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# Immune response of mature cows subjected to annual booster vaccination against neonatal calf diarrhoea with two different commercial vaccines: A non-inferiority study

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ABSTRACT

Neonatal calf diarrhoea can have important economic consequences. Scour vaccines are available against some of the most frequent pathogens responsible for this disease: Bovine Rotavirus (BoRV), Bovine Coronavirus (BoCV) and *E. coli* K99. In this multi-centre, randomised, blinded study, adult cows vaccinated with a trivalent vaccine marketed for years (Rotavec™ Corona, MSD Animal Health - RC) prior to last parturition were re-vaccinated 12–15 months later, prior to the upcoming parturition, with either a single injection of a recently marketed vaccine (Bovigen™ Scour, Virbac - BS), or RC. The aim of this trial was to verify whether BS is not inferior to RC for the stimulation of the immune response and the passive transfer to calves in these conditions.

A total of 136 multiparous dairy cows, from 5 different herds and located in 3 countries (France, UK and Germany) were enrolled in the study. Sixty-five cows were vaccinated with BS and 71 with RC. Antibody levels, measured by competitive ELISA and represented as percentage of inhibition (PI), were assessed in the cow's serum (on the day of vaccination: D0 and on days 21, 42 and at calving), in the colostrum and in the serum of calves in the first week of life. Differences in means of PI between groups and the 95% confidence interval (CI) were calculated. The non-inferiority threshold was set at −10%. The relationships between antibody levels in the colostrum and the vaccination-calving interval (VCI) or the inter-booster vaccination interval (IBVI) were also analysed.

All the lower margins of the 95% CI of the difference in means of PI, in all samples and for the 3 pathogens assessed, were above −10%. This result shows that BS is not inferior to RC for the stimulation of the immune response against BoRV, BoCV and *E. coli* K99 and the passive transfer of immunity to calves when this vaccine is administered to their dams previously vaccinated with RC. Furthermore, no correlation was found between PI values in the colostrum and the VCI or IBVI. The ratio of animals with a PI ≥ 95% in the colostrum, among cows with similar intervals, was not significantly different between groups, for all antigens tested.

Therefore, this study shows that a single injection of the heterologous vaccine BS can be used as a booster in cattle previously vaccinated with RC.

## 1. Introduction

Neonatal calf diarrhoea (NCD) affects mainly calves under 4 weeks of age. It is characterised by a diarrhoea leading to dehydration and acidosis which can have systemic consequences and potentially lead to death (Millemann, 2009). The prevalence of NCD has been estimated around 20% and this disease is a major cause of death in young calves, responsible for more than 50% in some countries (Millemann, 2009;

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Bartels et al., 2010; Lorenz et al., 2011; Cho and Yoon, 2014; Meganck et al., 2015). The loss of calves, the management of the disease and the consequences on the health, growth rate and reproductive potential of surviving calves can lead to a significant economic loss (Millemann, 2009; Cho and Yoon, 2014). The environment, herd size and farm management practices, such as hygiene, feeding, routine use of antibiotics, neonatal care and colostrum management, can influence NCD prevalence in herds (Bendali et al., 1999; Bartels et al., 2010; Meganck et al., 2014). These factors should be evaluated and properly monitored to avoid NCD. The etiological diagnosis of calf diarrhoea can also be sought in order to limit the consequences with the appropriate etiologic or preventive treatment (Millemann, 2009; Meganck et al., 2014, 2015).

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 Enterotoxigenic *Escherichia coli* (ETEC), but other pathogens can also be responsible for this disease (Acres et al., 1977; Athanassious et al., 1994; Millemann, 2009; Cho and Yoon, 2014; Meganck et al., 2015). The clinical features and analysis of the environment can help determine the origin of the disease and several diagnostic tools are now available to confirm the presence of one or more suspected pathogens (Bendali et al., 1999; Millemann, 2009; Cho and Yoon, 2014).

Different strains and serotypes of the NCD causative agents have been identified. Rotaviruses are double-stranded RNA viruses which can be classified by groups according to the genotype of their inner capsid protein VP6. They can be further classified according to their outer capsid proteins, mainly VP7 (glycoproteins, G) and VP4 (protease-sensitive, P) proteins (Matthijnssens et al., 2011). NCD is mainly induced by group A rotaviruses with G6 and G10 and P[1], P[5] and P[11] genotypes being the predominant ones in cows (Snodgrass et al., 1990; Cho and Yoon, 2014; Collins et al., 2014). In Europe, the prevalence of BoRV in diarrheic calves can vary from 30% to 60% but a higher prevalence can be seen in other areas (Bartels et al., 2010; Meganck et al., 2014). They are mainly found in calves at 1–2 weeks of age (Millemann, 2009; Bartels et al., 2010; Cho and Yoon, 2014).

Bovine coronavirus (BoCV), an enveloped and single-stranded RNA virus, also generally affects calves under 2 weeks of age (Millemann, 2009; Boileau and Kapil, 2010; Cho and Yoon, 2014). The pathology of BoCV in NCD is often more severe than that of rotavirus, resulting in a mucohaemorrhagic enterocolitis (Boileau and Kapil, 2010). The prevalence of this pathogen in calves with diarrhoea is generally around 8% in Europe (Bartels et al., 2010). Hemagglutinin-esterase (HE) and spike (S) proteins are important for the fusion with the host intestinal cells and these proteins (and others) contain important neutralizing epitopes with a generally low antigenic variability (Clark, 1993; Tsunemitsu et al., 1995; Boileau and Kapil, 2010; Cho and Yoon, 2014).

Among the six major diarrhoeagenic *E. coli* pathotypes, ETEC is the confirmed main causative agent of NCD (Kolenda et al., 2015). This pathogen is responsible for less than 10% of NCD cases in Europe but higher prevalence have been recorded elsewhere (Acres et al., 1977; Bartels et al., 2010; Meganck et al., 2014). The virulence factors of ETEC are its specific cytotoxic toxins and adhesins (DebRoy and Maddox, 2001; Kolenda et al., 2015). It has been shown recently, in a systematic review, that the fimbrial adhesins F5 (or K99), F17, and F41 fimbriae and the heat-stable enterotoxin (ST) were significantly associated with calf diarrhoea (Kolenda et al., 2015). This type of *E.coli* usually affects very young calves (under 5 days of age) and leads to a rapid dehydration.

*Cryptosporidium parvum* is a protozoan parasite frequently associated with NCD (in 30–60% cases) although infection can also be asymptomatic (Bartels et al., 2010; Cho and Yoon, 2014; Meganck et al., 2014). The oocysts excreted into the faeces can survive for months in the environment and are resistant to most disinfectant. Eliminating this pathogen can therefore be challenging (Cho and Yoon, 2014; Meganck et al., 2015).

Several vaccines have been developed against ETEC, rotavirus and coronavirus but no commercial vaccine is available against *C. parvum* yet (Cresswell et al., 2014). Vaccinating pregnant cows before parturition usually triggers an immune response which leads to the presence of protective immunoglobulins in the colostrum. The calf can then be passively protected if it stands rapidly and is allowed to nurse to satiety, or if the colostrum is fed properly (Kohara et al., 1997; Weaver et al., 2000; Meganck et al., 2015). The vaccines available for calf scours can contain different serotypes, strains or epitope presentations of BoCV, BoRV and ETEC. However, previous studies have shown that the different serotypes of Group A BoRV share similar epitopes and that heterotypic immune response can occur in already exposed cattle. This means that vaccination of adult cows with a single bovine rotavirus serotype will protect against all serotypes to which the cattle has been exposed (Snodgrass et al., 1984, 1991; Brussow et al., 1991; Taniguchi et al., 1991). A cross-reaction between BoCV strains is even more likely since this virus presents a low antigenic variation (Clark, 1993; Tsunemitsu et al., 1995; Boileau and Kapil, 2010). Finally, all vaccines contain the F5 adhesin, the main virulent factor found in calf ETEC responsible for NCD (Nagy and Fekete, 1999; DebRoy and Maddox, 2001; Kolenda et al., 2015; Picco et al., 2015). Therefore, although not demonstrated yet, it should be possible to use a different scour vaccine than the one used for the previous vaccination.

The aim of this multi-centre, randomised, blinded study was to assess antibody responses to BoRV, BoCV and F5 adhesin in adult cows that were vaccinated with a vaccine marketed for years prior to last parturition and revaccinated 12–15 months later, prior to the upcoming parturition, with a single booster-injection of either the same vaccine or a recently marketed new vaccine. This study aimed to evaluate antibody levels were evaluated in the serum of vaccinated dams, in the colostrum and in the serum of new-born calves, in order to evaluate the immune response in cows and the passive transfer in calves. The results of this study should provide evidence for the possibility to efficiently use these two different vaccines on successive vaccination courses.

## 2. Material and methods

### 2.1. Ethical approval

This study was approved by the Internal Ethical Review Committee of Virbac (approval # EU-ERC/201602-01)

### 2.2. Study design

This was a multi-centre, randomized, blinded, reference-controlled study. Animals vaccinated at last parturition with RC were revaccinated prior to the upcoming parturition with either BS or RC. The immune response 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.

For randomisation, animals were allocated to either treatment group according the last digit of their ear tag. Since both vaccines require a different volume of administration, the investigator vaccinating the pregnant cows could not be blinded. Blinding was achieved by assigning a different investigator to collect the samples.

### 2.3. Animals

Healthy pregnant cows from 5 different herds located in 3 countries (France, UK, Germany), with a parity of 2 or more and vaccinated against NCD with RC during the previous pregnancy (generally 12–15 months before depending on the calving-conception interval) were included in the study. Cows enrolled were expected to be due 12 to 3 weeks after vaccination (cows expected to be due outside this time range were not included). Animals were then allocated to vaccination group BS or group RC.

No exclusion was made after enrolment even if one or more samples were lacking. If calving occurred less than 10 days after vaccination, no samples were taken after D0. When twins were born, they were analysed independently.

### 2.4. Vaccines

Rotavec™ Corona and Bovigen™ Scour are tri-valent vaccines formulated to immunise pregnant cows against BoRV, BoCV and the F5-adhesin of *E.coli*.

The immunogenic ingredients of BS 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 F5 (K99) adhesin. The adjuvant used in this vaccine is a water-in-oil-in-water commercial preparation (Montanide ISA 206 VG, Seppic). Two injections during the last trimester of pregnancy are required for the primary vaccination course but a single injection is sufficient to boost the immune status in previously vaccinated cows.

The active substances of RC are the bovine rotavirus strain UK-Compton, serotype G6 [P5] (inactivated); the bovine coronavirus strain Mebus (inactivated); and the E. *coli* F5 (K99) adhesin (antigen). The adjuvants in this vaccine are aluminium hydroxide and an emulsifier. The vaccination regimen requires a single injection in the last trimester of each pregnancy.

In this study, one injection (IM) of vaccine (2 mL for RC and 3 mL for BS) was administered 12 to 3 weeks before calving was expected.

For the passive transfer of immunity, it was recommended that all calves receive sufficient amounts of colostrum (2–4 l according to weight) from their dams within 6 h of birth (Weaver et al., 2000; Conneely et al., 2014).

### 2.5. Collection of samples

Blood samples were collected from the coccygeal vein on day 0 (D0, vaccination day), day 21 (D21), day 42 (D42) and at parturition (calving). Calves were sampled during the first week of life. Blood was collected in labelled 5 mL tubes with a clot activator and clot separator (BD Vacutainer SST II Advance). In order to prevent haemolysis, only frozen serum (collected after maintenance of the blood sample tubes in vertical position for 6–12 h) were shipped to the laboratory for analysis, without additive, in labelled tubes.

Colostrum was collected at the milking parlour at the first milking, into labelled and sterile 100 mL plastic jars. A minimum of 50 mL of colostrum was required. In order to prevent development of bacterial contaminants, colostrum samples were supplemented with 18 mg bronopol (1 tablet/50 mL) and frozen before being sent for analysis.

### 2.6. 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 (Institute of Virology, Centre for Infectious Diseases, University of Leipzig, Leipzig Germany).

### 2.7. Statistical analyses

The only variable recorded during the study was the percentage of inhibition (PI) of optical density assessed by competitive ELISA.

The non-inferiority of BS *vs.* RC for the PI values was assessed using 95% confidence intervals (CI) of the difference between the former vaccine and the latter. As the assumption of normality does not hold for inhibition, the 95% CI of the difference between treatment groups was estimated with the bootstrap method. The bootstrap was performed with repeated samples with the same size as the original one and was done with replacement, using 5000 bootstrap replications (SAS 9.3 software). To calculate the 95% CI with the bootstrapped data, the bootstrap estimates corresponding to the 2.5th percentile and 97.5th percentile were selected. The non-inferiority of BS compared with RC was inferred if the lower bound of the 95% CI of the difference between groups at the end of the study was greater than the non-inferiority margin $\Delta = -10\%$.

Intra-group comparisons were performed using a Wilcoxon signed-rank test. The correlation between PI values in different samples or between PI values and intervals (inter-booster vaccination or vaccination-calving intervals) were assessed according to the Spearman's rank correlation coefficient ($\rho$). A Fisher's exact test was used to compare percentage of animals with specific PI values. For all the tests, an $\alpha$ level of 0.05 two-sided was used. When multiple comparisons were performed, the $\alpha$ level was adjusted using Bonferroni's method.

## 3. Results

### 3.1. Vaccination, parturition and intervals

All cows were vaccinated on D0 (n = 65 in the group 1, and n = 71 in the group 2). The median (min; max) interval between this vaccination and the previous one (inter-booster vaccination interval or IBVI) was 371 (311; 581) days and 388 (300; 580) days in vaccination group BS and group RC, respectively (n = 63 and 69, date of previous vaccination missing for two cows in each group). The median (min; max) interval between this vaccination and calving (vaccination-calving interval or VCI) was of 49 (11; 90) days in vaccination group BS (n = 60, date missing for 4 cows and 1 exclusion due to calving less than 10 days after vaccination) and of 53 (20; 85) days in group RC (n = 66, date missing for 5 cows). Three pairs of twins were born in group 1 and one pair was born in group 2.

### 3.2. Antibody levels in the different samples

All median PIs measured at D0 from cow's serum were > 75% of inhibition in both groups and similar values and ranges were observed between groups (Table 1). At D21, the individual values (% of inhibition or PI) were significantly higher compared to D0 ($p < 0.001$, Wilcoxon signed-rank test), except for BoRV in the vaccination group RC for which no significant difference was observed (Table 1). On D21, the lowest medians were observed for BoRV antibodies (85.3% and 79.4%) and the highest medians were observed for BoCV and F5 antibodies (medians > 98%, Table 1). Due to the high PIs on D0, the medians of individual variations between D21 and D0 were generally low ( < 13% of inhibition, Table 1).

A decrease of median PIs was observed on D42 and at parturition compared to D21 (significant difference at calving *vs.* D21 for all antigens and vaccines except for BoCV in vaccination group RC, Table 1). For BoRV, PI values at calving were even significantly lower than on D0, while for F5 antibodies, PI values were still higher than on D0 (Table 1).

In the colostrum, all median PIs were above 95%, including for BoRV antibodies (Table 1). No significant correlation was found between the IBVI and the PI values in the colostrum for any vaccine and antigen tested (Spearman's coefficient $\rho$ comprised between −0.23 and 0.17, n = 58 and 66, NS) or between the VCI and the PI values in the colostrum ($\rho$ comprised between −0.22 and 0.17, n = 58 and 66, NS). Tables 2, 3 show that, in vaccination group BS, all median PIs in the colostrum were > 95%, independently of the IBVI or VCI. Similar

**Table 1**
Levels of anti-bovine rotavirus (BoRV), anti-bovine coronavirus (BoCV) and anti-F5-adhesin (F5) antibodies, expressed as % of light inhibition measured by competitive ELISA, on D0, D21, D42 and calving time (dams' sera), in the colostra, and in calves' sera. Data are presented as median (min; max) and mean.

| | | D0 | D21 | D42 | Calving | Variation at D21 *vs.* D0 | Colostrum | Calf |
|---|---|---|---|---|---|---|---|---|
| BoRV | Vaccination group BS | 75.6% | 85.3%* | 80.9%# | 61.2%*,# | 6.7% | 97.2% | 87.0% |
| | | (34.7%; 97.2%) | (40.3%; 98.3%) | (14.3%; 98.7%) | (16.8%; 95.3%) | (−17.2%; 54.8%) | (57.5%; 99.1%) | (36.0%; 98.7%) |
| | | Mean: 72.5% | Mean: 80.9% | Mean: 76.2% | Mean: 57.9% | Mean: 8.3% | Mean: 93.7% | Mean: 82.6% |
| | | n = 63 | n = 62 | n = 48 | n = 59 | n = 60 | n = 61 | n = 61 |
| | Vaccination group RC | 78.9% | 79.4% | 75.8% | 58.7%*,# | 1.6% | 96.7% | 82.0% |
| | | (32.8%; 97.9%) | (8.1%; 97.5%) | (8.0%; 97.6%) | (7.9%; 95.3%) | (−39.8%; 39.9%) | (40.2%; 99.3%) | (14.0%; 98.5%) |
| | | Mean: 74.4% | Mean: 75.7% | Mean: 71.5% | Mean: 56.9% | Mean: 2.4% | Mean: 92.9% | Mean: 77.1% |
| | | n = 69 | n = 68 | n = 50 | n = 66 | n = 66 | n = 68 | n = 66 |
| BoCV | Vaccination group BS | 98.4% | 98.8%* | 98.7% | 98.5%# | 0.2% | 98.6% | 98.6% |
| | | (40.9%; 99.5%) | (90.3%; 99.8%) | (70.0%; 99.4%) | (69.5%; 99.5%) | (−4.1%; 58.0%) | (87.6%; 99.5%) | (90.5%; 99.6%) |
| | | Mean: 96.1% | Mean: 98.2% | Mean: 97.8% | Mean: 97.0% | Mean: 2.2% | Mean: 97.9% | Mean: 97.9% |
| | | n = 63 | n = 62 | n = 48 | n = 59 | n = 60 | n = 61 | n = 61 |
| | Vaccination group RC | 98.5% | 98.9%* | 98.8%* | 98.7% | 0.3% | 98.5% | 98.7% |
| | | (64.7%; 99.5%) | (93.7%; 99.5%) | (92.2%; 99.5%) | (90.7%; 99.7%) | (−4.0%; 33.6%) | (86.2%; 99.5%) | (77.1%; 99.5%) |
| | | Mean: 95.2% | Mean: 98.5% | Mean: 98.4% | Mean: 98.0% | Mean: 3.2% | Mean: 97.3% | Mean: 97.6% |
| | | n = 69 | n = 68 | n = 50 | n = 66 | n = 66 | n = 68 | n = 66 |
| F5 | Vaccination group BS | 83.5% | 98.5%* | 98.5%*,# | 97.4%*,# | 12.5% | 97.8% | 98.4% |
| | | (4.4%; 99.3%) | (16.0%; 99.4%) | (15.0%; 99.6%) | (9.3%; 99.6%) | (−2.0%; 90.4%) | (23.8%; 99.2%) | (27.5%; 99.5%) |
| | | Mean: 69.4% | Mean: 91.5% | Mean: 91.3% | Mean: 86.8% | Mean: 23.7% | Mean: 93.7% | Mean: 95.5% |
| | | n = 63 | n = 62 | n = 48 | n = 59 | n = 60 | n = 61 | n = 61 |
| | Vaccination group RC | 88.9% | 98.5%* | 98.2%* | 98.0%*,# | 8.6% | 97.8% | 98.6% |
| | | (0.0%; 99.4%) | (35.8%; 99.7%) | (33.1%; 99.5%) | (23.1%; 99.7%) | (−4.0%; 85.4%) | (68.8%; 99.5%) | (39.9%; 99.5%) |
| | | Mean: 71.8% | Mean: 94.5% | Mean: 93.4% | Mean: 92.4% | Mean: 23.0% | Mean: 95.9% | Mean: 95.1% |
| | | n = 69 | n = 68 | n = 50 | n = 66 | n = 66 | n = 68 | n = 66 |

* Significant difference compared to D0 ($p < 0.008$ - Wilcoxon signed rank test - Bonferroni correction applied)
\# Significant difference compared to D21 ($p < 0.008$ - Wilcoxon signed rank test - Bonferroni correction applied)

**Table 2**
Levels of anti-bovine rotavirus (BoRV), anti-bovine coronavirus (BoCV) and anti-F5-adhesin (F5) antibodies in the colostrum, expressed as % of light inhibition measured by competitive ELISA, depending on the inter-booster vaccination interval (IBVI). Data are presented as median (min; max); n = number of animals with the indicated IBVI ( ± 2 weeks); and the % of animals with a PI ≥ 95% in brackets.

| | | IBVI ≤ 12 months* | IBVI > 12 months* |
|---|---|---|---|
| BoRV | Vaccination group BS | 97% (57%; 99%) | 98% (60%; 99%) |
| | | n = 37 (70%) | n = 22 (86%) |
| | Vaccination group RC | 95% (40%; 99%) | 97% (53%; 99%) |
| | | n = 32 (59%) | n = 34 (79%) |
| BoCV | Vaccination group BS | 99% (94%; 99%) | 99% (88%; 100%) |
| | | n = 37 (95%) | n = 22 (82%) |
| | Vaccination group RC | 99% (93%; 100%) | 97% (86%; 99%) |
| | | n = 32 (94%) | n = 34 (74%) |
| F5 | Vaccination group BS | 98% (70%; 99%) | 98% (46%; 99%) |
| | | n = 37 (84%) | n = 22 (77%) |
| | Vaccination group RC | 98% (74%; 100%) | 97% (69%; 99%) |
| | | n = 32 (88%) | n = 34 (71%) |

* IBVI comprised between 10 and 19 months ( ± 2 weeks)

results were obtained in group RC except for BoRV when calving occurred less than 4 weeks after vaccination (median PI: 88%, n = 3, Table 3). The ratio of animals with similar intervals having a PI ≥ 95% in the colostrum was not significantly different between groups, for all antigens tested (Tables 2, 3).

In the calf's serum, the median PIs were more variable but all above 80% (Table 1). The lowest medians were observed for BoRV antibodies (87% and 82%) while the medians for BoCV and F5-adhesins antibodies were all above 98%. The ranges of PI values were wide however (BoRV antibody PI values varying from 14.0% to 98.5%, for instance, in vaccination group RC, Table 1), suggesting important individual variations, as in the serum of cows at D21 (Table 1).

A positive correlation was found between the PI values obtained in the cow's serum at D21 and the values obtained in calf's serum, for all antigens assessed and with both vaccines (Spearman's coefficient ρ comprised between 0.41 and 0.77, n = 54 and 64, $p < 0.01$ for BoRV, BoCV and F5 antibodies). A positive correlation was also found between the PI values found in the colostrum and those found in the calf's serum (ρ comprised between 0.45 and 0.71, n = 58 and 65, $p < 0.01$ for BoCV

**Table 3**
Levels of anti-bovine rotavirus (BoRV), anti-bovine coronavirus (BoCV) and anti-F5-adhesin (F5) antibodies in the colostrum, expressed as % of light inhibition measured by competitive ELISA, depending on the vaccination-calving interval (VCI). Data are presented as median (min; max); n = number of animals with the indicated VCI ( ± 3–4 days); and the % of animals with a PI ≥ 95% in brackets.

| | | VCI ≤ 3 weeks* | 4 ≤ VCI ≤ 6 weeks | 7 ≤ VCI ≤ 9 weeks | VCI ≥ 10 weeks* |
|---|---|---|---|---|---|
| BoRV | Vaccination group BS | 96% (57%; 98%) | 96% (60%; 99%) | 98% (58%; 99%) | 98% (81%; 99%) |
| | | n = 4 (75%) | n = 18 (78%) | n = 25 (80%) | n = 11 (64%) |
| | Vaccination group RC | 88% (88%; 98%) | 96% (40%; 99%) | 98% (55%; 99%) | 97% (53%; 98%) |
| | | n = 3 (33%) | n = 20 (65%) | n = 30 (77%) | n = 13 (62%) |
| BoCV | Vaccination group BS | 98% (94%; 99%) | 99% (95%; 99%) | 99 (88%; 100%) | 98% (89%; 99%) |
| | | n = 4 (75%) | n = 18 (94%) | n = 25 (88%) | n = 11 (91%) |
| | Vaccination group RC | 99% (95%; 99%) | 99% (93%; 99%) | 98% (86%; 100%) | 98% (90%;.99%) |
| | | n = 3 (67%) | n = 20 (90%) | n = 30 (80%) | n = 13 (85%) |
| F5 | Vaccination group BS | 99% (98%; 99%) | 98% (46%; 99%) | 98% (70%; 99%) | 98% (90; 99%) |
| | | n = 4 (100%) | n = 18 (89%) | n = 25 (84%) | n = 11 (64%) |
| | Vaccination group RC | 98% (95%; 99%) | 98% (74%; 99%) | 98 (86%; 100%) | 96 (69%; 99) |
| | | n = 3 (67%) | n = 20 (85%) | n = 30 (87%) | n = 13 (54%) |

* VCI comprised between 2 and 13 weeks ( ± 3–4 days)

**Table 4**
Difference in means of % inhibition found in the different samples from vaccination groups BS and RC, for anti-BoRV, anti-BoCv and anti-F5-adhesin antibodies. Data are presented as difference of the means [95% CI].

| | D21 | D42 | Calving | Variation at D21*vs.* D0 | Colostrum | Calf |
|---|---|---|---|---|---|---|
| BoRV | 5.2% [−0.1%; 10.4%] | 4.8% [−3.8%; 13.2%] | 1.0% [−7.2%; 8.9%] | 5.9% [1.3%; 10.7%] | 0.8% [−2.7%; 4.3%] | 5.5% [−0.5%; 11.6%] |
| BoCV | −0.3% [−0.8%; 0.3%] | −0.6% [−2.1%; 0.4%] | −1.0% [−2.5%; 0.3%] | −1.0% [−3.7%; 1.9%] | 0.6% [−0.3%; 1.5%] | 0.3% [−0.6%; 1.3%] |
| F5 | −3.1% [−9.1%; 2.6%] | −2.1% [−9.1%; 4.4%] | −5.6% [−1.3%; 1.4%] | 0.7% [−8.4%; 10.1%] | −2.2% [−6.1%; 1.1%] | 0.4% [−3.4%; 3.9%] |

and F5 antibodies with any vaccine and for BoRV in vaccination group RC) except for BoRV antibodies in group BS ($\rho = 0.24$, $p = 0065$).

### 3.3. Non-inferiority tests

To establish the non-inferiority of BS compared to RC, the difference between mean PIs observed with either vaccine (see Table 1) was calculated for each antigen in cow's serum at D21, D42 and at calving, for the variation at D21 *vs.* D0, and in the colostrum and calf's serum. The 95% CI was also calculated (Table 4). For both viruses and F5-antigen antibodies and in all samples or variation assessed, the lower value of the 95% CI was $> -10\%$ of inhibition (Table 4), showing that BS was not statistically inferior to RC.

## 4. Discussion

In this study we assessed if cows vaccinated with RC at the previous pregnancy could be efficiently revaccinated with a single injection of BS and induce an immune response not inferior to the one found in the group of cows revaccinated with RC. The immune response was evaluated in the serum of cows and calves and in the colostrum to evaluate the passive transfer, assuming calves were fed properly with the colostrum. The percentage of inhibition (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 (Taniguchi et al., 1991; Weaver et al., 2000; Ohlson et al., 2010).

Despite some individual PI values below 10% for F5-adhesin antibodies at D0, the median values for all antibody levels assessed (BoRV, BoCV and F5-adhesin antibodies) were above 75% of inhibition at D0. The maintenance of a high immune status may be the consequence of the previous vaccination (around 12–15 months before) as well as frequent contacts with the pathogens in the environment.

Boosting immunity with either vaccine resulted in a significant increase of PI values at D21 (except for BoRV antibodies in vaccination group RC – no significant increase). These results show that a boost of immunity against BoRV, BoCV and ETEC F5-adhesins can be achieved in group BS cows previously vaccinated with RC, despite the differences in serotypes (BoRV), strains (BoCV) and antigen presentations (F5 adhesin) between vaccines. These results can be explained by the shared antigens and the cross-reactivity between BoRV serotypes and between BoCV strains (Snodgrass et al., 1984; Chiba et al., 1986; Brussow et al., 1991; Clark, 1993; Tsunemitsu et al., 1995). Indeed, previous studies had found that vaccinating against one BoRV serotype increase the immune response against all serotypes the cow had been in contact with (Snodgrass et al., 1984, 1991; Taniguchi et al., 1991). Concerning F5 antibodies, the fact that a heterologous primo-vaccination, with the same antigen but a different presentation, is possible with F5-adhesin is not surprising and similar results have been obtained with other antigens in humans (Lu, 2009).

By comparing the means at D21 or the means of the variation D21-D0, BS was found to be non-inferior to RC. The lower limit of the 95% CI of the difference in means of variation was even $> 0$ for BoRV antibodies (Table 2), suggesting that BS might even be superior to RC to stimulate the immune response against this virus. Such a difference could be explained, for example, by the use of a different serotype for revaccination. The better efficiency of a heterologous prime-boost vaccination over an homologous one has been observed previously with viral vectors (Lu, 2009; Zhou et al., 2011; Choi and Chang, 2013). The difference in adjuvants could also explain the difference in immune response stimulation. This would suggest that the Montamide™ ISA adjuvant (based on purified squalene and squalane) present in BS would be more efficient to stimulate BoRV immune response than those present in RC (aluminium hydroxide and light mineral oil/emulsifier). The advantages of the former adjuvant have been demonstrated previously with other antigens (Patil et al., 2002; Jang et al., 2010; Khorasani et al., 2016).

Antibody levels in cow's serum at D42 and at calving were lower than at D21. The decrease observed in BoRV antibodies was greater than the one observed with the other antibodies so that the median PI at calving was even lower than at D0 while the median PIs were still $>$ 95% for BoCV and F5 antibodies. This result suggests a less intense and more labile immune response against BoRV than against the other antigens assessed. The decrease of antibodies in cow's serum before calving has been observed previously and is due to the transport of immunoglobulins from the blood stream into the lacteal secretion during colostrogenesis (Kohara et al., 1997; Weaver et al., 2000; Murphy et al., 2005). Indeed, in the colostrum, all the median PIs were above 95% in both groups while some median values in the serum of cow at calving were below 65% (for BoRV). Therefore, the results obtained in this study confirm that high antibody levels can be reached in the colostrum with both vaccines when they are administered at the right time (12 to 3 weeks before parturition). A minimum of 2–3 weeks is usually necessary between vaccination and parturition to allow the immune response to reach a maximum level (in around 3 weeks) when the passage of immunoglobulins to the mammary gland is at its peak 1–3 days before parturition) (Radostits, 1991; Weaver et al., 2000).

In this study, vaccination-calving intervals (VCI) ranged between 2 and 13 weeks with a median of 7 or 8 weeks and no significant correlation was found between VCI and antibody levels in the colostrum. However, the BoRV antibody levels obtained in the colostrum of cows vaccinated with RC less than 4 weeks before calving were lower than when the VCI was longer. Consistently with the lack of a significant increase at D21 compared to D0, this result suggests that more than 3 weeks is required for the immune response against BoRV to fully develop with this vaccine. Analysing the correlation between IBVI and antibody levels in the colostrum also showed that these two parameters were not linked for any of the antigens assessed. This means that a single injection of BS or RC at least 3 weeks before parturition increases the concentration of specific immunoglobulins in the colostrum regardless of the cow's vaccination history 10–19 months before in this study).

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 (Radostits, 1991). The passive transfer is also highly dependent on the amount of colostrum ingested by the calf and the time after birth it is ingested. It is advised (and such recommendations were given to farmers in this trial) to feed the calf with 2–4 litters of colostrum according to the calf's weight, in the first 4–6 h of life, when the transfer of macromolecules (including antibodies) across epithelial cells is optimal (Radostits, 1991; Weaver et al., 2000; Trotz-Williams et al., 2008; Conneely et al., 2014; Meganck et al., 2015). It has been estimated that

20–40% or even more calves could suffer from failure of passive transfer (FPT), which is a risk factor for NCD (Weaver et al., 2000; Cho and Yoon, 2014; Meganck et al., 2014; Raboisson et al., 2016). Other parameters such as the colostrum management and feeding practices are also involved in FPT (Weaver et al., 2000; Meganck et al., 2014). For instance, FPT is less likely when known amount of colostrum are given (with a nipple bottle, an oesophageal tube or in a bucket) instead of leaving the calves to suckle colostrum from their mothers (Filteau et al., 2003; Trotz-Williams et al., 2008). In the farms included in the study, the colostrum was generally given in buckets or *via* oesophageal tube, limiting the risks of FPT. Indeed, according to the high levels of antibodies found in the calf's serum (median PIs > 80%) and the good correlation between PIs found in the serum of cows at D21 or in the colostrum and those found in the serum of calves, it can be assumed that the colostrum was given properly. The variability in antibody levels found in the serum of calves can therefore be attributed to the variability found in the dams and in the colostrum rather than to the variability in colostrum feeding processes. Like in the other samples, the mean PIs found in the serum of calves was similar between groups for all antigens assessed and the BS vaccine was statistically not inferior to RC for this parameter.

Since calf diarrhoea is not only due to BoRV, BoCV and ETEC infections but can also be due to infections by other viruses (like bovine viral diarrhoea virus), bacteria (like salmonella) or parasites (like C. *parvum)* (Millemann, 2009; Cho and Yoon, 2014), the prevalence of calf diarrhoea in offspring of vaccinated cows was not recorded. However, the high PI values found in the calves of vaccinated cows in this study suggest that the calves will be protected from affections induced by BoRV, BoCV and ETEC (Radostits, 1991). Therefore, although vaccination should not be used as a sole preventive intervention, especially because it does not protect against all pathogens, it remains an efficient way to protect calves against the main pathogens of NCD (Radostits, 1991; Kohara et al., 1997; Bendali et al., 1999; Cho and Yoon, 2014; Meganck et al., 2014, 2015).

## 5. Conclusion

The data obtained in this study show that vaccinating multiparous pregnant dairy cows with either RC or BS while they were vaccinated with RC at the previous pregnancy gives similar results in terms of antibody levels in the serum of cows and calves and in the colostrum. The BS vaccine was found to be statistically non-inferior to RC for these parameters, despite the difference in serotypes, strains and antigen presentations between the two vaccines. This study also confirms that an efficient passive transfer of immunity can be achieved when vaccination of dams and colostrum intake are properly managed. The finding that a single injection of BS can be used as a booster in cattle previously vaccinated with another scour vaccine should facilitate the prophylactic vaccination campaigns in the field, especially when some vaccine shortage occurs or when another vaccine than the one previously used must be chosen for financial reasons.

## Disclosure statement

The first author (L. Durel) as well as C. Rose and J. Bennemann are ~~an~~ employees of Virbac Santé Animale or its affiliates. Virbac produces a commercially licensed vaccine for the control of NCD.

## Conflict of interest statement

This research being reported in this paper was supported by Virbac Santé Animale, Carros, France, a company with financial interest in the subject matter discussed in this manuscript.

The first author (L. Durel) as well as C. Rose and J. Bennemann are an employees of Virbac Santé Animale or its affiliates. Virbac produces a commercially licensed vaccine for the control of NCD.

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