Serum Antibody Response in Calves Receiving Modified Live and/or Inactivated Vaccines Containing Bovine Herpesvirus-1, Bovine Viral Diarrhea Virus, Parainfluenza-3 Virus, and Bovine Respiratory Syncytial Virus Immunogens

Robert W. Fulton, *DVM, PhD*
Lurinda J. Burge, *MS*
J.M. d'Offay, *DVM, PhD*
Mark E. Payton, *PhD*
Department of Infectious Diseases and Physiology
College of Veterinary Medicine, and Department of Statistics
Oklahoma State University, Stillwater, OK 74078

Abstract

Serum antibody responses to bovine herpesvirus-1(BHV-1), bovine viral diarrhea virus (BVDV), parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV) by calves receiving viral vaccines were evaluated. Calves were vaccinated at day 0 with one of four commercial vaccines containing BHV-1, BVDV, PI-3V, and BRSV immunogens. Selected vaccines were given a second time at day 28. Calves were revaccinated at day 140 with the respective vaccine given at day 0. The vaccines contained modified live virus (MLV) and/or inactivated components. Serums were collected at day 0 and at various intervals through day 196, and assayed for viral neutralizing antibodies. Between various vaccine groups there were differences in onset and duration of serum antibodies to each immunogen postvaccination. Anamnestic responses after day 140 revaccination to some but not all viruses were sometimes dependent on the type vaccine and level of antibodies at revaccination.

There are several viruses associated with respiratory and/or reproductive diseases of cattle.[12] These viruses include: bovine herpesvirus-1 (BHV-1) also known as infectious bovine rhinotracheitis virus (IBRV); bovine viral diarrhea virus (BVDV); parainfluenza-3 virus (PI-3V); and bovine respiratory syncytial virus (BRSV). Controlling these viral infections/diseases via vaccination programs is critical to preventive healthy programs for beef and dairy production. There are more than 160 commercial vaccines available for cattle containing BHV-1, BVDV, PI-3V, and BRSV immunogens.[6,20] Viral components may be given as a single vaccine alone, or in a variety of combinations with the other viral components as well as with bacterial vaccines/bacterins/toxoids.[6,20] These viral vaccines may be modified live virus, inactivated, or chemically altered live virus.[6,11,20] Benefits/risks of various vaccines along with efficacy against viral challenges are issues of concern in vaccine selection.[13,17] Duration of immunity in vaccinated cattle is an important point not always addressed in published studies relating to vaccine efficacy. Veterinarians and producers may have assumed that vaccines provide life-long immunity after initial vaccination. However, many vaccines have manufacturers' recommendations for annual vaccination after initial vaccination.[6,11,20]

The purpose of this study was to determine the antibody response of calves receiving commercial vaccines containing BHV-1, BVDV, PI-3V and BRSV components after initial vaccination at day 0 and revaccination at day 140.

Materials and Methods

Animals

Healthy beef calves (Hereford or Angus/Hereford cross) of mixed sex were used. The calves were weaned and less than 1 year of age.

Cells and Viruses

Madin-Darby bovine kidney (MDBK) monolayer cultures (BVDV free) were used in this study and were grown as described.[10] Viruses for serotesting were:

Address of correspondence:
Dr. Robert W. Fulton, Department of Infectious Diseases and Physiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, Phone: 405-744-6737, FAX: 405-744-8263, E-mail: RFulton@OKWAY.OKSTATE.edu

BHV-1, Cooper strain; BVDV, Singer strain; PI-3V, SF-4 strain; and a BRSV vaccinal strain.[10]

Serotests

A plaque reduction assay in 24-well plates was used to detect BHV-1 neutralizing antibodies.[10] Antibody titers of 1:10 or higher were considered positive in this study. A microtitration virus neutralization test (VNT) in 96-well plates was used to quantitate neutralizing antibodies to BVDV, PI-3V, and BRSV.[10] Antibody titers of 1:4 or higher were considered positive. Positive and negative control serums for all viruses were included in the respective serotest.

Vaccines and groups

The type of viral component, vaccine manufacturer and days of administration are summarized in Table 1.

Table 1. Vaccines and Groups.

Groups and Vaccines*	Vaccination Times
Vaccine Group 1	
- Tandem®SV+3†	Days 0 and 140
- MLV:BHV-1, BVDV, PI-3	
- Inactivated BRSV	
- Tandem®SV†	Day 28
- Inactivated BRSV	
Vaccine Group 2	
- Tandem®SV+3 IBR Plus†	Days 0, 28, and 140
- MLV:BHV-1, BVDV, PI-3V	
- Inactivated BRSV and BHV-1	
Vaccine Group 3	
- ViraShield®5‡	Days 0, 28, and 140
- Inactivated:BHV-1, BVDV, PI-3V, BRSV	
Vaccine Group 4	
- BoviShield®4§	Days 0 and 140
- MLV:BHV-1, BVDV, PI-3V, BRSV	
- BoviShield®BRSV§	Day 28

*The vaccines were administered intramuscularly.
†Sanofi Animal Health, Inc., Lenexa, Kansas.
‡Grand Laboratories, Inc., Freeman, South Dakota.
§SmithKline Beecham Animal Health, Pennsylvania.

Serum collection

The calves were bled at the dates indicated in Tables 2-5. The sera were collected, frozen, and stored at -20°C until assayed for antibodies.

Statistics

Geometric mean titers (GMT) for each treatment group were determined from the endpoint titers of the animals in each group. The data were analyzed by one-way analysis of variance (completely randomized design). The antibody levels against each virus were compared by Fisher's least significant difference (LSD) procedure using alpha - 0.05.[10]

Results

Statistical analysis and control group surveillance

The geometric means for each treatment group were compared by two different methods. First, antibody titers for the treatment groups were compared for significant differences at given collection dates. Second, antibody titers for the treatment groups were compared for significant differences between collection date (7,10,14,21,28,35,42,56,84,112, and 140) and day 0, the initial vaccination date; or between collection dates 154,168, and 196 and day 140, the revaccination date. During the experiment the calves remained free of clinical signs of viral disease. There was no serologic evidence of infection to BHV-1. The calves had preexisting PI-3V antibodies at time of vaccination due to a prior inapparent infection. There was no rise in PI-3V antibody titers in controls during the study indicative of active infection. There was no serologic evidence of infection in the BVDV nonvaccinated controls, except at day 84 when one animal developed BVDV antibodies which persisted throughout day 196. There were five BRSV seronegative control calves in the study. On isolated dates, one or more calves had low BRSV titers (4 or 8), however these titers then declined to <4 and did not persist. Possibly these low neutralizing titers were laboratory variations of the test, and did not represent those induced by active infection.

Antibody response to BHV-1

All four vaccines induced increased serum BHV-1 antibodies compared to day 0 after initial vaccination (Table 1). Vaccines I,II, and III, and IV contained: I, MLV; II, MLV/inactivated combination; III, inactivated, and IV, MLV. After a second recommended dose of vaccines II and III on day 28, each group responded with increased antibody titers at day 35: 46.4 to 176.8 for vaccine II; and 8.8 to 414.0 for vaccine III. The highest antibody titers were recorded on day 21 for vaccine 1; day 35 for vaccines II and III, and day 14 for vaccine IV. There were significant differences of antibody titers induced by the different vaccines at a given date (Table 2). For example, at day 14, vaccine II (MLV/inactivated combination) induced higher antibody titer after one dose than vaccine I (one dose MLV) and vaccine III (one dose, inactivated). Two doses of vaccine II and III induced higher antibodies than vaccines I and IV from days 35-112. There was a decline in antibodies until day 140. Vaccines II and III induced significantly higher titers from day 10 to day 140 compared to day 0; and vaccines I and IV induced antibody titers were higher for a shorter time: day 14 to day 56 for vaccine 1, and day 10 to day 42 for vaccine III. Some of the vaccinated animals became seronegative prior to day 140. By day

Table 2. Antibody response to GHV-1 after vaccination.

Serum Collection Day	Vaccine									
	I. MLV		II. MLV/ Inactivated		III. Inactivated		IV. MLV		Controls	
	Geometric Mean*	Number of Calves with titer ≥ 1:10	Geometric Mean	Number of Calves with titer ≥ 1:10	Geometric Mean	Number of Calves with titer ≥ 1:10	Geometric Mean	Number of Calves with titer ≥ 1:10	Geometric Mean	Number of Calves with titer ≥ 1:10
0†	0.0	0/6	0.0	0/6	0.0	0/4	0.0	0/3	0.0	0/6
7	0.0	0/6	0.0	0/6	0.0	0/4	0.0	0/3	0.0	0/6
10	$1.5^{a,b}$	1/6	$8.0^{a,c}$	4/6	4.2^{1}	2/4	$11.4^{b,d,1}$	2/3	0.0^{c}	0/6
14	$10.9^{a,b,c,1}$	5/6	$56.1^{a,d,e,1}$	6/6	$12.2^{d,f,1}$	3/4	$57.8^{b,g,1}$	3/3	$0.0^{c,e,f,g}$	0/6
21	$21.9^{a,1}$	5/6	$69.2^{b,c,1}$	6/6	$16.0^{b,d,1}$	4/4	$55.5^{e,1}$	3/3	$0.0^{a,c,d,e}$	0/6
28‡	$15.9^{a,1}$	5/6	$46.4^{b,c,1}$	6/6	$8.8^{b,d,1}$	3/4	$27.5^{e,1}$	3/3	$0.0^{a,c,d,e}$	0/6
35	$16.6^{a,b,c,1}$	5/6	$176.8^{a,d,e,1}$	6/6	$414.0^{b,f,g,1}$	4/4	$22.3^{d,f,h,1}$	3/3	$0.0^{c,e,g,h}$	0/6
42	$12.4^{a,b,c,1}$	5/6	$144.5^{a,d,e,1}$	6/6	$375.0^{b,f,g,1}$	4/4	$23.1^{d,f,h,1}$	3/3	$0.0^{c,e,g,h}$	0/6
56	$3.9^{a,b,1}$	3/6	$93.0^{a,c,d,1}$	6/6	$241.6^{b,e,f,1}$	4/4	$3.8^{c,e}$	1/3	$0.0^{d,f}$	0/6
84	$1.6^{a,b}$	1/6	$49.5^{a,c,d,1}$	6/6	$90.7^{b,e,f,1}$	4/4	$3.1^{c,e}$	1/3	$0.0^{d,f}$	0/6
112	$1.5^{a,b}$	1/6	$39.4^{a,c,d,1}$	6/6	$62.1^{b,e,f,1}$	4/4	$4.1^{c,e}$	1/3	$0.0^{d,f}$	0/6
140†	$0.0^{a,b,c}$	0/6	$34.2^{a,d,1}$	6/6	$54.8^{b,e,f,1}$	4/4	$11.2^{c,e,g}$	2/3	$0.0^{d,f,g}$	0/6
154	$396.5^{a,b,c,2}$	6/6	$312.3^{d,e,2}$	6/6	$1483.7^{a,d,f,g,2}$	4/4	$184.2^{b,f,h,2}$	3/3	$0.0^{c,e,g,h}$	0/6
168	$330.2^{a,b,2}$	6/6	$238.6^{c,d,2}$	6/6	$913.7^{a,c,e,f,2}$	4/4	$138.5^{e,g,2}$	3/3	$0.0^{b,d,f,g}$	0/6
196	$175.3^{a,b,2}$	6/6	$149.4^{c,d,2}$	6/6	$473.3^{a,c,e,f,2}$	4/4	$112.2^{e,g,2}$	3/3	$0.0^{b,d,f,g}$	0/6

*** 0.0 geometric mean indicates none of the calves had antibody titers of ≥ 1:10.**

Superscripts with the same letter within a row indicate significant differences at 0.05 level between groups for that collection date.

Superscripts with number 1 indicate significant differences at 0.05 level between the respective collection day and day 0 for that group.

Superscripts with number 2 indicate significant differences at 0.05 level between the respective collection day and day 140 for that group.

†Vaccines I, II, III, and IV administered.

‡Vaccines II and III.

56, 3/6 calves given vaccine I were seronegative, and 1/3 calves given vaccine IV were seronegative. By day 140, all 6 calves given vaccine I were seronegative.

Revaccination at day 140 with the respective vaccine induced higher BHV-1 antibody titers at day 154, 168, and 196 compared to day 140. At day 154, vaccine III (inactivated) induced the highest titer among the four vaccines. Antibody titers at day 140 were not predictive of the degree of immune response upon revaccination at day 140. The six calves receiving one dose of MLV vaccine I were seronegative at day 140; but they responded by day 154 with 396.5 geometric mean titer. The others' responses from day 140 to 154 were: 34.2 to 312.3 for MLV/inactivated combination vaccine II; 54.8 to 1483.7 for inactivated vaccine III; and 11.2 to 184.2 for MLV vaccine IV.

Antibody response to BRSV

The four vaccines induced increased BRSV serum antibodies in the seronegative calves after initial vaccination at day 0 (Table 3). All vaccine groups received a second dose at day 28. For vaccine I, the BRSV inactivated component was included in the multivalent vaccine given at day 0. The day 28 second dose was the monovalent inactivated BRSV vaccine. The vaccine II contained a BRSV inactivated vaccine in the multivalent vaccine given at both days 0 and 28. All four vaccine groups responded with increased BRSV serum antibodies after one dose: day 10 for vaccine I (inactivated); day 21 for vaccine II (inactivated); day 14 for vaccine III (inactivated); and day 10 for MLV vaccine IV. The MLV vaccine IV induced higher BRSV antibody titers at day 7 than the inactivated vaccines I, II, and III; and was the group at day 7 having higher titers than the control group. All four vaccine groups responded with higher BRSV serum antibody titers at day 35 after day 28 revaccination: 2.0 to 20.2 for inactivated vaccine I; 6.1 to 27.9 for inactivated vaccine III; and 4.0 to 64.0 for MLV vaccine IM. At day 42, after revaccination at day 28, the MLV vaccine IV had induced higher BRSV antibody titers than the inactivated vaccines.

All four vaccines responded with increased BRSV serum antibodies in the day 154 collections upon revaccination at day 140. Two weeks after revaccination, the antibody titers were increased: 2.5 to 10.1 for inactivated vaccine I; 2.3 to 10.6 for inactivated vaccine II; 3.4 to 32.0 for inactivated vaccine III; and 5.7 to 128.0 for MLV vaccine IV. There were no differences in antibody titers between vaccine groups at day 168.

Antibody response to PI-3V

All calves were seropositive at the onset of the study (Table 4). However, all four vaccine groups responded with increased PI-3V serum antibodies after

Table 3. Antibody response to BRSV after vaccination.

Serum Collection Day	Vaccine									
	I. Inactivated		II. Inactivated		III. Inactivated		IV. MLV		Controls	
	Geometric Mean*	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4
0†	0.0	0/6	0.0	0/5	0.0	0/4	0.0	0/2	0.0	0/5
7	1.8^{a}	2/6	0.0^{b}	0/5	1.4^{c}	1/4	$5.7^{a,b,c,d}$	2/2	0.0^{d}	0/5
10	$4.0^{a,b,c,d,1}$	6/6	$1.3^{a,e}$	1/5	$2.0^{b,f}$	2/4	$11.3^{c,e,f,g,1}$	2/2	$0.0^{d,g}$	0/5
14	$5.0^{a,b,1}$	6/6	$1.3^{a,c}$	1/5	$2.8^{d,e,1}$	3/4	$11.3^{c,d,f,1}$	2/2	$0.0^{b,e,f}$	0/5
21	$2.0^{a,b,c}$	3/6	$5.3^{a,d,1}$	5/5	$6.7^{b,e,1}$	4/4	4.0^{f}	2/2	$0.0^{c,d,f}$	0/5
28†	$2.0^{a,b}$	3/6	$6.1^{a,c,1}$	4/5	$9.5^{b,e,1}$	4/4	4.0	2/2	$1.3^{c,e}$	1/5
35	$20.2^{a,b,c.1}$	6/6	$27.9^{d,e,1}$	5/5	$45.3^{a,f,1}$	4/4	$64.0^{b,d,g,1}$	2/2	$0.0^{c,e,f,g}$	0/5
42	$28.5^{a,b,c,1}$	6/6	$5.3^{a,d,e,f,1}$	5/5	$19.0^{d,g,h,1}$	4/4	$90.5^{b,e,g,1}$	2/2	$1.3^{c,f,h,i}$	1/5
56	$6.3^{a,1}$	6/6	$2.6^{b,c,1}$	3/5	$9.5^{b,d,1}$	4/4	$16.0^{c,e,1}$	2/2	$1.7^{a,d,e}$	2/5
84	$6.3^{a,1}$	6/6	$4.6^{b,c,1}$	5/5	$9.5^{d,1}$	4/4	$16.0^{b,e,1}$	2/2	$1.3^{a,c,d,e}$	1/5
112	3.2^{1}	5/6	3.5^{1}	4/5	3.4^{1}	3/4	$8.0^{a,1}$	2/2	1.5^{a}	1/5
140†	2.5^{1}	3/6	2.3^{1}	3/5	3.4^{1}	3/4	5.7	2/2	2.0	2/5
154	$10.1^{a,b,c,2}$	6/6	$10.6^{d,e,2}$	5/5	$32.0^{a,f,2}$	4/4	$128.0^{b,d,g,2}$	2/2	$1.5^{c,e,f,g}$	1/5
168	$7.1^{a,2}$	5/6	$8.0^{b,2}$	5/5	$13.5^{c,2}$	4/4	$32.0^{d,2}$	2/2	$1.3^{a,b,c,d}$	1/5
196	$4.5^{a,2}$	6/6	3.0	4/5	5.7^{b}	4/4	$5.7^{c,2}$	1/2	$1.3^{a,b,c}$	1/5

* 0.0 geometric mean indicates none of the calves had antibody titers of ≥ 1:4.
Superscripts with the same letter within a row indicate significant differences at 0.05 level between groups for that collection date.
Superscripts with number 1 indicate significant differences at 0.05 level between the respective collection day and day 0 for that group.
Superscripts with number 2 indicate significant differences at 0.05 level between the respective collection day and day 140 for that group.
† Vaccines I, II, III, and IV.

Table 4. Antibody response to BRSV after vaccination.

Serum Collection Day	Vaccine									
	I. MLV		II. MLV		III. Inactivated		IV. MLV		Controls	
	Geometric Mean*	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4	Geometric Mean	Number of Calves with titer ≥ 1:4
0†	50.8^{a}	6/6	80.6	6/6	107.7	4/4	128.0^{a}	3/3	80.6	4/4
7	$181.0^{a,b,1}$	6/6	$362.0^{c,d,1}$	6/6	$2896.3^{a,c,e,f,1}$	4/4	$256.0^{e,g}$	3/3	$71.8^{b,d,f,g}$	4/4
10	$287.4^{a,b,1}$	6/6	$362.0^{c,d,1}$	6/6	$2896.3^{a,c,e,f,1}$	4/4	$406.4^{e,g,1}$	3/3	$80.6^{b,d,f,g}$	4/4
14	$287.4^{a,b,1}$	6/6	$287.4^{c,d,1}$	6/6	$4096.0^{a,c,e,f,1}$	4/4	$406.4^{e,g,1}$	3/3	$101.6^{b,d,f,g}$	4/4
21	$228.1^{a,1}$	6/6	$203.2^{b,1}$	6/6	$2896.31^{a,b,c,d,1}$	4/4	256.0^{e}	3/3	114.0^{d}	4/4
28‡	$143.7^{a,1}$	6/6	101.6^{b}	6/6	$2435.5^{a,b,c,d,1}$	4/4	161.3^{c}	3/3	101.6^{d}	4/4
35	$181.0^{a,1}$	6/6	161.3^{b}	6/6	$2435.5^{a,b,c,d,1}$	4/4	128.0^{e}	3/3	101.6^{d}	4/4
42	$114.0^{a,b}$	6/6	$256.0^{a,c,d,1}$	6/6	$4096.0^{b,c,e,f,1}$	4/4	128.0^{e}	3/3	$90.5^{d,f}$	4/4
56	$64.0^{a,b}$	6/6	$181.0^{a,c,d,1}$	6/6	$2048.0^{b,c,e,f,1}$	4/4	80.6^{e}	3/3	$57.0^{d,f}$	4/4
84	45.3^{a}	6/6	101.6^{b}	6/6	$608.9^{a,b,c,d,1}$	4/4	64.0^{e}	3/3	45.3^{d}	4/4
112	45.3^{a}	6/6	101.6	6/6	$304.4^{a,b,c,1}$	4/4	64.0^{b}	3/3	50.8^{c}	4/4
140†	$35.9^{a,b}$	6/6	$101.6^{a,c}$	6/6	$304.4^{b,c,d,e,1}$	4/4	80.6^{d}	3/3	57.0^{e}	4/4
154	$57.0^{a,b,c}$	6/6	$143.7^{a,d}$	6/6	$1217.8^{b,d,e,f,2}$	4/4	$203.2^{c,e,g}$	3/3	$64.0^{f,g}$	4/4
168	$45.3^{a,b,c}$	6/6	$143.7^{a,d}$	6/6	$724.1^{b,d,e,f,2}$	4/4	$203.2^{c,e,g}$	3/3	$64.0^{f,g}$	4/4
196	$45.3^{a,b}$	6/6	$114.0^{a,c}$	6/6	$608.9^{b,c,d,e,2}$	4/4	128.0^{d}	3/3	64.0^{e}	4/4

* Superscripts with the same letter within a row indicate significant differences at 0.05 level between groups for that collection date.
Superscripts with the number 1 indicate significant differences at 0.05 level between the respective collection date and day 0 for that group.
Superscripts with the number 2 indicate significant differences at 0.05 level between the respective collection date and day 140 for that group.
† Vaccines I, II, III, and IV administered.
‡ Vaccines II and III administered.

vaccination at day 0: by day 7 for MLV vaccines I and II and inactivated vaccine III; and day 10 for MLV vaccine IV. In the case of MLV vaccines I, II, and IV, there was a transient, yet significant increase in PI-3V serum antibodies postvaccination after day 0. Revaccination at day 28 with MLV vaccine III, induced increased PI-3V serum antibodies at day 42 and 56 over day 28. Inactivated vaccine III induced increased PI-3V serum antibodies from day 7 through 140 compared to day 0. In almost all collections from day 7 through 140, the PI-3V serum antibody titers induced by inactivated vaccine III were higher than those induced by MLV vaccines, I,II, and IV after vaccination at day 0.

The only vaccine to induce increased PI-3V serum antibodies after revaccination at day 140 was the inactivated vaccine III. The day 154, 168, and 196 PI-3V antibody titers induced by vaccine III were higher than those induced by the MLV vaccines I,II, and IV.

Antibody response to BVDV

The number of seronegative BVDV calves vaccinated with MLV and inactivated vaccines was limited (Table 5). BVDV seropositive calves were given these vaccines, but the results are not reported. The four seronegative calves receiving the inactivated vaccine III developed BVDV serum antibodies. One calf became seropositive at day 21, and another at day 28; whereas the other two calves developed BVDV antibodies by day 35, one week after receiving the second dose. One calf, no. 27 developed BVDV antibodies which were transient, lasting from day 35 to 56. The two BVDV seronegative calves receiving MLV vaccine, I and II, each developed BVDV serum antibodies, MLV vaccine I; day 35 after vaccination; and MLV vaccine II, day 21 after vaccination. The calf receiving a second dose of MLV vaccine II, developed an 8-fold increase in BVDV serum antibodies, 32 to 256 from day 28 to 35. For the inactivated vaccine III, with one exception, calf 27 which received 2 doses, the BVDV antibody titers induced after initial vaccination remained elevated until day 140.

Revaccination at day 140 with MLV or inactivated vaccines, did not always induce increased BVDV serum antibodies (Table 5). The two calves receiving MLV vaccine, I and II, had preexisting BVD antibody titers: 64 and 256. There was only a one dilution increase in BVDV antibodies for one calf (64 to 128) and a one dilu-

Table 5. Antibody response to BVDV after vaccination.

Serum Collection Day	Vaccine							
	I. MLV	II. MLV	III. Inactivated		Calf Number			
	Antibody titer†	Antibody titer	Geometric Mean*	Number of Calves with titer ≥ 1:4	Antibody titer			
					27	41	49	55
0‡	0.0	0.0	0.0	0/4	0	0	0	0
7	0.0	0.0	0.0	0/4	0	0	0	0
10	0.0	0.0	0.0	0/4	0	0	0	0
14	0.0	0.0	0.0	0/4	0	0	0	0
21	0.0	8.0	1.7	1/4	0	0	0	8
28§	0.0	32.0	3.4	2/4	0	0	4	32
35	32.0	256.0	22.6	4/4	4	32	32	64
42	64.0	256.0	26.9	4/4	4	64	32	64
56	32.0	128.0	45.3	4/4	8	64	64	128
84	32.0	64.0	26.9	4/4	0	64	128	64
112	64.0	128.0	26.9	4/4	0	256	64	32
140‡	64.0	256.0	64.0	4/4	4	512	128	64
154	64.0	256.0	152.2	4/4	32	512	128	256
168	128.0	128.0	152.2	4/4	64	512	128	128
196	128.0	128.0	152.2	4/4	16	512	128	256

*** 0.0 geometric mean indicates none of the calves had antibody titers of ≥ 1:4.**

† 0.0 titer indicates no neutralization of BVDV at 1:4, the lowest dilution tested.

‡ Vaccines I, II, and III administered.

§ Vaccines II and III administered.

tion decline for the other calf (256 to 128). Two calves receiving inactivated vaccine III developed four-fold or greater BVDV serum antibody titers after day 140 revaccination; calf 27, 4 to 64; and calf 55, 64 to 256. Two calves, no. 41 and 49, had titers of 512 and 128 respectively; but did not have increased BVDV antibody titers after day 140 revaccination.

Discussion

Results of the study indicate neutralizing serum antibody titers in calves receiving commercially available USDA licensed vaccines by the intramuscular route were increased after the initial vaccination. There were differences regarding the onset and duration of antibodies which were dependent on vaccine type. After the highest level of antibodies was attained to each virus after initial vaccination, there was general decline of antibodies until day 140. Revaccination at day 140 generally indicated an anamnestic response with increased serum antibodies to the respective virus. However, not all animals had this anamnestic response. Perhaps this lack of anamnestic response was due to antibody level at day 140, and/or vaccine type.

Summary Points of this Current Study

1. A multivalent vaccine containing both MLV and inactivated BHV-1 components induced higher BHV-I antibodies after only one dose than a MLV vaccine at day 10, 14, and an inactivated vaccine at day 14,21, and 28. All four vaccines, two MLV, an inactivated, and a MLV/inactivated combination vaccine induced increased BHV-I serum antibodies after the initial vaccination(s).
2. BHV-1 serum antibodies induced in calves by two doses of either an inactivated vaccine or a MLV/inactivated combination were higher and had longer duration of elevated BHV-I antibodies than those antibody titers induced by only one dose by two different MLV vaccines.
3. Three inactivated BRSV vaccines and a MLV BRSV vaccine induced increased BRSV serum antibodies after two doses. However, MLV BRSV induced more rapid and higher antibody levels than the inactivated vaccines.
4. An inactivated PI-3V vaccine induced higher PI-3V serum antibodies in PI-3V seropositive calves than did MLV vaccines.
5. An inactivated BVDV vaccine induced increased BVDV serum antibodies after two doses. Most, but not all of these BVDV antibody titers persisted until day 140.
6. All four BHV-I vaccines induced increased BHV-I antibodies after revaccination at day 140. Presence of actively induced BHV-I antibodies at day 140 did not prevent an anamnestic response after revaccination.
7. Revaccination of calves with low geometric mean BRSV serum antibody-titers (2.3 to 5.7) responded with increased BRSV titers. All four vaccines, three inactivated and one MLV induced increased BRSV antibodies. There were some animals with preexisting BRSV serum antibodies that did not develop increased BRSV antibodies after revaccination at day 140.
8. Revaccination of calves with PI-3V antibodies with an inactivated PI-3V vaccine induced increased PI-3V antibodies; whereas three MLV vaccines did not induce increased PI-3V antibodies.
9. Revaccination of calves at day 140 with inactivated or MLV BVDV vaccines gave variable results in a limited study. Two calves with BVDV antibody titers of 64 and 256 did not develop an anamnestic response after receiving MLV vacccine. Two calves previously vaccinated with an inactivated vaccine developed increased antibodies: 0 to 32 and 64 to 256. Two calves with higher antibodies, 512 and 128, did not develop an anamnestic response with increased antibodies.

The antibody responses to the vaccines in this study were similar to a companion study reported prior by this laboratory.[10] This current protocol used three different vaccines plus one MLV vaccine used in the prior study. This latter MLV vaccine contained MLV BHV-I, BVDV, PI-3V, and BRSV and served as another control to determine interexperiment ability of the cattle to respond to the same viral vaccine. The antibody responses to this MLV vaccine were comparable to the antibody responses in the prior study with one exception. In the current study, the calves (2) responded with increased BRSV antibody titers at day 154 after revaccination at day 140. The calves had BRSV antibody titers of 8 and 4 at day 140, with both calves having titers of 128 at day 154. In the prior study, five calves with BRSV antibody titers of 4 and 8 did not have four-fold or greater increase in BRSV antibody titers after revaccination. Potentially, the lower numbers of calves receiving MLV BRSV vaccine in the current study is a possible explanation for these different responses.

Other studies have investigated the ability of commercial vaccines to induce antibodies in calves after vaccination.[5,7,8,9,15,18,19] Certain studies examined the antibody duration of only a few weeks or as long as 30 months. In some instances the animals may have had preexisting antibodies to some of the immunogens.

A recent study examined the antibody response in calves given inactivated or MLV BRSV vaccines.[8] The calves received the respective vaccines on days 0 and 14. The serums assayed for viral neutralizing antibodies were from the day 24 collection, 10 days after the

second dose. The vaccine containing MLV BRSV and an inactivated BRSV vaccine induced higher geometric antibody titers than three other vaccines containing inactivated BRSV. The results of the current study were similar to this cited study.

The antigenic diversity of BVDV is applicable in discussing the results of the assay for BVDV antibodies in this study.[2,21] The Singer strain was used in the VNT. BVDV are now classified as Type 1 and 2 based on genotypic and antigenic differences.[14] The Singer strain is classified as a Type I.[14] The vaccines in this current study contained BVDV strains other than Singer: vaccines I and II contained the Type 1 NADL strain[1]; and vaccine III contained both Type 1 and 2 strains. Bolin and Ridpath reported in two separate studies that calves receiving MLV or inactivated BVDV vaccines responded with a range of neutralizing antibodies to several BVDV strains.[3,4] Ongoing studies are determining the antibody levels to several Type 1 and 2 strains including cytopathic and noncytopathic biotypes.

This study indicates differences in serologic responses by calves receiving BHV-I, BVDV, PI-3V, and BRSV immunogens in MLV or inactivated vaccines. These differences may not reveal variation in vaccine efficacy as challenge *in vivo* was not performed. Vaccine efficacy should include experimental challenge with the homologous agent, and the vaccines should provide protection against the homologous agent under natural exposure conditions. Serologic results of this current study from these *in vitro* challenges have limitations compared to *in vivo* studies.

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