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Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus core-like particle (CLP) or virus-like particle (VLP) vaccines

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Heterotypic passive immunity to IND (P5/G6) bovine rotavirus (BRV) was evaluated. Three groups of calves (n = 5 per group) were fed 1% pooled colostrum supplements (birth to 7 days of age) from BRV seropositive cows vaccinated with recombinant SA11(P2/G3) rotavirus-like particles (VLPs), recombinant SA11 rotavirus core-like particles (CLPs), or inactivated SA11 rotavirus (SA11). Control calves (n = 5 per group) received either pooled colostrum from unvaccinated (BRV field exposure seropositive) control cows, or no colostrum. IgG1 antibody titers to IND BRV for the pooled colostrum were: 1048576 (VLP); 1048576 (CLP); 262144 (SA11); and 16384 (control colostrum). Elevated titers of BRV neutralizing (VN) antibodies were present in VLP colostrum (98000), and SA11 colostrum (25000), but not in CLP colostrum (1400), compared to colostrum from nonvaccinates (2081). Calves were orally inoculated with virulent IND BRV at 2 days of age and challenged at post-inoculation day (PID) 21. Calves were monitored daily for diarrhea and faecal BRV shedding through PID 10 and post-challenge day (PCD) 10. After colostrum feeding, the IgG1 antibody titers were highest in serum and faeces of calves fed VLP and CLP colostrum, but VN and IgA antibodies were highest in calves fed VLP colostrum. After BRV inoculation, calves fed colostrum from vaccinated cows had significantly fewer days of BRV-associated diarrhea and BRV shedding than control calves. All calves fed VLP colostrum were protected from diarrhea after BRV inoculation; two calves shed BRV. In the CLP colostrum group, one calf developed BRV-associated diarrhea and all calves shed virus. In the SA11 colostrum group, three calves developed BRV-associated diarrhea and four calves shed virus. BRV-associated diarrhea and shedding occurred in 9 of 10 control calves. Active IgM antibody responses occurred in faeces and/or serum of most calves after BRV inoculation. However, the highest active antibody responses (IgM and IgG1 in serum, and IgM, IgG1 or IgA in faeces) after BRV inoculation were in calves fed control or no colostrum, in association with clinical diarrhea in most of these calves. After challenge at PID 21, BRV-associated diarrhea and shedding were of short duration or absent, in all groups. These results demonstrate the efficacy of colostrum from VLP vaccinated cows to provide heterologous, passive protection against BRV diarrhea and shedding in calves. In comparison, calves fed CLP or SA11 colostrum were only partially protected against BRV diarrhea or shedding. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: recombinant rotavirus subunit vaccines; core-like particles; virus-like particles; passive immunity in calves; bovine rotavirus

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Bovine rotavirus (BRV) is an important cause of diarrhea in calves under 3 weeks of age. Protection against BRV diarrhea and infection in calves during the nursing period has been correlated with high levels of neutralizing antibodies in the calf's intestine, mainly associated with IgG1, passively acquired via colostrum and milk. Although most cows are seropositive to BRV due to field exposure, in unvaccinated cows, antibody titers in milk decline to unprotected levels after parturition. Parenteral vaccination of cows with experimental rotavirus vaccines effectively enhances IgG1 BRV antibodies in serum which are selectively transported into colostrum and milk. However, the success of commercial maternal rotavirus vaccines for elevating colostral and milk antibody titers and providing passive immunity against rotavirus to calves has been variable in the field, necessitating the development of improved vaccines. Production of double-layered rotavirus core-like particles (CLPs) composed of rotavirus inner capsid proteins VP2/6 or triple-layered rotavirus-like particles (VLPs) composed of inner and outer capsid proteins VP2/4/6/7, by the co-expression of rotavirus genes, in recombinant baculovirus expression systems represents a new strategy for the development of rotavirus subunit vaccines. Previous studies have indicated that the VLPs are antigenically authentic, highly immunogenic and induce neutralizing and ELISA antibody responses in guinea pigs, rabbits, mice and cattle. An SA11 VLP vaccine administered parenterally to rotavirus seronegative rabbits induced total to partial protection against infection after virus challenge. Recently, Fernandez et al. reported that ELISA antibody titers to the IND (P[5]G6) strain of BRV were significantly enhanced in serum, colostrum and milk of cows vaccinated with the heterologous simian rotavirus SA11 (P[2]G3) VLPs, CLPs and inactivated virus vaccines. However, neutralizing antibody titers were significantly elevated only in the cows vaccinated with SA11 VLPs or inactivated virus.

The objectives of this study were twofold: first, to evaluate protection against BRV-induced disease in newborn calves fed supplemental pooled colostrum from cows vaccinated with recombinant VLP or CLP vaccines, inactivated SA11 rotavirus, or unvaccinated cows (controls); and second, to determine the BRV antibody isotype titers in serum and faeces of these calves. We also examined if calves that were passively protected after the initial inoculation with BRV were immune to a subsequent BRV challenge exposure 21 days later.

MATERIALS AND METHODS

Recombinant vaccines

Recombinant VLP were prepared by the coexpression in baculovirus of Rp VP2 and SA11 VP4, VP6, and VP7. Recombinant CLP were produced by the coexpression of Rp VP2 and SA11 VP6.

Colostrum pools

To produce the immune colostrum, three groups of pregnant Holstein cows were vaccinated with one of three SA11 rotavirus vaccines in Incomplete Freund's adjuvant (IFA) by the combined intramuscular and intramammary routes, as described previously. Briefly, cows received 100 µg or 250 µg of VLP antigen, or 250 µg of CLP antigen, or 5 ml of BE1 inactivated SA11 rotavirus (1x10^6 PFU ml^-1 preinactivation titer). Vaccines were administered in 5 ml doses with an equal volume of incomplete Freund's adjuvant, intramuscularly one week before drying off, and intramammary 2 weeks later. Four separate whole colostrum pools were prepared (first-milking colostrum) from six cows inoculated with the recombinant VLP vaccine; three cows inoculated with the recombinant CLP vaccine; five cows inoculated with the 2-bromothymidine-1-namine (BEI)-inactivated SA11 vaccine, and from four uninoculated control cows. The entire first milking colostrum was pooled for each separate group and mixed thoroughly. Pooled colostrums were then aliquoted and stored at −20°C for the passive protection studies. Why was obtained from each colostrum pool as described previously and used in assays to determine the virus neutralization and the isotype (ELISA) antibody titters to IND BRV.

Colostrum feeding and calf inoculation

Unsuckled, newborn, colostrum-deprived (CD), male Holstein calves were procured and maintained as described previously. Five calves were assigned to each of four colostrum feeding groups (VLP, CLP, inactivated SA11, or control colostrum) and were fed 1% supplemental pooled colostrum (20 ml of colostrum in 21 of cows milking infant formula) from each of the respective colostrum pools twice a day from birth through 7 days of age. Five control calves were not fed colostrum, but maintained on infant formula. All calves (between 20 and 30 hours of age) were orally inoculated with a suspension of 5 ml of IND BRV (titer ≅1x10^6 FFU ml^-1) in 20 ml of phosphate-buffered saline solution (PBS), pH 7.4, prepared from the intestinal contents of an infected gnotobiotic calf. IND BRV (titer ≅1x10^6 FFU ml^-1) for oral challenge 21 days later, was prepared and administered in a similar manner.

Clinical observations and sample collection.

Daily records (birth to PID 10 and PCD 0–10) were maintained of clinical signs and observations on stool color and consistency (faecal scores 0 = normal to 3 = liquid, a score of ≥2 indicating diarrhea). Faecal samples were collected daily from birth through PID 10 and from PCD 0 to 10. Serum samples were collected before the initiation of colostrum feeding (within 2 h after birth), at inoculation (~20–30 h of age) and weekly through post-inoculation day (PID) 35. For detection of coproantibodies, faecal samples were suspended in PBS (dilution 1:16), centrifuged 15 min at 800g and filtered through 0.45 µm syringe filters. Serum samples were inactivated at 56°C for 30 min, then stored frozen at −20°C for subsequent testing.

Detection of rotavirus

To detect rotavirus shedding, faecal samples were examined by immunoelectron microscopy.
culture immunofluorescence (CCIF)\textsuperscript{16} and antigen capture ELISA\textsuperscript{15}, as previously described. A sample was considered rotavirus positive if it was positive in any one of the three assays. Rotavirus positive samples were also evaluated by polyclonal gel electrophoresis (PAGE)\textsuperscript{16} to confirm the dsRNA electropherotype of the challenge strain of BRV (IND).

Isotype antibody ELISA

IgG1, IgG2, IgA and IgM antibody responses to IND BRV (P\textsuperscript{[5]}G\textsuperscript{[6]}) were analysed in all serum and weekly faecal specimens using an indirect isotype-specific ELISA as previously described\textsuperscript{17}. Briefly, polystyrene, 96-well microtiter plates were coated with 7 \(\mu\)g ml\(^{-1}\) (in 0.06 M bicarbonate buffer, pH 9.6) of hyperimmune antiserum against BRV produced in a gnotobiotic pig (capture antibody). Plates were stored for up to 7 days at 4°C. Plates were washed using PBS containing 0.05% Tween 20 (PBS-T) prior to the addition of standard volumes of 0.1 ml well\(^{-1}\) of each reagent (diluted in PBS-T) followed by 1 h sequential incubations of each reagent at 37°C in a humid CO\textsubscript{2} atmosphere. Semi-purified IND BRV from infected MA104 cell cultures or mock-infected MA104 cell culture control fluids were added to each well followed by addition of the test serum or faecal samples (serial fourfold dilutions starting at 1:16) to antigen-coated and control (mock antigen) wells. Secondary antibodies consisting of MAb (from ascites) to bovine IgG, IgM (1:10000) and IgA (1:5000) were added, each to separate plates followed by the indicator antibody, goat anti-mouse IgG (H+L), F(ab\textsuperscript{\prime})\textsubscript{2}, conjugated to alkaline phosphatase (1:10000), and the substrate, p-nitrophenyl phosphate in 10% diethanolamine buffer (pH 9.6). The absorbance was read in an ELISA reader at 405 nm. The ELISA antibody titers were expressed as the reciprocal of the highest sample dilution which had an absorbance of \(> 3 \text{ SD}\) above the background control (samples in mock antigen wells).

Virus neutralization test

Colostral whey from each colostrum pool were processed\textsuperscript{11} and along with calf sera and faeces\textsuperscript{25} were tested in plaque reduction virus neutralization tests to detect neutralizing antibody titers against IND BRV\textsuperscript{17}. Antibody titers were expressed as the reciprocal of the highest sample dilution which resulted in an 80% reduction in plaques\textsuperscript{15}.

Statistical analyses

Statistical analyses were performed on the log transformed values of the reciprocal IgG1 and IgA antibody titers. Days of BRV shedding, days of BRV-associated diarrhea, and cumulative faecal scores were also analysed. Diarrhea was considered BRV-associated if rotavirus antigen or virus was detected at any time during the period of diarrhea. Cumulative faecal scores were calculated for 0–10 PID inclusive, this time interval was used as most representative of the protective effects of oral colostral supplements fed up to and including PID 6.

Analyses of variance (ANOVA) were performed to detect significant differences among experimental groups. When such differences were noted, the least significant difference (LSD) test was applied. A significance level of \(p \leq 0.05\) was used throughout.

RESULTS

Virus neutralization and ELISA isotype antibody titers in colostrum pools

Virus neutralization and ELISA isotype antibody titers in colostrum pools against IND BRV are summarized in Table 1. The VLP colostrum pool had the highest VN antibody titer (~47-fold higher than the control colostrum pool), followed by the inactivated SA11 colostrum pool (~12-fold higher than the control colostrum pool). The VN antibody titers of the CLP and control colostrum pools were similar. In ELISA, IgG1 antibody titers to BRV were highest in the VLP and CLP colostrum pools (~4 and 64-fold higher than SA11 and control colostrum pools, respectively) and were the predominant antibody isotype in colostrum. The IgA antibody titer of the VLP colostrum pool was ~16-fold higher than the CLP and inactivated SA11 colostrum pools and ~1000-fold higher than the control colostrum pool. Higher, but low IgM antibody titers were detected in vaccinated colostrum pools compared with control colostrum, and low IgG2 antibody titers to BRV were detected only in the VLP colostrum pool.

Protection against BRV shedding and diarrhea after inoculation

BRV shedding in faeces, when detected, was detected by all 3 assays (IEM, ELISA and CCIF) with one exception; in calf 288 (CLP colostrum group), BRV was detected after challenge only by ELISA. The rotavirus dsRNA electropherotypes detected by PAGE in faeces of inoculated calves shedding BRV were consistent with the electropherotype of the IND BRV strain used for inoculation and challenge.

Table 1. Virus neutralization (VN) and ELISA isotype antibody titers to IND BRV in bovine colostrum pools from cows vaccinated intramuscularly and intramammarily with SA11 VLPs, CLPs or inactivated rotavirus vaccines, or from non-vaccinated controls

| Vaccine colostrum group | VN | IgG1 | IgG2 | IgA | IgM |
|-------------------------|----|------|------|-----|-----|
| VI P (n = 6)\textsuperscript{a} | 98 000 | 1 048 576 | 16 | 65 536 | 64 |
| CLP (n = 3) | 1400 | 1048 576 | <4 | 4096 | 16 |
| SA11 inact. (n = 5) | 25 000 | 262 144 | <4 | 4096 | 64 |
| Control (n = 4) | 1800 | 16 384 | <4 | 64 | <4 |

\textsuperscript{a}n = Number of cows in each group from which entire first milking colostrum (ranging from 2 to 18 l from each cow) was collected, pooled, and mixed thoroughly. Geometric mean antibody titers of samples from cows contributing to the pools were published previously\textsuperscript{15}.

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significantly fewer days of BRV-associated diarrhea than calves receiving control colostrum or no colostrum (Table 2). In addition, diarrhea was significantly less severe (as measured by the cumulative faecal scores) in calves receiving VLP and CLP colostral supplements than in calves in control groups. Although differences among the three vaccine groups were not statistically significant, calves in the VLP colostrum group had the lowest mean days of virus shedding, and the lowest mean cumulative faecal score. None of the VLP colostrum calves experienced BRV-associated diarrhea; however one calf developed BRV-associated diarrhea at PID 12 for 3 days, 6 days after colostrum supplementation ended (data shown are for PID 0–10). The onset PID of BRV diarrhea and shedding for each calf are shown (Table 3).

**Passive antibodies to BRV**

Antibody titers in serum and faeces of the calves in the different colostrum feeding groups are summarized (Table 3, Figures 1 and 2). At the initial sampling (~2 h after birth), all calves were negative for rotavirus antibodies (titers <16) in serum and faeces (data not shown). Serum and faecal antibody titers of calves fed colostrum from vaccinated cows increased after the first feeding, but peak faecal antibody titers were not attained in some calves until 6 days of age (PID 5), possibly due to delayed passage of gastrointestinal contents in these individuals (Table 3). Serum and faecal IgG1 antibodies were significantly higher in calves fed VLP and CLP colostrum than in calves in control groups; titers decreased after cessation of colostrum feeding (Figures 1 and 2). Titers of serum and faecal IgA antibodies were significantly higher in calves receiving VLP colostrum, than in calves in the other four groups. Virus neutralizing antibody titers were low (GMT <100) in faeces of calves in all groups, within 5 days of BRV inoculation, with the exception of faeces from calves fed VLP colostrum (GMT = 1660 data not shown), corresponding to the highest VN antibody titers detected in the pooled VLP colostrum (Table 1).

**Protection against BRV shedding and diarrhea after challenge**

We attempted to clarify whether calves which were passively protected after inoculation with BRV develop immunity to subsequent challenge. After challenge (PID 21) there were no significant differences in the number of days of BRV shedding, or in days of BRV-associated diarrhea among the vaccine colostrum and control groups (Table 2). Virus shedding was of short duration post-challenge; mean days of shedding were below 1.4 days for all groups. Similarly BRV-associated diarrhea was infrequent (a total of three calves in three different groups) and of short duration (≤2 days in all cases, Table 4). However, significant differences were evident in IgG1 antibody GMT in serum and faeces at the time of challenge at PID 21, with the highest GMT in the control groups comprised of calves recovered from BRV shedding and diarrhea after inoculation (Table 4).

**Active immune responses in BRV inoculated and challenged CD calves**

In calves that received control colostrum or no colostrum, peak IgM antibody responses to BRV were detected in serum and faecal specimens at PID 7–14, preceding IgG1 and IgA antibody responses which occurred at PID 14–21 (Figures 1 and 2). IgG2 antibody titers to BRV were detected only in serum after challenge, at PCD 7–14. IgA antibody titers to BRV increased in serum and faeces of control and no colostrum fed calves after BRV challenge. The highest active antibody responses (IgM and IgG1 in serum, and IgM, IgG1 and IgA in faeces) occurred in these two groups (Figures 1 and 2).

In VLP colostrum fed calves, a low IgM antibody response to BRV was detected in serum and faeces after inoculation, and an increase in IgM and IgG1 antibodies was detected in faeces after challenge (Figures 1 and 2). In the CLP colostrum fed calves, a transient IgM antibody response was detected in faeces after BRV inoculation, but no increased IgG1 or IgA antibody responses were detected after BRV inocula-

| Table 2 | Summary of responses to rotavirus inoculation and challenge of calves fed colostrum pools from cows vaccinated with SA11 VLP, CLP or inactivated rotavirus vaccines, or non-vaccinated controls |
|---------|----------------------------------------------------------------------------------------------------------|
| Colostum feeding group | Mean days rotavirus shedding | Mean days rotavirus diarrhea (PID 0–10)* | Mean cumulative faecal score* (PID 0–10) | Mean days rotavirus shedding | Mean days rotavirus diarrhea* (PCD 0–10) |
| VLP colostrum | 1.6** | 0* | 0.8* | 0.8 | 0 |
| CLP colostrum | 3.4* | 0.6* | 1.7* | 1.4 | 0.2 |
| Inact. SA11 colostrum | 3.2* | 1.2* | 6.6** | 0 | 0 |
| Control colostrum | 5.8* | 3.6* | 9.4* | 0.8 | 0.5 |
| No colostrum | 6.0* | 7.6* | 17.4* | 0.5 | 0.5 |

*Unsuckled newborn calves were fed 1% colostrum pools (twice daily from birth through 7 days of age) from the indicated groups and orally inoculated with IND BRV at 20–30 hours of age, and orally challenged with IND BRV at 21 days of age.

Days of diarrhea from PID 0 to PID 10 were analysed as the most representative of protective effects of oral colostral supplements fed up to and including PID 6.

Stool consistency was scored daily (0 = normal to 3 = liquid). Cumulative scores for PID 0 to PID 10 were calculated as a measure of severity of diarrhea.

Six calves experienced mild diarrhea in the absence of rotavirus shedding, possibly related to diet or other unidentified infections, and were not considered to have rotavirus diarrhea for purposes of calculation. Two of these calves were in the CLP colostrum group, two were in the SA11 colostrum group, and two were in the no colostrum group.

Groups in the same column with different letter superscripts differ significantly (p < 0.05); groups in the same column with the same letter superscript do not differ significantly; groups in the same column but lacking letter superscripts do not differ significantly.
tion or challenge (Figures 1 and 2). In the inactivated SA11 colostrum fed calves, peak IgM responses were detected in serum and faeces after inoculation, preceding peak IgA antibody responses in faeces (Figures 1 and 2). The IgG1 antibody titers in serum and faeces remained constant or decreased after challenge.

**DISCUSSION**

Previous studies have demonstrated that calves fed colostrum from cows immunized with experimental rotavirus vaccines are protected against rotavirus infection and diarrhea.\(^{14,19}\) Protection in calves correlated with the presence of high levels of neutralizing rotavirus antibodies in the colostrum, mainly associated with IgG.\(^{14,17}\) Because variable results have been reported in field trials using inactivated or live vaccines, it is essential to develop ways to enhance passive immunity to improve the efficacy of rotavirus vaccines. Recently, Fernandez et al.\(^{14}\) reported significantly increased BRV antibody titers, associated with mainly IgG1 in colostrum and milk of cows vaccinated with VLP and CLP subunit vaccines or inactivated SA11 rotavirus, confirming previous results using rabbits and mice.\(^{12,20}\) In this study we analysed the efficacy of the 7 day feeding of colostrum supplements obtained from cows vaccinated with SA11 rotavirus VLP and CLP recombinant vaccines or an inactivated vaccine, for protection of unsuckled colostrum-deprived calves against BRV challenge.

IgG1 antibodies to BRV were predominant in the colostrum pools used in these experiments, followed by IgA (16–256-fold lower). However IgA antibody titers were at least 16-fold higher in the VLP colostrum pool, compared to the CLP or inactivated SA11 colostrum pools. Although the IgG1 titers were higher in the

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**Table 3** Responses to rotavirus inoculation of newborn calves fed colostrum pools from cows vaccinated with SA11 VLP, CLP or inactivated rotavirus vaccines or non-vaccinated controls

| Colostrum feeding group | Peak antibody titer to BRV \(^{a}\) | Rotavirus shedding (No. pos/total no.) | Rotavirus diarrhea (No. pos/total no.) | Cumulative fecal score \(^{b}\) (PID 0–10) |
|-------------------------|-------------------------------|---------------------------------|---------------------------------|--------------------------|
| VLP colostrum           |                               |                                 |                                 |                          |
| 256                     | 1024                          | 65536                           | 64                              | 4096                                   |
| 267                     | 4096                          | 65536                           | 16                              | 256                                    |
| 268                     | 1024                          | 65536                           | 16                              | 269                                    |
| 269                     | 16384                         | 65536                           | 16                              | 1024                                   |
| 286                     | 1024                          | 16384                           | 64                              | 256                                    |
| GMT                     | 2353                          | 49667                           | 28                             | 588                                    |
| CLP colostrum           |                               |                                 |                                 |                          |
| 258                     | 1024                          | 16384                           | 4                               | 236                                    |
| 269                     | 4096                          | 65536                           | 4                               | 5                                       |
| 290                     | 4096                          | 16384                           | 4                               | 3                                       |
| 291                     | 4096                          | 16384                           | 4                               | 4                                       |
| 293                     | 4096                          | 16384                           | 4                               | 3                                       |
| GMT                     | 3104                          | 21619                           | 4                               | 9                                       |
| Inact. SA11 colostrum   |                               |                                 |                                 |                          |
| 283                     | 256                           | 1024                            | 16                              | 4                                       |
| 284                     | 256                           | 256                             | 4                               | 4                                       |
| 285                     | 256                           | 256                             | 4                               | 4                                       |
| 294                     | 64                            | 16                              | 4                               | 3                                       |
| 296                     | 4096                          | 4096                            | 4                               | 16                                      |
| GMT                     | 446                           | 336                             | 5                               | 5                                       |
| Control colostrum       |                               |                                 |                                 |                          |
| 272                     | 64                            | 64                              | 4                               | 2                                       |
| 274                     | 64                            | 4                               | 4                               | 2                                       |
| 278                     | 64                            | 16                              | 4                               | 4                                       |
| 290                     | 64                            | 16                              | 4                               | ND                                     |
| 281                     | 16                            | 4                               | 4                               | 3                                       |
| 282                     | 16                            | 64                              | 4                               | 2                                       |
| GMT                     | 37                            | 10                              | 4                               | 2                                       |
| No colostrum            |                               |                                 |                                 |                          |
| 262                     | 4                             | 4                               | 4                               | ND                                     |
| 265                     | 4                             | 4                               | 4                               | 1                                       |
| 271                     | 4                             | 4                               | 4                               | 2                                       |
| 298                     | 4                             | 4                               | 4                               | 3                                       |
| 299                     | 4                             | 4                               | 4                               | 2                                       |
| GMT                     | 4                             | 4                               | 4                               | 4                                       |

\(^{a}\)Serum samples were collected at PID 0. Antibody titers for fecal samples represent the peak between PID 0 and 5; fecal antibody titers showed a delayed increase within this time in some calves, possibly due to delayed passage of intestinal contents

\(^{b}\)Unsuckled newborn calves were fed 1% colostrum pools (twice daily from birth through 7 days of age) from the indicated groups and orally inoculated with IND BRV at 20–30 hours of age. The post-inoculation day of onset and the duration of BRV shedding and diarrhea are noted

\(^{c}\)The sum of the daily scores for each group is shown for each group

\(^{d}\)Calves with antibody titers of < 16 were designated as 4 for calculations of GMT. ND = not done

\(^{e}\)Groups in the same column with different superscripts differ significantly (p < 0.05)
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colostrum pools from VLP, CLP and inactivated SA11 vaccinated cows than in the control colostrum pool, the highest IgG1 titers were detected in the VLP and CLP colostrum pools. In contrast, the VN antibody titer for the CLP colostrum pool was similar to control colostrum, confirming the inability of VP2 and VP6 proteins to induce VN antibodies even in field exposed, rotavirus seropositive cows. Others have reported similar failure of VP2 and VP6 proteins to induce VN antibodies in other experimental animals14-23, although VP6 constructs induced antibodies that provided partial passive immunity to mice22,23. Experimental work using mice has demonstrated that SA11 rotavirus, VP4 peptides or constructs coupled to or assembled with VP6 and consisting of VP6, VP6/7 or VP6/4/7 induced formation of neutralizing antibodies affording passive protection to newborn mice; however, the best passive protection was induced by VP6/4/722-24. Moreover, administration of purified VP7, baculovirus-expressed VP7 or a VP7 peptide alone failed to increase neutralizing antibody titers in cows25 or mice22,23.

Studies indicate that there is a positive association between antibody titers in colostrum and antibody

Serum Antibody Titers

![Graphs showing geometric mean isotype antibody titers (GMT) to BRV in serum of unsuckled calves supplemented with colostrum from cows vaccinated with SA11 VLPs, CLPs or inactivated rotavirus vaccines, or from nonvaccinated cows, or with no colostrum. Calves received colostrum twice daily from birth through 7 days of age. The day of BRV inoculation (PID 0) is indicated by a single arrow, and the day of BRV challenge (PID 21, PCD 0) by double arrows.](image)

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titers in serum and faeces of calves after colostrum feeding. In this study, calf serum IgG1 antibody titers to BRV at the time of inoculation reflected the titers of the colostrum that was fed. Antibody titers in calves receiving VLP colostrum and CLP colostrum were similar and were significantly higher than titers in calves receiving inactivated SA11, control or no colostrum. Calves fed inactivated SA11 colostrum had significantly higher titers than calves receiving control colostrum or no colostrum. Faecal antibody titers were highly variable within groups at the time of inoculation (20–30 hours of age); stools collected at this time were meconium-like in some calves, suggesting that ingested colostrum supplements had not yet passed the length of the intestinal tract. Antibody titers in faeces in some calves did not peak until PID 5; it is unclear whether some of this antibody was transudated from the serum of the calf into the intestine, as was previously proposed in studies of passive immunity to BRV by Besser et al. Peak faecal IgG1 antibody titers (PID 0–5) were significantly higher in calves receiving VLP or CLP colostrum (coinciding with the highest IgG1

![Fecal Antibody Titers](image)

**Figure 2** Geometric mean isotype antibody titers (GMT) to BRV in faeces of unsuckled calves supplemented with colostrum from cows vaccinated with SA11 VLPs, CLPs or inactivated rotavirus vaccines, or from nonvaccinated cows, or with no colostrum. Calves received colostrum twice daily from birth through 7 days of age. The day of BRV inoculation (PID 0) is indicated by a single arrow, and the day of BRV challenge (PID 21, PCD 0) by double arrows.
antibody titers in these two groups), than in calves in the other three groups.

Calves receiving colostrum supplements from vaccinated cows had significantly fewer days of BRV diarrhea and shedding following inoculation, than calves in control groups. It has been reported previously that high VN titers are necessary for complete protection, and that an association exists between VN antibody titers and protection in calves. Investigators studying mice and piglets have reported a correlation between faecal IgA antibodies or intestinal IgA antibody secreting cells, respectively and protection against rotavirus challenge. Calves in the present study which received VLP colostrum had high titers of IgG1 antibodies in serum and faeces, and high titers of IgA antibodies and VN antibodies in faeces. This group of calves had the lowest mean days of BRV shedding, and no BRV diarrhea while the colostrum supplement was being fed. It is unclear whether VN antibodies or IgA antibodies (or both) were instrumental in this protection, as both were elevated in this group.

Calves which received CLP colostrum had similar titers of IgG1 antibodies in serum and faeces (PID 0–5) as calves in the VLP group, but low titers of IgA and VN antibodies. Although all five calves in the CLP group shed BRV, only one calf experienced diarrhea while receiving the colostrum supplement, indicating at least partial protection. Studies of mice suggest that high titers of non-neutralizing antibodies to the major structural protein, VP6 can contribute to partial passive protection against rotavirus diarrhea. In calves, passively-derived IgG1 is transferred from the serum into the gastrointestinal tract after birth, and these serum-derived IgG1 antibodies may contribute to passive intestinal immunity to BRV. It is unclear whether the mechanism of passive protection afforded by non-neutralizing IgG1 antibodies in calves is

### Table 4 Responses to rotavirus challenge of 22-day-old (PID 21) calves, fed colostrum pools from cows vaccinated with SA11 VLP, CLP or inactivated rotavirus vaccines or non-vaccinated controls

| Colostrum feeding group | Antibody titer to BRV at challenge | Rotavirus shedding (No. pas/total no.) | Rotavirus diarrhea (No. pas/total no.) |
|-------------------------|-----------------------------------|---------------------------------------|----------------------------------------|
| VLP colostrum           |                                    |                                       |                                        |
| 208                     | 1024                               | 16                                    | 4b                                     |
| 209                     | 4096                               | 64                                    | 4                                     |
| 210                     | 286                                | 266                                   | 16                                     |
| GMT                     | 1351                               | 21b                                   | 12a                                    |
| CLP colostrum           |                                    |                                       |                                        |
| 208                     | 1024                               | 16                                    | 4                                     |
| 209                     | 4096                               | 64                                    | 4                                     |
| 210                     | 286                                | 4                                     | 4                                     |
| GMT                     | 588                                | 16b                                   | 5a                                     |
| Inact. SA11 colostrum   |                                    |                                       |                                        |
| 208                     | 64                                 | 4                                     | 4                                     |
| 209                     | 4096                               | 64                                    | 2                                     |
| 210                     | 286                                | 4                                     | 4                                     |
| GMT                     | 111                                | 256                                   | 4                                     |
| Control colostrum       |                                    |                                       |                                        |
| 208                     | 16384                              | 256                                   | 4                                     |
| 209                     | 4096                               | 256                                   | 4                                     |
| 210                     | 4096                               | 64                                    | 16                                    |
| GMT                     | 7132                               | 256                                   | 4                                     |
| No colostrum            |                                    |                                       |                                        |
| 208                     | 16304                              | 256                                   | 4                                     |
| 209                     | 16384                              | 64                                    | 4                                     |
| 210                     | 16384                              | 256                                   | 4                                     |
| GMT                     | 54051                              | 256                                   | 4                                     |

<sup>a</sup>Unsuckled newborn calves were fed 1% colostrum pools (twice daily from birth through 7 days of age) from the indicated groups and orally inoculated with IND BRV at 20–30 hours of age. Calves were challenge exposed to IND BRV at PID 21. The post-challenge day of onset and the duration of BRV shedding and diarrhea are noted.

<sup>b</sup>Calves with antibody titers of < 16 were designated as 4 for calculations of GMT.

<sup>c</sup>Groups in the same column with different superscripts differ significantly (p < 0.05).

<sup>d</sup>These calves experienced mild diarrhea not associated with rotavirus shedding, but possibly related to diet or other unidentified infections persisting for 1–3 days.

<sup>e</sup>ND = not done.
analogous to that recently proposed for non-neutralizing IgA monoclonal antibodies in mice (intracellular neutralization of rotavirus in intestinal epithelial cells during dimeric IgA transport)\textsuperscript{30}. Whether a receptor for IgG1 transport across intestinal epithelial cells exists in calves, comparable to the polymeric Ig receptor for dimeric IgA transport is unknown. Although Fc-specific receptors for IgG1 exist on the alveolar epithelial cells of the bovine mammary gland, and contribute to the selective transport of IgG1 from serum into bovine mammary secretions\textsuperscript{31}, the presence of comparable Fc receptors for IgG1 on intestinal epithelial cells of calves has not been investigated.

Calves which received colostrum with inactivated SA11 vaccine, shed virus for a comparable length of time as calves in the CLP group, but had the highest mean days of rotavirus diarrhea, and the highest cumulative faecal scores of calves in the three vaccine groups. Although moderate titers of IgG1 antibodies were present in serum and faeces of these calves at PID 0-5, IgA and VN antibody titers were low. The higher VN antibody titers in the VLP colostrum calves probably relate to the consistently higher VP4 and VP7 antigen concentrations in the VLP vaccine\textsuperscript{14} and their presentation in an immunogenic form in association with intact triple-layered particles. The lower concentrations of VP4 and VP7 in the inactivated SA11 rotavirus vaccine\textsuperscript{12} may be a major factor limiting the effectiveness of this type of conventional vaccine, or the process of inactivation may alter the structural or antigens of the native virus particles, decreasing their immunogenicity.

In the present study, calves receiving no colostrum or control colostrum developed active immune responses after rotavirus inoculation, with IgM antibodies present in serum and faeces by PID 7. IgA and IgG1 antibodies were evident in faeces by PID 14, and IgG1 antibodies appeared in serum at the same time. Active immune responses in calves receiving colostrum from vaccinated cows were of lower magnitude, developed later and (when present) consisted mainly of IgM, findings consistent with suppression of active immune responses by maternal antibodies\textsuperscript{10,32}. Surprisingly, although control calves had strong serological evidence of active immune responses to BRV by the time of challenge, and the other three groups did not, there was little BRV diarrhoea or shedding in any of the groups. The possibility that immune priming occurred in the presence of maternal antibodies deserves further investigation. An alternative explanation would be that sufficient residual passive antibodies (reserected from the serum into the intestine) were present at PID 21 to provide the observed level of protection against BRV challenge exposure.

Our results support the ability of a heterotypic SA11 VLP vaccine or SA11 CLP and inactivated vaccines (P[2]G3) to induce complete or partial passive protection in calves, against a serotypically unrelated BRV strain (P[5]G6). Such VLP vaccines possess potential advantages over existing BRV vaccines including safety (due to inability to replicate and a lack of adventitious agents potentially present in live vaccines), and the consistent expression of VP4 and VP7 on VLP in a stable and immunogenic form. Additional studies are needed to analyse the role and mechanism of passive immunity associated with the CLP vaccine which induced non-neutralizing antibodies to the major structural protein VP6, but nevertheless mediated at least partial passive protection in calves.

ACKNOWLEDGEMENTS

Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center (OARDC), The Ohio State University. Approved as OARDC manuscript #48-97. This work was supported in part by USDA NRICGP Competitive grant No. 93-37204-9201 and a Texas Applied Technology Program Grant No. 004949-029.

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