Leukocyte dynamics in vaccinated and unvaccinated animals following dual challenge with Bovine Viral Diarrhea Virus-*Mannheimia haemolytica* model to compare vaccine efficacy in calves

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Introduction

Bovine respiratory disease continues to be the greatest threat to calf health. One of the common recommendations is to vaccinate young beef and dairy calves at 30-60 days of age to provide protection at weaning or when commingled. The study objective was to determine whether there were any differential leukocyte changes that could be measured following a dual challenge with Bovine Viral Diarrhea Virus (BVDV) and *M. haemolytica* ~5 months after vaccination.

Materials and methods

In this study colostrum-fed dairy cross beef calves were vaccinated at ~30 days of age with either a placebo (CON), an adjuvanted parenteral vaccine containing modified live BVDV type 1 and type 2, bovine herpesvirus 1 (BHV-1), bovine parainfluenza virus (PI3) and bovine respiratory syncytial virus (BRSV) and *M. hemolytica* toxoid (Vac 1) or concurrently with intranasal temperature-sensitive (TS) BHV-1-BRSV-PI3 and a parenteral vaccine containing modified live BVDV type 1 and type 2 and *M. haemolytica* toxoid (Vac 2). The calves were challenged ~150 days post vaccination with BVDV 1b and then 7 days later with *M. haemolytica*. The calves were than euthanized 6 days after the *M. haemolytica* challenge. Whole blood was collected from all groups for complete blood count (CBC), and BVDV viremia from buffy coats by PCR, on days 0, 3, 5, 7, 9 and 12. Blood was also collected for blood leukocyte differential (BLD) count using the QScout chute side system (Applied Animal Diagnostics, Morrisville, NC) on days 0, 3-10 and 12. At necropsy, lungs were scored.

Results

Comparisons were made between the 3 treatments at each time point using both absolute counts and percent leukocytes. BLD cell types identified were segmented neutrophils, band neutrophils, lymphocytes, eosinophils and monocytes. The total white blood cells count (WBC) using the QScout system was comparable to those obtained using a clinical cytometer. Comparing the absolute WBC total counts there was a significant ($P < 0.05$) decrease in both Vac 2 and control compared to Vac 1 from days 3-7 following BVDV challenge and days 9-10 following the *M. haemolytica* challenge. The number of segmented neutrophils was significantly lower in the control group vs the Vac 1 group following BVDV infection on days 3, 4, 6 and 7, but there was no significant difference between 2 and control groups for days 3-7. Following *M. haemolytica* challenge, there was a significant decrease in the control group vs. the 2 vaccine groups on days 8 and 10. Interestingly, the only difference in differential percent was segmented neutrophils on days 4 and 10. The lymphocyte numbers were significantly lower in the control and Vac 2 groups than Vac 1 group on days 3-7 following BVDV challenge. The lymphocyte numbers following the *M. haemolytica* challenge were lower in the Vac 2 than Vac 1 group on days 8-10 and 12. Similar to segmented neutrophils, the only difference in percent of lymphocytes was day 4 when the control group was higher than Vac 1 group. The number of monocytes was significantly lower in the control group vs. the Vac 1 group following BVDV infection on days 3-7, but there was no significant difference between Vac 2 and control groups for days 4-6. Following *M. haemolytica* challenge, monocytes in the control group were significantly higher than the 2 vaccine groups on day 8. The differences in percent of monocytes was higher in controls on days 9, 10 and 12 than the vaccinates. The number of eosinophils was significantly lower in the control group vs. both of the vaccine groups following BVDV infection on days 3-7. Following *M. haemolytica* challenge, eosinophils were significantly lower in the control group than the 2 vaccine groups on days 9, 10 and 12. The percent eosinophils in the control group were significantly lower on days 3-6, 9-10 and 12 than Vac 2 group. Band neutrophils were the same in all 3 groups following BVDV infection. Following *M. haemolytica* challenge, band neutrophils were significantly higher on days 8-9 in the control groups than Vac 1 group. The percent band neutrophils in the control group were significantly higher on days 9 and 12 than the 2 vaccine groups. One additional comparison was done where all the animals were grouped by the presence or absence of lung lesions. The number of animals with lung lesions was similar in all 3 groups – 9-10 animals per treatment group had lesions. Unlike the comparisons between the 3 treatment groups, there were similar patterns between the absolute counts and percent differential. All of the differences occurred following *M. haemolytica* challenge. Animals with lung lesions had significantly higher absolute segmented neutrophil numbers on days 8 and 9 and higher segmented neutrophil differential percent on days 8-10 and 12. There was no difference in lymphocytes absolute numbers in animals with or without lung lesions. There were significantly lower lymphocyte differential percent in animals with lung lesions on

days 8-10 and 12. The pattern with monocytes was similar to segmented neutrophils with significantly higher absolute numbers on days 8-10 and 12 and significantly higher monocyte differential percent on days 10 and 12 in animals with lung lesions. Although there were higher numbers and percent differential of eosinophils in animals with no lung lesions, there was only a statistically difference on day 5. Like the segmented neutrophils and monocytes, band neutrophils had significantly higher absolute numbers on days 8-10 and 12 and a significantly higher band neutrophil differential percent on days 8-9 and 12 in animals with lung lesions.

Significance

In this study, the major innate pro-inflammatory cells, segmented neutrophils, monocytes and band neutrophils and the major anti-inflammatory cell the eosinophil, were good indicators of the vaccine status of the animal. Control animals had lower absolute segmented neutrophils and lower monocytes following BVDV infection compared to vaccinates while percent differential was unaffected. Following *M. haemolytica* challenge, there was an absolute increase in segmented neutrophils, monocytes and band neutrophils and a decrease in eosinophils in the controls compared to Vac 1 group. The Vac 2 group had similar pattern with Vac 1 group, but the differences often were not significant compared to the control group. The BLD correlated with the clinical, virological and pathology data that was previously published for these animals. In that study, Vac 1 group had less clinical signs, viremia and lesions than both Vac 2 and control groups. The comparison with BLD and presence or absence of lung lesions indicated that lung lesions were seen in animals with higher segmented and band neutrophils and monocytes and lower numbers of lymphocytes and eosinophils. This is consistent with our understanding that higher number of proinflammatory cells and the lower number of anti-inflammatory cells are associated with lung damage. Although peripheral blood is not the lung inflammatory cell milieu, the BLD provided surrogate indicators of protection from respiratory disease in this model system.

