Comparison of humoral and T-cell-mediated immune responses to a single dose of Bovela® live double deleted BVDV vaccine or to a field BVDV strain

Ratree Platt  
*Iowa State University*

Lyle Kesl  
*Veterinary Resources, Inc.*

Christian Guidarini  
*Boehringer Ingelheim Animal Health GmbH*

Chong Wang  
*Iowa State University, chwang@iastate.edu*  

Follow this and additional works at:  
http://lib.dr.iastate.edu/vmpm_pubs

Part of the  
Comparative and Laboratory Animal Medicine Commons, Veterinary Microbiology and Immunobiology Commons, Veterinary Pathology and Pathobiology Commons, and the  
Veterinary Preventive Medicine, Epidemiology, and Public Health Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/vmpm_pubs/135. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Veterinary Microbiology and Preventive Medicine at Iowa State University Digital Repository. It has been accepted for inclusion in Veterinary Microbiology and Preventive Medicine Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Comparison of humoral and T-cell-mediated immune responses to a single dose of Bovela® live double deleted BVDV vaccine or to a field BVDV strain

Ratree Platta, Lyle Kestb, Christian Guidarini, Chong Wangc, James A. Rotha,⁎

a College of Veterinary Medicine, Iowa State University, Ames, IA, USA
b Veterinary Resources, Inc., Ames, IA, USA
c Boehringer Ingelheim Animal Health GmbH, Ingelheim am Rhein, Germany

A B S T R A C T

The objective of this study was to determine and compare the humoral and cellular immune responses of calves exposed to a single dose of Bovela® bovine viral diarrhea virus (BVDV) live double deleted vaccine or a field strain virus (FSV) of BVDV type 2 (strain 890). Thirty seronegative, colostrum-deprived 5 month-old Holstein steer calves that tested negative for persistent BVDV by ear notch immunohistochemistry and seronegative to BVDV types 1 and 2 were used. Calves were screened by multi-parameter flow cytometry (MP-FCM) 1 week before vaccination to ensure that they were negative for T cell responses to the BVDV types 1 and 2 viruses in the Bovela® vaccine. Calves were assigned to 3 treatment groups: control (PBS), FSV inoculated, and Bovela® vaccinated. The humoral response was tested by standard serum virus neutralization (SVN) test to BVDV types 1 (Singer strain) and 2 (strain 125). The response by CD4, CD8, and gamma delta (γδ TCR) T cells was evaluated by MP-FCM using individual BVDV types 1 and 2 from Bovela® vaccine as recall antigens at 5, 6, and 7 weeks after vaccination. Activation markers used were upregulation of surface CD25 (IL-2R), intracellular interferon gamma (IFNγ) and intracellular interleukin 4 (IL-4). Each T cell subset was evaluated for increased expression of each activation marker compared to non-antigen stimulated cells of the same animal. All Bovela® vaccinated and FSV inoculated calves produced SVN antibodies to both BVDV types 1 and 2 while control animals remained seronegative throughout the study. The mean (weeks 5, 6, and 7) T cell recall responses to Bovela® BVDV type 1 and type 2 recall antigens were numerically higher in all three T cell subsets (CD4, CD8, and γδ TCR) for all three activation markers (CD25, IFNγ, and IL-4) when compared to either the control animals or to the FSV inoculated animals. These differences were often, but not always, statistically significant (P < 0.05).

1. Introduction

Bovine viral diarrhea virus (BVDV) causes a variety of clinical syndromes in cattle including respiratory disease, reproductive dysfunction, immunosuppression, persistent infection and mucosal disease resulting in significant economic losses (reviewed in Lanyon et al., 2014). A large number of vaccines for BVDV are available in the U.S. These vaccines are either conventional inactivated and adjuvanted vaccines or modified live virus (MLV) vaccines with reduced virulence (Ridpath, 2013; Griebel, 2015). Vaccination of cows to prevent the fetus from infection is essential to prevent abortion, fetal malformations, and the birth of persistently infected (PI) calves (Griebel, 2015). PI animals are the major source of transmission of BVDV within and between herds (Moennig and Becher, 2015). Killed vaccines are viewed as being safer than MLV vaccines but are not as effective at inducing aspects of T cell-mediated immunity and providing protection from a diversity of BVDV strains (Platt et al., 2008; Ridpath, 2013; Griebel, 2015; Downey-Slinker et al., 2016). MLV vaccines have been shown to induce a more complete T cell-mediated immune response (Platt et al., 2006) and to be more capable of protecting pregnant animals and their fetuses from infection (Newcomer et al., 2015; Griebel, 2015). They can also be effective in inducing protection from challenge and memory CD4, CD8 and γδ TCR T cell responses, but not antibody, in young calves in the presence of maternal antibody (Platt et al., 2009) However, most MLV BVD vaccines are not safe for use in pregnant animals that are not already immune and should not be used in calves nursing pregnant animals that are not immune. Some MLV vaccines in the US are approved for use in pregnant animals that have previously been vaccinated. However, since vaccines are not capable of protecting 100% of vaccinated animals, there is some concern associated with using MLV vaccines in pregnant animals, even if they have previously been vaccinated.

⁎ Corresponding author.
E-mail address: jaroth@iastate.edu (J.A. Roth).

http://dx.doi.org/10.1016/j.vetimm.2017.03.003
Received 20 December 2016; Received in revised form 13 March 2017; Accepted 13 March 2017

0165-2427/ © 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).
A new BVDV vaccine has been developed based on genetic modification of the vaccine virus to inactivate the E\textsuperscript{neo} and N\textsuperscript{neo} genes of both the BVDV types 1 and 2 strains of virus in the vaccine (Meyer et al., 2007). These viruses have been tested for safety in seronegative pregnant heifers and cows and shown to be incapable of crossing the placenta (Meyer et al., 2007). These viruses have been tested for safety in seronegative pregnant heifers and cows and shown to be incapable of crossing the placenta (Meyer et al., 2007). The objective of the research reported here was to determine if this genetically modified BVDV MLV vaccine is capable of inducing SVN antibodies and CD4, CD8 and γδ T cell responses as other MLV BVDV vaccines have been shown to do.

2. Materials and methods

2.1. Animals and experimental design

Thirty 5 month-old colostrum-deprived Holstein steer calves that tested negative for persistent BVDV by immunohistochistry on ear notches and seronegative (SVN titers < 2) to BVDV types 1 (Singer strain) and 2 (strain 125) viruses were used. The calves were housed in open front sheds near Ames, IA, USA in March through May 2016. They were allowed a 2-week acclimation period prior to the experiment. The timeline, treatment and tests performed are outlined in Table 1. Calves were assigned a number using the Excel random number generator, then sorted by ascending random number to assign 10 animals each to the three treatment groups: PBS control group; then sorted by ascending random number to assign 10 animals each to the three treatment groups: PBS control group; field strain BVDV type 2 strain 890 (FSV) inoculated group and Bovela\textsuperscript{®} vaccinated group. All treatments were administered intramuscularly. Each treatment group was housed separately in a single pen with no contact with other treatment groups. Calves were provided water, ad libitum from the facility site well and ad libitum access to grass hay. Calves were fed a commercial complete pelleted ration (Purina Calf Growena) at a rate of approximately 5 lbs. per head per day. Calves were observed daily from week 1 through week 7 by a trained animal observer who was blinded to treatment groups. Parameters observed and recorded included: attitude, appetite, nasal discharge, ocular discharge, respiratory character, cough and fecal consistency. All animal related procedures were approved by the Veterinary Resources, Inc. Animal Care and Use Committee (Table 2).

2.2. Vaccine and viruses

The vaccine used was Bovela\textsuperscript{®} live double deleted BVDV vaccine (serial # 241-013) containing both BVDV types 1 and 2 strains at a minimum titer of 10\textsuperscript{7} TCID\textsubscript{50} of each strain per 2 mL dose. The Bovela\textsuperscript{®} vaccine was not currently licensed by the USDA Center for Veterinary Biologics and is not currently available for use in the United States. The 2 BVDV strains in the Bovela\textsuperscript{®} vaccine were non-cytopathic double deleted mutants. The parent strain for the BVDV type 1 fraction was KE-9 originally isolated in Germany and for the BVDV type 2 fraction was NY-93 originally isolated in the U.S. Both strains had identical genetic modification by deletion of the coding region of amino acid 349 located in the coding sequence for E\textsuperscript{neo}, and deletion of the coding region for the whole N\textsuperscript{neo} protein, except the N-terminal four codons. Both the E\textsuperscript{neo} and N\textsuperscript{neo} proteins were known to be important virulence factors due to their inhibition of interferon production by the infected cell.

The field strain virus used for intramuscular inoculation of the FSV group was BVDV type 2 strain 890 (provided by Dr. Julia Ridpath at the USDA National Animal Disease Center in Ames, IA) at 2 × 10\textsuperscript{7} TCID\textsubscript{50}/animal.

The viruses used as recall antigens to stimulate PBMCs in the MP-FCM assay were BVDV type 1 Bovela\textsuperscript{®} vaccine strain 241-033115-1 and BVDV type 2 Bovela\textsuperscript{®} vaccine strain 241-033115-2, both used at 10\textsuperscript{5.5} TCID\textsubscript{50}/mL.

In addition, EDTA blood samples were collected on days −3, −2, −1, 4, 6, 8, 11, 13, and 15 for total and differential white blood cell counts (conducted by the Clinical Pathology Laboratory, College of Veterinary Medicine, Iowa State University).

2.3. Serum virus neutralization antibody assays

Serum samples for the SVN tests were separated on collection day and frozen at −20 °C until tested. Specific SVN antibody to cytopathic BVDV type 1 (Singer strain) and type 2 (strain 125) were assayed by the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University.

2.4. Multi-parameter flow cytometry (MP-FCM)

Whole blood samples (10 mL) were collected in 2 mL of 2 x acid citrate dextrose (0.15 M sodium citrate, 0.076 M citric acid monohydrate, and 0.287 M dextrose). Buffy coats were collected and peripheral blood mononuclear cells (PBMC) were isolated as previously described (Platt et al., 2006). The MP-FCM assay was performed as previously described (Platt et al., 2009). Briefly, buffy coats were collected by centrifugation at 1,000g for 20 min. PBMC were isolated by lysing the contaminating RBC with 2 vols of buffered water (deionized water with 0.15 M Na2HPO4 and 0.15 M KH2PO4) for 1 min followed by 1 vol of 3xPBS and centrifuged at 400g for 10 min. Then resuspended in RPMI + + (RPMI with 15% FBS and 1.5% penicillin/streptomycin solution) to 5 × 10\textsuperscript{6} cells/mL. Two hundred μL of each PBMC suspension containing 10\textsuperscript{6} cells, were added to each of 7 wells of a 96-well flat-bottomed tissue culture microtiter plate. Two wells each received 50 μL RPMI + + as a non-antigen stimulated control. Two wells each received 50 μL of Bovela\textsuperscript{®} BVDV type 1, and Bovela\textsuperscript{®} BVDV type 2. Another well received 50 μL of conjugated secondary antibody (Con A) at 5 μg/well as the mitogen control. The plates were incubated in a 5% CO\textsubscript{2} humidified incubator at 37 °C for 4 days. After 4 days incubation, PBMC were washed with 50 μL of phosphate-buffered saline (PBS) and transferred to

### Table 1

Timeline, Treatment, and Tests. MP-FCM = Multi-Parameter Flow Cytometry. SVN = Serum Virus Neutralization.

<table>
<thead>
<tr>
<th>Week</th>
<th>Treatment (all groups)</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>−2 to 0</td>
<td>Acclimation period</td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>Collect serum and whole blood</td>
<td>MP-FCM</td>
</tr>
<tr>
<td>0</td>
<td>Administer treatments:</td>
<td></td>
</tr>
<tr>
<td>−2 to 7</td>
<td>Daily animal observations</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Collect serum and whole blood</td>
<td>MP-FCM &amp; SVN tests</td>
</tr>
<tr>
<td>6</td>
<td>Collect serum and whole blood</td>
<td>MP-FCM &amp; SVN tests</td>
</tr>
<tr>
<td>7</td>
<td>Collect serum and whole blood</td>
<td>MP-FCM &amp; SVN tests</td>
</tr>
</tbody>
</table>
96-well round-bottomed tissue culture microtiter plates. The plates were incubated in the same condition for an additional 4 h. PBMC were washed once with 150 μL PBS + + (PBS with 0.5% bovine serum albumin, and 0.1% sodium azide). All microplate wash cycles were performed at 300 × g for 1 min. The list of antibodies and reagents used in this study are summarized in Table 1. Primary monoclonal antibody mix (50 μL) was added to all wells. The monoclonal primary antibody mix consisted of mouse anti-bovine CD4 isotype IgG2a (clone IL11A), mouse anti-bovine CD8 isotype IgM (clone BAQ111A), mouse anti-bovine γδ TCR isotype IgG2b (clone GB21A), and mouse anti-bovine CD25 isotype IgG3 (clone LCTB2A). All primary monoclonal antibodies were purchased from Washington State University (WSU).

Fig. 1. Means (± SEM) total blood cell counts by type. Blood samples were collected on days −3, −2, −1, 4, 6, 8, 11, 13, and 15 of the experiment. Each cell type was counted and averaged by day. Statistically significant differences (P < 0.05) were analyzed between treatment groups. A indicates the differences between Bovela® and FSV groups compared to PBS group. B indicates the differences between Bovela® group compared to FSV group.

Fig. 2. Means (± SEM) Log2 SVN titers to cytopathic BVDV type 1 (Singer strain) and BVDV type 2 (strain 125) from samples collected on weeks −1, 5, 6, and 7 of the experiment. Values within the same week, not connected by the same letter are significantly different (P < 0.05).
Monoclonal Antibody Center (Pullman, WA). All antibodies were diluted to their previously titrated optimum dilutions. The plates were incubated at room temperature (RT, approximately 25 °C) for 15 min. After two wash cycles, the secondary antibody conjugate mix (50 μL) was added to all wells. The secondary antibody conjugate mix consisted of goat anti-mouse IgG2a-Phycoerythrin-Texas Red (PE-TR) (cat #1080-10, Southern Biotech., Birmingham, AL), rat anti-mouse IgM-PerCP-Cy5.5 (cat # 550881, BD Biosciences, San Jose, CA), goat anti-mouse IgG2b-Alexa Fluor 647 (AF647) (cat # A-21242, Thermo Fisher Scientific, Waltham, MA) and goat anti-mouse IgG3-R-Phycoerythrin (R-PE) (cat # 1100-09, Southern Biotech., Birmingham, AL). The plates were incubated at RT for 15 min. After three wash cycles, 100 μL of cell fixing and permeabilizing solution (BD cytofix-cytoperm, BD Biosciences Pharmingen, San Diego, CA) was added to all wells and the plates were incubated at RT for 30 min. The plates were washed once with BD wash solution (BD Biosciences, San Diego, CA). The anti-cytokine antibody mix (50 μL) was added to all wells. The primary antibody mix for intracellular staining consisted of mouse anti-bovine IFNγ-biotin (cat # MCA1783B, Bio-Rad, Raleigh, NC) and rabbit anti-bovine IL-4 (cat # PBOIL41, Thermo Fisher Scientific, Waltham, MA). The plates were incubated at RT for 15 min. After two wash cycles with BD wash solution, the secondary reagents mix (50 μL) was added to all wells. The secondary reagent consisted of streptavidin-PE-Cy 7 (cat # SA1012, Thermo Fisher Scientific, Waltham, MA) and goat anti-rabbit IgG-Alexa Fleur 700 (AF700) (cat # A-21038, Thermo Fisher Scientific, Waltham, MA). The plates were incubated at RT for 15 min. Three more wash cycles with BD wash solution were performed and 1% ultra-pure formalin in PBS (125 μL) was added to all wells. Stained PBMCs were transferred to flow tubes and kept in the dark at 4 °C until flow cytometric analysis was performed.

Flow cytometric analysis was performed by personnel of the Cell and Hybridoma Facilities at Iowa State University using a Canto cytometer (BD Biosciences, San Diego, CA). FlowJo cell analysis software (Ashland, OR) was used to analyze flow cytometry data. The live cells were gated and 3 major bovine T cell subsets, CD4, CD8, and γδ TCR were identified. The percentages of each T cell subset expressing CD25 (%CD25+) and their geometric mean fluorescent intensities (MFI) were collected. The CD25 EI (expression index) of each T cell subset was calculated by dividing the averaged product (%CD25+ x MFI) from 2 wells of antigen-stimulated cells by the averaged product from 2 wells of non-antigen stimulated cells of the same subset of the same animal. The IFNγ EI and IL-4 EI were calculated the same way as CD25 EI.

2.5. Statistical analysis

The responses were analyzed using repeated measures analysis of variance (ANOVA). Group, day and their interaction were used as fixed effects, whereas calf was the subject of repeated measures. The antibody data were log-transformed before analyses. Comparison among groups were performed using F-test followed by post-hoc Tukey’s t-tests for pairwise comparisons. A probability of $p < 0.05$
was considered statistically significant for all tests.

3. Results

3.1. Clinical observations

Animals remained healthy throughout the study. The only abnormal clinical signs observed were mild, transient ocular discharge. The Bovela® group had two incidences of one day each and the FSV group had five incidences of one day each of mild transient ocular discharge in a total of 10 animals observed daily for 7 weeks after vaccination or FSV inoculation.

3.2. Total and differential white blood cell counts

The means (± SEM) of total and differential white blood cell counts are found in Fig. 1. The FSV group had significantly (P < 0.05) decreased total WBC counts compared to the PBS group and the Bovela® group on most days when samples were evaluated from days 4–15 after inoculation. The Bovela® group had significantly (P < 0.05) lower WBC counts than the PBS group on days 6 and 8 after inoculation. Both the FSV and Bovela® groups had significantly lower neutrophil counts than the PBS group on some occasions between days 4 and 11 after inoculation. The FSV inoculated cattle had significantly (P < 0.05) lower lymphocyte counts than the PBS and the Bovela® group from days 4 through 13 after inoculation. The lymphocyte counts for the Bovela® group were very similar to the PBS group and were significantly higher than the FSV group for days 3 through 15. The means (± SEM) of platelet counts are shown in Fig. 4. The FSV group had significantly lower platelet counts than the Bovela® group on days −1 through −3, apparently due to random chance. After virus inoculation, the platelet counts for the FSV group decreased and were significantly lower than the values for the PBS group on days 4 through 8. The platelet counts for the Bovela® group also decreased on days 4 through 8 after inoculation, but they were never significantly lower than the values for the PBS group. By day 11, the platelet counts for the FSV and Bovela® groups increased and were numerically higher than the values for the PBS group. At the end of the experiment, the platelet counts for the FSV group (day 15) and Bovela® group (days 13 and 15) were significantly higher than the values for the PBS group.

3.3. Serum virus neutralization titers

The SVN titers to BVDV types 1 and 2 are shown in Fig. 2. The PBS group remained seronegative throughout the observation period. The FSV and Bovela® groups had similar SVN antibody titers against BVDV type 1. However, the FSV group had titers to BVDV type 2 that were several fold higher (P < 0.05) than the Bovela® group.

3.4. T cell responses to BVDV recall antigens as measured by multi-parameter flow cytometry

Sample plots showing gating strategies to identify T cell subsets and to detect up-regulation of the 3 activation markers are shown in Figs. 3 and 4 respectively.

The MP-FCM results following PBMC stimulation with the 2 individual strains of Bovela® BVDV are found in Figs. 5 through 7. For all T cell subsets combined, the Bovela® group had significantly higher EI values for all three T cell activation markers when BVDV type 1 and type 2 were each used as recall antigens. There were no instances where the FSV group had higher EI values than the PBS group for all T cell subsets combined.

The mean CD25 EI data is shown in Fig. 5. There were no significant differences in T cell subset responses between treatment groups when
evaluated before the experiment. The CD25 EI was always significantly (P < 0.05) higher for the Bovela® group than the PBS group for each T cell subset evaluated. The Bovela® group results were also significantly (P < 0.05) higher than the FSV group when the Bovela® BVDV type 1 virus was used as the recall antigen. When the Bovela® BVDV type 2 virus was used as the recall antigen, the values for the Bovela® group were significantly higher than the FSV group for the CD4T cell subset only. The FSV group did not have detectable CD25 responses to the Bovela® BVDV type 1 virus. It did have significantly higher responses to the Bovela® BVDV type 2 virus for the CD8 and γδ TCR T cells compared to the PBS group.

The mean IFNγ EI data is shown in Fig. 6. There were no significant differences between groups before animal inoculation. The Bovela® group had significantly higher IFNγ EI for all T cell subsets when stimulated with the Bovela® BVDV type 2 virus and for the CD4 and CD8T cell subsets when stimulated with the Bovela® BVDV type 1 virus. The FSV group had significantly higher IFNγ EI for CD8T cell responses to the Bovela® BVDV type 2 virus compared to the PBS group only.

The mean IL-4 EI data is shown in Fig. 7. There were no significant differences between groups before the experiment. The Bovela® group had significantly higher IL-4 EI responses in CD4 and CD8T cell subsets when stimulated with Bovela® BVDV type 1 virus, and CD4T cell subset responses when stimulated with Bovela® BVDV type 2 virus. The FSV group did not have significantly higher IL-4 EI responses to any recall viruses for any T cell subsets.

4. Discussion

The Bovela® vaccine has significant safety advantages compared to other MLV BVD vaccines. It has previously been shown to be safe in pregnant heifers and cows and to be incapable of crossing the placenta (Meyer et al., 2007). In this manuscript we demonstrated an additional safety advantage, that Bovela® vaccine did not induce lymphopenia. Virulent BVDV and a MLV BVDV vaccine have previously been shown to decrease both lymphocyte and neutrophil numbers in blood (Roth et al., 1981; Roth and Kaeberle, 1983). Inoculation with FSV induced a decrease in both neutrophils and lymphocytes. The Bovela® vaccine also induced a numerical decrease in platelet numbers, but it was not significantly different (P < 0.05) from the values for the PBS group. The FSV induced a decrease in platelet count, which were significantly lower than the PBS group. This has previously been shown with BVDV challenge (Marshall et al., 1996; Platt et al., 2009). The platelet counts
for the FSV group were significantly lower than the values for the Bovela® group both before and after virus inoculation. The lower counts before vaccination/FSV inoculation were apparently due to random chance, therefore the values after vaccination/FSV inoculation are difficult to interpret.

The Bovela® vaccine induced SVN antibody titers to both BVDV types 1 and 2. The FSV inoculation induced SVN antibody titers to BVDV type 1 similar to the titers induced by Bovela® vaccine. The antibody titers to BVDV type 2 were much higher for the FSV group than for the Bovela® group. This may be due to the much higher titer of BVDV type 2 FSV administered (2 × 10⁷ TCID₅₀) to the calves as compared to the dose of virus in Bovela® vaccine (10³ TCID₅₀ of each strain). The SVN titers were to heterologous viruses in all cases. The relatedness of the Bovela® vaccine viruses and FSV to the heterologous viruses used in the SVN assay may have influenced the observed titers.

The Bovela® vaccine has efficacy advantages as compared to killed vaccine. It induced memory CD4, CD8, and γδ TCR T cells to homologous BVDV types 1 and 2 when evaluated using surface expression of CD25 as the activation marker. Other MLV BVD vaccines have previously been shown to induce all three types of memory T cells (Platt et al., 2006; Platt et al., 2009). A killed BVDV vaccine failed to induce detectable CD8 T cell responses (Platt et al., 2008), apparently because the killed virus did not induce synthesis of BVD virus proteins in the cytoplasm which is important for antigen presentation on MHC I molecules for recognition by CD8 T cells. The Bovela® vaccine also induced significant upregulation of intracellular expression of IFNγ in both CD4 and CD8 T cells in response to homologous BVDV types 1 and 2 recall viruses. The γδ TCR T cells only had significant upregulation of IFNγ when restimulated with homologous BVDV type 2. Induction of memory CD4, CD8, and γδ TCR T cells by virulent virus (Endsley et al., 2004) and by an MLV BVD vaccine (Platt et al., 2009), in the presence of maternal antibody has previously been shown to induce all three types of memory T cells without inducing measurable antibody responses and to provide protection from challenge with virulent type 2 BVD virus (Ridpath et al., 2003).

5. Conclusions

The Bovela® live double deleted vaccine combines some of the safety advantages of killed BVDV vaccines and some of the efficacy advantages of MLV BVD vaccines. In contrast to other MLV BVD vaccines, it has previously been shown to be safe for use in seronegative pregnant heifers and cows (Meyer et al., 2007) and we demonstrated here that it does not induce a lymphopenia after vaccination. In contrast to a killed
BVDV vaccine, Bovela® is capable of inducing memory CD8 (cytotoxic) T cells to the homologous BVDV types 1 and 2 viruses. The Bovela® live double deleted vaccine provides a potentially important new tool for control of BVD virus infections in pregnant animals and their fetuses.

Acknowledgements

This work was supported by a grant from Boehringer Ingelheim Animal Health GmbH, Ingelheim am Rhein; Registergericht Mainz: HR B 23097, Germany

References


Fig. 7. Mean (±SEM) IL-4 Expression Indices of each treatment group were analyzed and compared by T cell subset. Results from weeks 5, 6, and 7 were combined. Each error bar is constructed using 1 standard error of the mean. Levels, within the same subset, not connected by same letter are significantly different (p < 0.05).