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Research Article

Immune Response and Efficacy of a New Calf Scour Vaccine Injected Once during the last Trimester of Gestation

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Abstract

Objectives: The objectives of this study were to 1) evaluate the immune response induced by a single dose of a brand new vaccine against neonatal calf diarrhoea (NCD) in seronegative pregnant cows and 2) verify that calves born from these cows and fed with their colostrum are immunised against pathogenic strains of Rotavirus, Coronavirus and E. coli expressing F5 adhesin.

Materials and methods: The study included 45 seronegative pregnant cows. Control animals (n=15) received a placebo and vaccinated animals (n=30) received a single dose of the test vaccine (Bovigen®Scour, Virbac, France) between 12 and 3 weeks before calving. Serological monitoring of animals was done using an ELISA test kit specific for each antigen. Within 2 hours of calving, all calves were fed with colostrum from their own mother. Newborn calves were then challenged with either an infective dose of E. coli O101:K30, F5 + before 12 hours after birth (n=15), or Rotavirus, or Coronavirus at between 5 and 7 days after birth (two groups of n=15). The calves were monitored for 7-10 days (clinical signs and excretion of pathogens).

Results and discussion: A single injection induced a potent seroconversion, which was then reflected in the concentration of specific antibodies in the colostrum, and in the serum of calves fed this colostrum. Colostrum from vaccinated animals also had significantly ($P<0.01$) higher antibody titres for Rotavirus (96.1% ± 1.1), Coronavirus (82.3% ± 5.8) and E. coli F5 (81.0% ± 6.8) than that from control animals (13.8% ± 2.9, 12.4% ± 2.9 and 1.4% ± 1.7 respectively). Furthermore, calves that drank colostrum from vaccinated cows had durations of diarrhoea and clinical diarrhoea scores significantly ($P<0.01$) lower than calves who drank colostrum from the placebo mothers. The calf mortality rate in the control groups ranged from 20% - 40%, while no mortalities were observed in calves from vaccinated cows. Calves of the vaccinated group were also less likely to excrete pathogens, had significantly shorter ($P<0.01$) pathogen excretion times and excreted significantly less ($P<0.01$) pathogen than those from the control group.

Conclusion: This study demonstrated that a single injection of the test vaccine administered to seronegative pregnant cattle 12 to 3 weeks prior to calving significantly increased colostrum antibodies against the 3 antigens contained in the vaccine. When properly fed to calves, the colostrum was highly protective against challenge by three major causes of NCD.

Keywords: Neonatal Calf Diarrhoea; Rotavirus; Coronavirus; K99; vaccine; colostrum

Introduction

Neonatal calf diarrhoea (NCD) affects mainly calves under 4 weeks of age. It is characterized by a diarrhoea leading to dehydration and acidosis which can have systemic consequences and potentially lead to death [1]. The main causative infectious agents of NCD, especially in calves under 12 days of age, are the bovine rotavirus (BoRV), Cryptosporidium parvum, the bovine coronavirus responsible for calf diarrhoea (BoCV-CD) and several

enterotoxigenic *Escherichia coli* (ETEC), but other pathogens can also be accountable for this disease [1-5]. Several commercial vaccines including various strains of ETEC, rotavirus, coronavirus as well as Clostridium perfringens, alone or in combination have been developed. Vaccination of pregnant cows before parturition have been long recognised as a reliable way to trigger an immune response which leads to the presence of protective immunoglobulins (Ig) in the dam's colostrum. The calf can then be passively protected if it stands rapidly and can nurse to satiety, or if the colostrum is fed properly [5-7].

During the process of colostrogenesis, i.e. the prepartum transfer of immunoglobulins from the maternal blood circulation into mammary gland secretions, Ig progressively concentrate over several weeks prior to parturition [8]. In bovine colostrum, the respective concentrations of IgG1 and IgG2 average 60-70 and 7-9 mg/mL, respectively, that indicates a very selective transfer of IgG1 accounting for a 10-fold enhancement in colostrum concentration followed by a similar decrease in mature milk, both relative to serum concentrations [9]. In heifers and other non-previously vaccinated animals, most commercial vaccines require an initial injection and a booster a couple of weeks apart. Handling cattle in the last trimester of gestation, and dosing it twice, particularly heifers is not that easy, potentially at risk of abortion, and may be considered as a significant welfare issue. Vaccination of pregnant cows prior to the last few weeks of gestation results in a dramatic increase in specific IgG in the serum (about the antigen). Beyond individual and unpredictable exceptions, and thanks to the active concentration of IgG1, high levels of these specific IgG should be transferred into the colostrum. It may be hypothesized that a single dose of a labelled vaccine with demonstrated immunogenic and protective properties should cause a enough immune response with high specific antibody titres in the colostrum.

The aim of this study was to assess antibody responses to BoRV, BoCV, and F5 adhesin in the colostrum of dams that have never been vaccinated before, and injected with a single dose of a new commercial calf scour vaccine some weeks prior to the due date, as well as the protective effectiveness of the colostrum in newborn calves challenged with pathogenic strains of either BoRV, BoCV, or *E. coli* K99.

Material and Methods

Ethical approval

Challenge tests have been performed after approval by the USKVBL (Institute for State Control of Veterinary Biologicals and Medicaments in the Slovak Republic - approval No 97/2014/OB/KS), and the approval of RVPS (Regional Veterinary and Food Administration - approval No 14/004509).

Study design

This was a randomized study with a vaccinated group and a placebo group. Animals non-previously vaccinated against major calf scour antigens (BoRV, BoCV, and *E. coli* K99) were vaccinated prior to the upcoming parturition with the test vaccine. The immune response in dams and passive immunisation of calves will be compared based on antibody levels (assessed by ELISA) found in the serum of cows and calves and in the colostrum. In a second step, calves fed with colostrum of their dam, were challenged with pathogenic strains of either BoRV, BoCV, or E. coli K99, and various clinical criteria were recorded.

Animals

A farm survey was carried out in Slovakia before the experiment (data not presented). The purpose of this survey was to identify farms where not any scour vaccine was routinely administered. Then animals of the reproductive herd were screened to confirm a low equable level of titre of antibodies to the three pathogens on the selected farm. Healthy pregnant cows (>6 months) and never injected with a vaccine containing BoRv, BoCV or *E. coli* F5 antigens, were included in the study. Cows enrolled were expected to be due 12 to 3 weeks after vaccination. Animals were then randomly allocated to vaccination group (BS) or to the control group (C). After birth, the offspring will be assigned to the same vaccination group as their mother (BS calf=calf born from BS vaccinated cow).

Vaccines and passive immunization

The test product is a tri-valent vaccine formulated to immunize pregnant cows against BoRV, BoCV and the F5-adhesin of *E. coli* (BOVIGEN SCOUR, Virbac, France). The immunogenic ingredients of the vaccine are the bovine rotavirus strain TM-91, serotype G6[P1] (inactivated); the bovine coronavirus strain C-197 (inactivated); and the *E. coli* strain EC/17 (inactivated) expressing the F5 (K99) adhesin. The adjuvant used in this vaccine is a water-in-oil-in-water commercial preparation (Montanide ISA 206 VG, Seppic, France). This vaccine was initially granted a marketing authorization requiring two injections during the last trimester of pregnancy for the primary course. However, further studies showed that a single injection was enough to boost the immune status in previously vaccinated cows, whatever the scour vaccine used [10]. In the current study, a single injection (IM) of vaccine (2 mL) was administered 12 to 3 weeks before calving was expected to naïve animals. Animals in the control group were dosed with 3 mL of a placebo formulation (MEM 30.6%, PBS 15.3%, Montanide ISA 206 53.9%, thiomersal 0.1 mg/mL, formaldehyde 0.45 mg/mL). For the passive transfer of immunity, all calves received colostrum from their dam (10% of the body weight within the first 24h of life) in 3 meals, the first one within 2 hours of birth. The next days, and until they turned 6 days-old, calves were fed twice-a-day on colostrum/milk (2-3L) from their dam.

Collection of samples

In selected farms, all cows were sampled at enrolment (screening) to identify a possible natural exposition to the three pathogens. Then cows were sampled just before vaccination, and

at calving. Calves were sampled during the first 48h of life. After collection blood samples were left at 15-25 °C for minimum 30 minutes and afterwards samples were stored at +2 to + 8 °C. Serum was took off the tube within 24 hours after sampling, and was heat inactivated at 56 °C for 30 min and frozen at -20°C for further testing. At the first milking, colostrum was collected into the sterile 100 ml jars closed with a lid. Milk was then sampled daily, up to 3 days after calving. Samples were then stored at +2 to +8 °C up to examination and processed within 48 hours after sampling. Colostrum was treated with enzyme LACTOCHYM in the proportion of 1ml of enzyme per 50ml of colostrum. After treatment the samples were centrifuged for 15 min. at 1000rpm. For subsequent examination supernatant only was used. Stoll samples were collected daily from all calves within 7 days after challenge. Collection staff wore sterile gloves, and samples were collected into the sterile plastic containers. Samples were frozen at -20 °C for further testing.

Tests and analyses

Titres in anti-BoRV, anti-BoCV and anti-F5 antibodies were assessed by measuring the inhibition of optical density (% of inhibition) by competitive enzyme-linked immunosorbent assay (ELISA), on serum and colostrum. The BIO K 126 - Monoscreen AbELISA Rotavirus bovin / Compétition; BIO K 295 - Monoscreen AbELISA E.coli F5 (K99) / blocage; and BIO K 392 - Monoscreen AbELISA Coronavirus bovin / Compétition (all from BIO-X Diagnostics, Belgium) were used for the indirect quantification of BoRV, F5 and BoCV antibodies, respectively. The tests were performed in an accredited laboratory (Pharmagal-Bio s.r.o.). Every cluster of newborn calves were then orally challenged with either a group A serotype G[6]P[1] BoRV strain (strain 4/A/2, 106.9FAID50/calf), a generic BoCV strain (strain B11, 106.6TCID50/calf), or an O101:K30, F5+ E. coli strain (strain EC42, 5.8x108CFU/calf), appropriate infectious load having been assessed elsewhere. Calves were challenged at 12 hours of life (maximum) for *E. coli*, and between 5 to 7 days of life for the viruses. Calves were then observed daily in order to assess the intensity of diarrhoea (0: no sign, 1: mild diarrhoea, 2: severe diarrhoea, 3: death), and the vitality of the animal (0: active, 1: slight apathy, 2: serious apathy). Daily scores were summed over the study period and individual cumulative scores were statistically processed. Clinical follow up of calves was carried out until day 7 post-challenge.

Finally faecal cultures were performed up to 10 days post-challenge to follow up the excretion of the enteropathogens involved in the infectious challenge, and to exclude the role of other pathogens as well,: *Salmonella spp*, Cryptosporidium parvum, and *Eimeria spp*. Elisa kit for antigenic diagnosis of *E.coli* F5 (K99), Multiscreen AgELISA BIO K 314, was used for the determination of the content of F5 antigen. A similar detection kits (BIO K 315) was used for the detection of coronavirus and rotavirus antigens, respectively. Quick immunochromatographic test strips for the detection of Cryptosporidium (BIO K 38) was performed according to label instructions (all test kits from BIO-X Diagnostics, Belgium). Presence of Salmonella spp. was assessed by a cultivation method, and diagnostic of *Eimeria spp*. in faeces by a sugar flotation method.

Judgement of results

Efficacy of the vaccine was judged according to Good Laboratory Practices and met the requirements of the related sections of the European Pharmacopeia (Ph. Eur. 1954, Ph. Eur. 1953, and Ph. Eur. 0961, section immunogenicity), and Directive 2009/9/EC. Vaccine will deemed to comply with the regulation if there is a significant reduction in clinical scores as well as a significant reduction in diarrhoea and virus excretion in calves given colostrum and milk from vaccinated cows compared to those fed on colostrum and milk from control dams. A P value of <0.05 was taken to indicate statistical significance.

Results

Forty-five pregnant cows were randomly enlisted in three groups of 15 for this study. For every single group, 10 were vaccinated and 5 were dosed with the placebo. All cows were dosed on the same day, and all cows calved between 60 and 70 days after the start of the study.

Antibody levels in the different samples

Before vaccination cows presented very low, if any, level of antibodies for the three pathogens, and they can be considered as seronegative (data not presented). Results of immunological tests in cows' and calves' sera, and colostrum are presented in Table 1, as percentage of inhibition ELISA (PI). At vaccination all average PIs measured from cow's sera were low or very low. At calving, PI were significantly higher (P<0.05, t-test) for the three antigens. High level of antibodies to BoRV, BoCV, and the F5-adhesin were also observed in the colostrum from the first milk out. In BS calf's serum high antibody levels to BoRV and BoCV were observed whereas control calves were seronegative. Since calves were inoculated very soon with E. coli K99 (<12h after birth), the immune status of calves to this pathogen has not been tested; it was assumed that the infectious load may interfere with the absorption of the specific antibodies, and then result in uninterpretable values.

Infectious challenge

Result of infectious challenges are presented in Table 2. In calves born from vaccinated dams, and fed on their dams' colostrum and milk, the incidence of post-challenge diarrhoea was significantly (P<0.05, c2) lower than in controls, and for the three pathogens. Also, a significant (P<0.05, t-test) reduction in clinical severity of diarrhoea was observed for the three pathogens with cumulative scores of 2.0 vs. 13.4, 2.8 vs. 10.4, and 1.1 vs. 11.6, for BoRV, BoCV and the *E. coli* K99, respectively. In BS calves, none of them died whereas 2 died from Rotavirus infection, 2 from Coronavirus, and 1 from *E. coli* K99 among the 15 control animals. All (100%) of control calves excreted pathogens over the follow up period, whereas only 50% of

BS calves shed viruses, and 60% of them shed *E. coli* K99; however, this difference is not significant. Average durations of excretion of the three pathogens were significantly shorter (P<0.05, c2) in BS calves than in controls. Amounts of pathogens excreted over the follow up period were also significantly reduced (P<0.05, t-test) in BS calves than they were in controls. Concurrent pathogens (*Salmonella spp, Eimeria spp, C. parvum,* nor BoRV, or BoCV, or *E. coli* K99, with consideration to the challenge group) were not detected, neither in BS calves nor in controls (data not presented) (Table 3).

Table 1: Mean titre (±SD) of antibodies to BoRV, BoCV or F5 adhesin in serum, colostrum and milk samples (values expressed as % of inhibition ELISA) in vaccinated and control animals, and their offspring.

	Dams								Calves	
	Serum at vaccination		Serum after vaccination (at calving)		Colostrum (first milking)		Milk (72 hours after calving)		Serum before infection	
	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls
BoRV	12.5[a] (±4.5)	10.2 (±4.1)	84.8[a] (±8.4)	9.2 (±3.9)	96.1[b] (±1.1)	13.8[b] (±2.9)	24.9[c] (±11.3)	0.2[c] (±0.4)	85.1 (±7.7)	0
BoCV	10.7[a] (±4.2)	12.2 (±3.5)	78.8[a] (±10.2)	8.4 (±3.1)	82.3[b] (±5.8)	12.4[b] (±2.9)	22.5[c] (±12.3)	0.4[c] (±0.5)	74.0 (±11.6)	0
F5-adhesin	0.9[a] (±1.0)	2.0 (±3.0)	71.9[a] (±15.8)	0.6 (±0.8)	81.0[b] (±6.8)	1.4[b] (±1.7)	25.9[c] (±8.3)	1.2[c] (±1.0)	N.A.*	

a, b, c: Figures with same superscripts within the row differ significantly (P<0.01, t-test)

*Calves were challenged on day 0 by oral route with *E. coli* K99, therefore antibody levels 48h after birth were considered as uninterpretable.

Table 2: Occurrence of diarrhoea, clinical severity, and duration of symptoms in animals born from vaccinated and control dams after infectious challenge with selected strains of BoRV, BoCV, and *E. coli* K99.

	Mortality rate(1)		Proportion of calves with diarrhoea(1)		Avg. clinical score of diarrhoea in calves(2)		Avg. duration of diarrhoea (days)(2)	
	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls
BoRV (4/A/2)	0/10	2/5	5/10	5/5	2.0 (±2.3)	13.4 (±2.6)	1.7	7.0
BoCV (B11)	0/10	2/5	7/10	5/5	2.8 (±2.1)	10.4 (±2.5)	2.5	5.6
E. coli K99 (EC42)	0/10	1/5	5/10	5/5	1.1 (±1.3)	11.6 (±8.7)	1.1	5.8

(1) Differences between vaccinated and control animals are not significant (Fisher's exact test)

(2) The numeric values for individual symptoms were summed over the study period to create a total clinical score for the animal. All differences are significant (P<0.01, Student's *t*-test)

(3) All differences are significant (P<0.01, χ 2)

Table 3: Pathogen shedding in vaccinated and control animals after infectious challenge with selected strains of BoRV, BoCV, and *E. coli* K99.

	Proportion of calves excreting pathogens(1)		Proportion of period of observation (days) that excretion was observed(2)		Avg. pathogen excretion as % inhibition ELISA (±SD)(3)	
	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls
BoRV (4/A/2)	5/10	5/5	20/70	30/30	13.0 (±12.9)	57.2 (±17.0)
BoCV (B11)	8/10	5/5	29/70	25/31	8.4 (±8.5)	27.5 (±18.7)
E. coli K99 (EC42)	6/10	5/5	21/100	22/43	6.6 (±5.8)	25.5 (±15.7)

(1) Differences between vaccinated and control animals are not significant (Fisher's exact test)

(2) The days with pathogen excretion were summed. The observation period was 7 days for the viruses, and 10 days for E. coli K99. All differences are significant (P<0.01, χ 2)

(3) All differences are significant (P<0.01, Student's *t*-test)

Discussion

In this study we assessed that vaccination of cows with a single dose of a brandnew inactivated vaccine containing a combination of BoRV, BoCV and a suspension of adhesins F5 of the E. coli, sharply increased the levels of the specific antibodies to these important pathogens in both serum and colostrum. Although not critical for the protection of the newborn calf, detection of antibodies to BoRV and BoCv in calves' serum indicated for the least that calves were fed with an enough colostrum of their dams soon after birth. These results agreed with those gained with a comparable commercial

single-injection trivalent vaccine that have been being marketed for years [11]. Level of antibodies to the F5 adhesin in the calf's serum was not measured in this study, but it was found to be high in another study with the same vaccine [10].

The percentage of inhibition of the antigens (PI, reflecting antibody concentrations) was assessed by competitive ELISA for BoRV, BoCV and F5-adhesin antibodies. This method was chosen as it is one of the most commonly used and most accurate ways to evaluate antibody concentrations in these different samples. All cows have neither been previously vaccinated nor repeatedly exposed to BoRV, BoCv or E. coli K99, and PI values were very low on day 0. PI values remained low in the control group, but a dramatic seroconversion was observed in vaccinated animal. However, results of the seroconversion were somewhat lower for the F5-adhesin than for the other pathogens assessed, suggesting a less intense immune response. It may also argued that the decrease of antibodies in cow's serum before calving has been observed by many authors, and is due to the transport of immunoglobulins from the blood stream into the lacteal secretion during colostrogenesis. Thus PIs at parturition may be difficult to interpret.

High PI values in the colostrum reflect values of the serum. As demonstrated by others [12], content in IgG of the blood serum declines from 4-5 weeks (even 8 weeks) before parturition until calving, and rate of IgG reduction correlates significantly with the IgG concentrations in the colostrum. Levels of antibodies in the colostrum are of great importance for the protection of calves by passive transfer and correlations between these levels and the protection of calves have been demonstrated previously [13]. The passive transfer is also highly dependent on the amount of colostrum ingested by the calf and the time after birth it is ingested. According to PIs found in the colostrum of cows (first milking) and those found in the serum of calves, it can be assumed that the colostrum was properly fed to calves.

The infectious challenge was carried out according to the applicable requirements of the European Pharmacopeia for infectious challenges with Rotavirus, Coronavirus and *E. coli*. Following a serious infectious challenge, the incidence of diarrhoea as well as the clinical score monitored over 7 to 10 days were significantly lower in calves from vaccinated cows than in control animals. Regarding BoRV, these results confirm that high IgG1 levels to BoRV antibody titres in colostrum correlate with passive protection against challenge with homotypic virus [14]. Although limited to the F5 fimbria, this study also supports the conclusion that passive protection with immune colostrum may be successful in preventing infections with ETEC strains bearing the classical fimbria types (K88, K99, 987P and F41) [15]. There are few reports on oral administration of antibodies to BoCV derived from serum or colostrum. It has been however shown that a significant protection may be achieved in calves treated with high titres colostrum antibodies against a BoCV challenge strain [16]. Results gained in this study do not refute this point.

Excretion of the pathogens was observed in both groups after calves were challenged. Nevertheless, feeding newborn calves with colostrum from vaccinated cows, sharply reduced the proportion of pathogen-excreting animals. In addition, calves fed with colostrum from their vaccinated mother excreted fewer pathogens, and for shorter periods than control calves. It has been substantiated decades ago that feeding calves, or even piglets, with bovine colostrum with a rotavirus-neutralizing activity resulted in a dramatic reduction of the virus excretion after an experimental challenge [17,18]. Regarding *E. coli* K99, our results are somewhat better than those published in an old study where the excretion rate was similar in both groups [19]. Concurrent pathogens were not detected in any of the samples. This is a major bias for assessing the effectiveness of the vaccine under real conditions, when animals are usually under pressure from several pathogens, and where co-infections is frequently observed in diarrheic calves [4], and C. parvum infections are particularly concerning [20].

Conclusion

The data gained in this study show that dosing cows in the last trimester of pregnancy with a single dose of the new vaccine described in this paper results in an indisputable seroconversion. The mechanism of colostrogenesis then leads to the accumulation of antibodies to BoRV, BoCV, and F5 adhesin into the colostrum. When properly fed to newborn calves, this colostrum gives to the young animal a strong passive immunity allowing it to quickly overcome the consequences of an infection by one of the three pathogens.

Disclosure Statement

The first author (L. Durel) as well as C. Rose are employees of Virbac Santé Animale or its affiliates. Tomáš ŽUFFA and Denisa SVITAČOVÁ are employees of Pharmagal-Bio. Both companies have commercial interests in the licensed vaccine described in this study.

Highlights

a. A new vaccine induces a seroconversion to Rotavirus, Coronavirus, and *E. coli* K99.

b. Seroconversion is observed in the pregnant dam's serum, and its colostrum.

c. Transfer of immunity is observed in calves fed on colostrum from vaccinated dams.

d. Calves fed on colostrum from vaccinated dams are less subjected to NCD.

e. NCD pathogen shedding is reduced in calves fed on colostrum from vaccinated dams.

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