaccine strain, and as such belonging to Group A BRV (10). Information on protective immunity against infection with Group A BRV having the same or different G/P serotype as the vaccine strain is incomplete (11). Some stud-

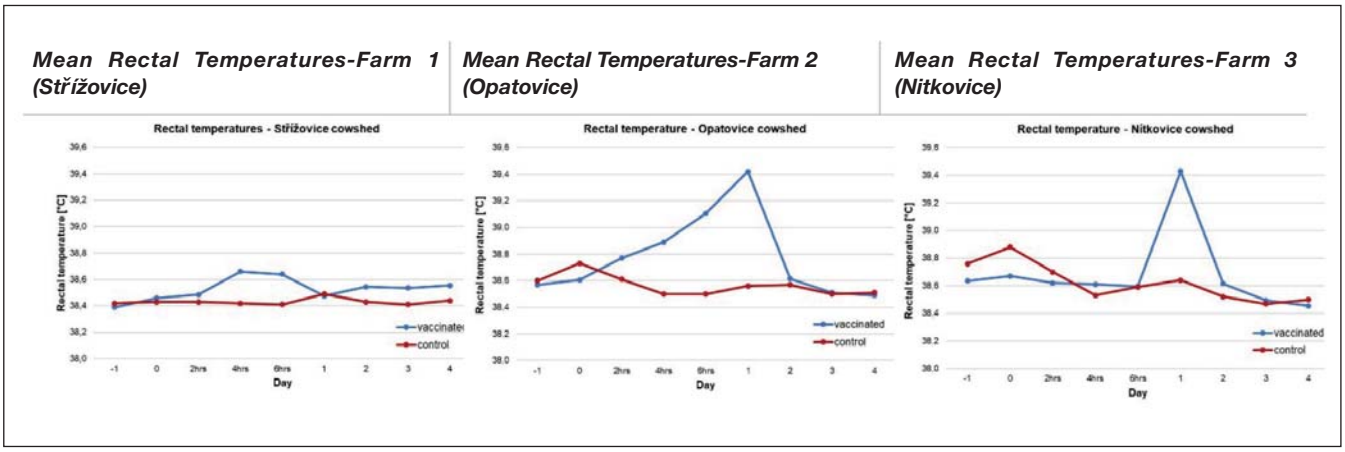


Figure 9 - Field Study: Mean Rectal Temperatures in cows/heifers following vaccination (n=20 vaccinates and 10 controls on each site).

ies hinted that certain strains could neutralise *in vitro* and protect challenge-exposed calves *in vivo* against BRV strains with a different G/P serotype. It has been shown that vaccination of a mature cow that had had natural rotavirus exposure led to cross-serotype stimulation of heterotypic antibodies (12) (13). Other publications indicated that the different serotypes of Group A BRV share similar epitopes which are recognised by cross-reacting antibodies (14). Thus, a single serotype vaccination of seropositive cows could stimulate antibody production to a wide range of BRV serotypes and genotypes, suggesting that this strategy may provide a means of enhancing passive protection against other potential serotype challenges (8) (11).

For BCV, all isolates appear to be of a single serotype (as observed by seroneutralisation). Thus, the existence of a single serotype of BCV and cross-protection among strains from the different clinical syndromes (such as winter dysentery, calf diarrhoea and bovine respiratory coronavirus) suggest that a single broadly cross-reactive strain of BCV may suffice for a vaccine (15).

For ETEC: the challenge strain serotype (O101:K99, F41) differed from the vaccine strain (O8: K99). The challenge strain expresses both F5 and F41 adhesins. Many ETEC strains that produce F5 (K99) also produce F41 (16). It has previously been shown in calves that a vaccine containing only F5 protected against a challenge with an ETEC strain B44 producing both F5 and F41 (17). Such protection could result from steric hindrance or agglutination of the bacteria so that F41 pili could not promote adhesion in vivo (18).

The efficacy of the vaccine tested may be due to appropriate concentrations of the antigens present in the vaccine formulation and the adjuvants used. Aluminium hydroxide is known to stimulate humoral immunity, and saponin-derived adjuvants such as Quil-A are potent enhancers of both humoral and cellular immunity (19). The adjuvant system may be at least partly responsible for the modest rise in rectal temperature observed in the vaccinated cows.

The efficacy described for all components of this maternal vaccine in the calf challenge studies was achieved using the feeding regimen stipulated in the EU Pharmacopeia monographs, ie. 7 days of colostrum/milk previously collected from vaccinated dams. Although this colostrum-feeding regimen does not accurately reflect commercial practices, this would presumably be the case for all cattle maternal vaccines approved in Europe.

Recommendations regarding colostrum-feeding and Transfer of Passive Immunity (TPI) in commercial herds have been developed for dairy herds, where it is customary to separate dams from calves at birth. Colostrum is collected from cows and ideally fed to calves (~10% BW) via an oesophageal tube or a nipple-bottle within 1 to 2 hours of birth (20) (21). The merits of a second colostrum meal (8 to 12 hours later) are also documented (22). There is scope for improvement in management of colostrum feeding in developed cattle industries, with NAHMS systematic surveys in the US dairy operations demonstrating an improvement in young calf mortality over the years between 1996 and 2014, although it remained above 5% at last count, of which 30% from digestive causes (23).

In beef dam-calf operations, calves are expected to suckle their dams from birth for several months. Human intervention is rarer and occurs in case of weak calves failing to get up, maternal inexperience or poor udder/teat conformation (24). Paradoxically, these actions are associated with a higher risk of ill-health in calves (25), perhaps because when all goes well, no intervention is necessary. Similarly to data reported in this paper, vaccination of pregnant beef heifers in the last third of pregnancy (prior to or simultaneous with colostrogenesis) has been shown to increase vaccinal antigen serum neutralising titres in the vaccinated heifers and their nursing calves-although not the calves overall serum IgG concentrations (26). This highlights the potential benefits of attention to colostrum management in beef systems.

Colostrum provides many vital services to the newborn calf in addition to the well-known and traditionally measured immunoglobulins (27) (28) (29) (30)). Its high fat content contributes to the metabolism of thermoregulation; cytokines and maternal leukocytes participate in immune defence and development; and various hormones (including growth hormone, insulin and insulin growth factor), as well as multiple oligosaccharides (OS), contribute to enterocyte maturation, GI tract development, glucose metabolism and a healthy microbiome. Indeed, colostrum contains up to 72 times more OS than whole milk (28).

CONCLUSIONS

The test vaccine demonstrated excellent clinical efficacy in the laboratory challenge studies against all vaccine components,

with calves from the 'vaccine' groups in the ooi studies demonstrating complete prevention of diarrhoea, whereas control calves were all affected with varying severity for several days.
In the BCV doi study, the protection was also significantly improved in 'vaccine' group calves compared with controls.
Virus excretion after challenge was significantly reduced in 'vaccine' calves, both in duration and virus load, which translates into lower contamination of the calf environment.
This efficacy was associated with a significant elevation of specific antibodies, first in the serum and colostrum of vaccinated cows and heifers, which transferred efficiently as antibodies in the serum of calves.
In conclusion, the data presented and discussed demonstrate the unique potential of the vaccine to prevent NCD symptoms linked with BRV, BCV and ETEC after a single injection of pregnant dams 12 to 3 weeks before expected parturition, as well as to reduce the viral challenge pressure faced by subsequent calves in the herd.
To capture the full potential, a dam vaccination program with an effective vaccine has to go hand in hand with 'good colostrum management practices' alongside veterinary guidance, thus resulting in high-quality colostrum being fed to newborn calves early enough (within 2 to 6 hours after birth for the first feed) and in sufficient quantity (10% bodyweight in the first 12 hours). Whilst the principles of these measures have been agreed for decades, their practical implementation may not always be straightforward.

Acknowledgements

All studies were financed by Bioveta and all authors are in the direct or indirect (MPT) employment of Boehringer Ingelheim or Bioveta (BM, JK).

References

1. Johnson K.F., Burn C.C., Wathes D.C. (2011). Rates and risk factors for contagious disease and mortality in young dairy heifers. CAB Reviews, 6:059.
2. Gomez D.E., Weese J.S. (2017). Viral enteritis in calves. Can Vet J., 58(12): 1267-1274.
3. Millemann Y. (2009). Diagnosis of neonatal calf diarrhea. Revue Med Vet, 160(8-9): 404-409.
4. Clark M.A. (1993). Bovine coronavirus. Br Vet J., 149(1): 51-70.
5. Constable P.D. (2009). Treatment of calf diarrhea: antimicrobial and ancillary treatments. Vet Clin North Am Food Anim Pract., 25(1): 101-120.
6. Amin N., Seifert J. (2021). Dynamic progression of the calf's microbiome and its influence on host health. Comput Struct Biotechnol J., 19: 989-1001.
7. Wooding F.B. (1992). Current Topic: The Synepitheliochorial Placenta of Ruminants: Binucleate Cell Fusions and Hormone Production. Placenta, 13: 101-113.
8. Gunn A., Izzo M., House J. (2020). Chapter 20. Manifestations and management of disease in neonatal ruminants. Diarrhea. In: Large Animal Internal Medicine, Eds Smith B., Van Metre D., Pusterla N., 6th ed, 351-378, Elsevier Health Sciences.
9. University of Wisconsin-Madison. Calf Health Scoring Criteria. Available at: https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf. Accessed 12.5.23.
10. Otto P.H., Rosenhain S., Elschner M.C., Hotzel H., Machnowska P., Trojnar E., Hoffmann K., Johne R. (2015). Detection of rotavirus species A, B and C in domestic mammalian animals with diarrhoea and genotyping of bovine species A rotavirus strains. Vet Microbiol., 179(3-4): 168-76.
11. Duhamel G. (2020). Chapter 48. Use of biologics in the prevention of infectious diseases. Neonatal calf enteric disease vaccines. In: Large Animal Internal Medicine, Eds Smith B., Van Metre D., Pusterla N., 6th ed, 1655-1660, Elsevier Health Sciences.
12. Snodgrass D.R., Ojeh C.K., Campbell I., Herring A.J. (1984). Bovine rotavirus serotypes and their significance for immunization. J Clin Microbiol., 20(3): 342-6.
13. Snodgrass D.R., Fitzgerald T.A., Campbell I., Browning G.F., Scott F.M., Hoshino Y., Davies R.C. (1991). Homotypic and heterotypic serological responses to rotavirus neutralization epitopes in immunologically naive and experienced animals. J Clin Microbiol., 29(11): 2668-72.
14. Brüssow H., Eichhorn W., Rohwedder A., Snodgrass D., Sidoti J. (1991). Cattle develop neutralizing antibodies to rotavirus serotypes which could not be isolated from faeces of symptomatic calves. J Gen Virol., 72. (2): 349–64.
15. Saif L.J. (2010). Bovine respiratory Coronavirus. Vet Clin Food Anim Pract., 26
16. Morris J.A., Thorns C., Scott A.CC, Sojka W.J., Wells G.A. (1982). Adhesion in vitro and in vivo associated with an adhesive antigen (F41) produced by a K99 mutant of the reference strain Escherichia coli B41. Infect Immun., 36(3): 1146-53.
17. Acres S.D. (1985). Enterotoxigenic Escherichia coli infections in newborn calves: a review. J Dairy Sci., 68(1): 229-56.
18. Runnels P.L., Moseley S.L., Moon H.W. (1987). F41 pili as protective antigens of enterotoxigenic Escherichia coli that produce F41, K99, or both pilus antigens. Infect Immun., 55: 555-8.
19. Rajput Z.I., Hu S.H., Xiao C.W., Arijo A.G. (2007). Adjuvant effects of saponins on animal immune responses. J Zhejiang Univ Sci B., 8(3): 153-161.
20. Lombard, J.E., Urie N., Garry F., Godden S., et al. (2020). Consensus recommendations on calf- and herd- level passive immunity in dairy calves in the United States. J. Dairy Sci., 103: 7611-7624
21. Robbers, L., Ruurd, J., Nielen, M., Koets, A. (2021). A Scoping Review of On-Farm Colostrum Management Practices for Optimal Transfer of Immunity in Dairy Calves. Front. Vet. Sci., 8:668639
22. Abuelo A., Cullens F., Hanes A., Brester J.L. (2021). Impact of 2 Versus 1 Colostrum Meals on Failure of Transfer of Passive Immunity, Pre-Weaning Morbidity and Mortality, and Performance of Dairy Calves in a Large Dairy Herd. Animals, 11: 782.
23. Morbidity and Mortality in U.S. Preweaned Dairy Heifer Calves NAHMS Dairy 2014 Study Calf Component Information Brief September 2021 - Morbidity and Mortality in U.S. www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy17/morb-mort-us-prewean-dairy-heifer-nahms-2014.pdf.
24. McGee M., Earley B. (2019) Review: passive immunity in beef suckler calves. Animal., 13(4): 810-825.
25. Bragg R., Macrae A., Lycett S., Burrough E., Russell G., Corbishley A. (2020). Prevalence and risk factors associated with failure of transfer of passive immunity in spring born beef suckler calves in Great Britain. Prev Vet Med., 181: 105059.
26. Reppert E. J., Chamorro M.F., Robinson L., Cernicchiaro N., Wick J., Weaber R. L., Haines D. M. (2019). Effect of vaccination of pregnant beef heifers on the concentrations of serum IgG and specific antibodies to bovine herpesvirus 1, bovine viral diarrhea virus 1, and bovine viral diarrhea virus 2 in heifers and calves. Can J Vet Res., 83(4): 313–316.
27. Blum J.W. (2006). Nutritional physiology of neonatal calves. J Anim Physiol Anim Nutr (Berl), 90(1-2): 1-11.
28. Fischer-Tlustos A.J., Lopez A., Hare K.S., Wood K.M., Steele M.A. (2021). Effects of colostrum management on transfer of passive immunity and the potential role of colostral bioactive components on neonatal calf development and metabolism. Can. J. Anim. Sci. 101: 405–426.
29. Ghaffari M.H., Sadri H., Steinhoff-Wagner J., Hammon H.M., Sauerwein H. (2021). Effects of colostrum feeding on the mRNA abundance of genes related to toll-like receptors, key antimicrobial defense molecules, and tight junctions in the small intestine of neonatal dairy calves. J Dairy Sci, 104(9): 10363-10373.
30. Godden S.M., Lombard J.E., Woolums A.R. (2019). Colostrum Management for Dairy Calves. Vet Clin Food Anim. 35: 535–556.