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Clostridial antibody response from injection-site lesions in beef cattle, long-term response to single or multiple doses, and response in newborn beef calves^{1,2}

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ABSTRACT: Experiments were conducted to compare clostridial antibody response of beef heifers that do and do not develop injection-site lesions, evaluate long-term antibody response of a single- and multiple-dose toxoid, and evaluate the ability of a clostridial toxoid to elicit an active antibody response in newborn calves. In Exp. 1, 37 weaned heifers were vaccinated (d 0) with a clostridial vaccine (Alpha-7, 2 mL, s.c.). Serum samples were collected on d 0, 28, 56, 84, and 112 to determine clostridial antibody titers. On d 28, heifers were visually inspected and palpated for injection-site lesions. The percentage of heifers that developed lesions was 64.9%. Lesioned heifers had elevated antibody titers for *Clostridium chauvoei* (CC) on d 28 ($P < 0.08$) and 84 ($P < 0.07$) compared with non-lesioned heifers. *Clostridium sordellii* (CS) and *perfringens* type D (CPD) antibody titers were greater in lesioned heifers than in non-lesioned heifers on d 28 and 56. In Exp. 2, long-term antibody response of Alpha-7 (A7) and Ultrabac 7 (UB7) was investigated in stocker heifers. The A7 heifers ($n = 15$) received one 2-mL vaccination (d 0), and the UB7 heifers ($n = 15$) received a 5-mL vaccination on d 0 and 28. Blood samples were collected on d

0, 28, 56, 84, 112, 140, and 180. *Clostridium chauvoei*, CPD, and *Cl. novyi* (CN) antibody titers from the A7 heifers were greater than those from the UB7 heifers on d 28. Due to the second UB7 injection, CC, CS, CN, and *Cl. perfringens* type C (CPC) antibody titers were greater in UB7 heifers than in A7 heifers on d 56. By d 112, titers were not different, and by d 140 all antibody titers were below detectable levels. In Exp. 3, 58 pregnant, mature, crossbred cows were vaccinated with A7 before calving. At birth, calves were carefully observed to ensure consumption of colostrum. Calves were blocked according to parturition date, and calves in each block were randomly allocated to receive A7 (s.c. at 3 ± 3 d of age) or remain unvaccinated controls. Calves were bled at the time of vaccination (d 0) and on d 28, 56, 84, and 112. Antibody titers for CC, CPC, and CPD were elevated on d 0 and decreased throughout the experimental period ($P < 0.01$), but no titer differences ($P > 0.10$) were detected between treatment groups on any of the days sampled. These data indicated that antibody titers against clostridial diseases are enhanced when injection-site lesions develop. One injection of Alpha-7 seemed to provide the same length of protection as two injections of Ultrabac 7.

Key Words: Antibodies, Beef Cattle, Calves, Clostridium, Lesions, Newborn Animals

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Introduction

Clostridial diseases can affect beef cattle of all ages but are a primary concern in cattle between 6 mo and

2 yr of age. Because feeder cattle are marketed by the time they reach 2 yr of age, vaccinating for clostridial diseases is a matter for cow-calf producers, stocker cattle operators, and feedlot managers. Although clostridial vaccinations are very effective, 5-mL clostridial bacterins injected 376 and 255 d before slaughter produced lesion incidence in the sirloin butts of 92.7 and 79.5%, respectively (George et al., 1995). Therefore, the National Cattlemen's Beef Association, Beef Quality Assurance Task Force concluded that all products labeled for s.c. administration should be administered s.c. ahead of the point of the shoulder using the tented method (NCBA, 1995). This method of administration

¹Mention of trade names or proprietary or specific equipment does not constitute a guarantee of warranty of the product by the University of Arkansas and does not imply approval to the exclusion of other products that may also be suitable.

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for clostridial vaccines causes visible injection-site lesions (Beecher 1995; McFarlane et al., 1996).

Some clostridial vaccines require revaccination 4 to 6 wk following the initial treatment. Many cattle producers fail to gather their cattle for revaccination, making the duration of the initial vaccination between a single or multiple dose toxoid important. Many stocker cattle grazing programs and feedlot feeding programs last 110 to 180 d, so long-term, single-dose clostridial protection would be very beneficial. Because many feedlot managers have limited knowledge about the vaccination history of calves entering their feedlots, clostridial vaccination is very inexpensive insurance (Galyean and Eng, 1998). Approximately 91% of feedlots with a capacity of greater than 8,000 animals vaccinated against one or more clostridial agents (NAHMS, 2000).

The objectives of these experiments were to evaluate the clostridial antibody responses of calves that develop lesions at the injection site, to evaluate the long-term immune response of single- and multiple-dose toxoid, and to evaluate the ability of a clostridial toxoid to elicit an active antibody response in very young calves.

Materials and Methods

Experiment 1. Thirty-seven weaned, crossbred heifers (approximately 8 mo of age) from two locations (Fayetteville, AR; n = 15 and Greenbrier, AR; n = 22) were vaccinated with a 2-mL clostridial vaccine (Alpha-7, Boehringer Ingelheim Vetmedica, St. Joseph, MO). Alpha-7 bacterium-toxoid contains an oil adjuvant and is labeled for a single 2-mL injection. Injections were administered s.c. on the left side of the neck using the tented technique with a pistol-grip syringe. Enough toxoid was drawn into the syringe to vaccinate 10 animals. After the pistol-grip syringe was expired, the old 16-gauge, 1.91-cm needle was replaced with a new sterile needle and enough toxoid was withdrawn from the vial to vaccinate 10 additional heifers. If a needle became bent or burred, it was immediately replaced. The vaccination area was not cleaned, and the hair was not clipped. On d 28, heifers were visually inspected and palpated for injection-site lesions. Heifers that developed lesions were designated as the lesioned group, and those that did not were designated as the non-lesioned group. Heifers from the Greenbrier location had received clostridial vaccinations as preweaned calves, but heifers from Fayetteville had not. Heifers in each location were pastured and managed together according to acceptable management practices.

Blood was collected via jugular venipuncture from each heifer immediately before Alpha-7 injection (d 0) and on d 28, 56, 84, and 112. Blood samples were placed in crushed ice immediately after collection. Serum was harvested and stored at -20°C until it was assayed. Agglutination titers were determined for *Cl. chauvoei* by the serum agglutination test modified from Claus and Macheak (1972) and Troxel et al. (1997). Antitoxin units were determined for *Cl. perfringens* type D and

Cl. sordellii by the antitoxin neutralization test as described by USDA:APHIS:VS (1993) and Troxel et al. (1997) and by USDA (1998), respectively.

Experiment 2. Thirty weaned stocker heifers (194 ± 28.5 kg) were randomly assigned to receive injections of either Alpha-7 (n = 15) or Ultrabac 7 (n = 15, SmithKline Beecham Animal Health). Ultrabac 7 is labeled for 5-mL injections with revaccination in 4 to 6 wk and uses an aluminum hydroxide adjuvant. Both products protect beef cattle against *Cl. chauvoei* (blackleg), *Cl. septicum* (malignant edema), *Cl. novyi* (black disease), and *Cl. perfringens* types C and D. Heifers that received Alpha-7 (d 0) were administered one 2-mL injection, and heifers that received Ultrabac 7 were administered a 5-mL injection on d 0 and 28. All heifers were vaccinated s.c. in the neck region as described in Exp. 1. All heifers were pastured and managed together according to acceptable management practices.

Blood was collected via jugular venipuncture from each heifer immediately before Alpha-7 or Ultrabac 7 injection (d 0) and on d 28, 56, 84, 112, 140, and 180. Blood samples were handled as previously described in Exp. 1. Serum was harvested and stored at -20°C until it was assayed. Agglutination titers were determined for *Cl. chauvoei* and antitoxin units were determined for *Cl. perfringens* type D as described in Exp. 1. Antitoxin units were determined for *Cl. perfringens* type C, *Cl. novyi*, and *Cl. septicum* by the antitoxin neutralization test as described by USDA:APHIS:VS (1985) and Troxel et al. (1997), USDA (1999), and British Pharmacopoeia (1993), respectively.

Experiment 3. Fifty-eight mature (4 to 10 yr of age), pregnant, crossbred cows located at the University of Arkansas Monticello research farm were vaccinated with Alpha-7 85 \pm 14 d before calving. Mature cows (> 3 yr of age) were used because older cows showed an increased response to clostridial vaccination compared with younger cows (< 3 yr of age; Troxel et al., 1997). All cows received Alpha-7 in the neck region using the tented technique. Pregnant cows were vaccinated in order to achieve high levels of maternal antibodies in colostrum following calving. At the time of birth, all calves were carefully observed to ensure an adequate opportunity for the consumption of colostrum. Passive transfer of antibodies, however, was not measured. Following calving, calves were blocked according to parturition date, and calves within each block were randomly allocated to receive Alpha-7 (n = 29) or remain unvaccinated controls (n = 29). The Alpha-7-treated calves were injected s.c. at 3 ± 3 d of age in the neck region using the tented technique. Cows and calves were vaccinated using disposable (polypropylene) syringes and 16-gauge, 1.91-cm needles. All calves were bled via jugular venipuncture for serum samples at the time of vaccination (d 0) and then on d 28, 56, 84, and 112. Blood samples were processed as described in Exp. 1. Serum was assayed for *Cl. chauvoei* agglutination titers and for *Cl. perfringens* type C and D antitoxin units as described in Exp. 1 and 2. All cattle were pastured and

managed together according to acceptable management practices.

Data Analysis. In Exp. 1 and 2, heifers served as experimental units, and in Exp. 3 calves served as experimental units. *Clostridium chauvoei* was measured in microagglutination titers, whereas the other clostridials were measured as antitoxin units. Therefore, the term "titer" will be used to denote levels of the antibody response for all clostridials. Data from all three experiments were tested for normality by the Shapiro-Will test (SAS, Inst. Inc., Cary, NC). The null hypothesis was rejected ($P < 0.05$) for all three experiments. Therefore, we concluded that the data were not normally distributed around the mean. Because the data were not normally distributed, the variation around each mean value is not reported. Titers were transformed to a natural logarithm before analysis for all three experiments. Experiment 1 was arranged in a completely randomized design with two locations. Experiment 2 was also analyzed as a completely randomized design. Experiment 3 was arranged as a randomized complete block design with parturition date as the blocking criterion. The GLM procedure of SAS (SAS Inst. Inc.) was used to determine the effects of location, treatment, and interactions in Exp. 1, treatment effects in Exp. 2, and block and treatment effects in Exp. 3. Non-transformed least squares means are reported.

Results and Discussion

Experiment 1. On d 28, 64.9% of the heifers ($n = 24$) developed injection-site lesions with an average lesion size (diameter) of 5.6 ± 1.9 cm. These calves were designated as the lesioned group, and those that did not develop injection-site lesions ($n = 13$) were designated as the non-lesioned group. There were no differences ($P > 0.10$) between locations in the number of heifers developing injection-site lesions or in lesion size. All heifers were examined again for injection-site lesions on d 112. Forty-five percent of the heifers still had detectable lesions with an average size of 3.3 ± 2.0 cm. Beecher (1995) reported an injection-site lesion percentage of 50, 50, and 30 on d 18, 33, and 54, respectively, on steer calves following Alpha-7 vaccination. In that study, all steers were vaccinated on the left side of the neck where no other vaccinations were given and a 58.0 cm² area of hair was removed with electric clippers. The area was cleansed with an alcohol-soaked cloth, and the injection was administered with an 18-gauge, 2.54-cm needle that had been cleaned with alcohol. The tenting method for s.c. vaccination was used. The majority of lesions ranged between 2 and 6 cm. In the present study, the injection-site area was not clipped or cleansed with alcohol, nor were the needles cleaned with alcohol between vaccinations. This could explain the higher incidence of injection-site lesions (64.9%), but even using more sanitary techniques a 50% injection-site lesion percentage on d 18 and 33 resulted (Beecher, 1995). Many clostridial vaccines require two injec-

tions at 4- to 6-wk intervals. Beecher (1995) reported that following the second injection the percentages of injection-site lesions and injection-site swelling were greater than those occurring after the first injection.

In this study and in that of Beecher (1995), all lesions felt hard to the touch and none felt fluid-filled. McFarlane et al. (1996) reported that the d-30 lesion was characterized as dermatitis/myositis/cellulitis. It was described as chronic, lymphoplasmacytic, and fibrosing with mineralization. The center of the lesion was composed of sheets of degenerated neutrophils surrounded by mixed mononuclear cells, then fibrous connective tissue with abundant neovascularization extending outward between the muscle bundles. Scattered degeneration of skeletal muscle had occurred throughout the lesion with minimal regeneration.

Injection-site lesions may be caused by many factors. Some factors may include the animal's sensitivity to the clostridial vaccines, the vaccination injury itself, the adjuvant used to enhance the immune response, and contamination (dirty needles, skin, etc.) at the time of vaccination. Oil adjuvant vaccines (such as Alpha 7) are more successful in stimulating antibody production (Straw et al., 1986), and higher antibody titers have been associated with greater disease protection (Henry, 1983). Vaccines containing an oil adjuvant produce larger and more persistent lesions in the muscle than vaccines produced with aluminum hydroxide (Straw et al., 1986). Some have suggested vaccinating cattle against clostridial diseases at the base of the ear. Coffey et al. (1998) reported injection-site lesion percentages of 68 and 54% on d 20 and 34, respectively, following an Alpha-7 CD injection at the base of the ear in preweaned calves. The lesions averaged 1.3×4.1 cm (height \times length) and 3.0×6.1 cm on d 50 and 34, respectively. Chirase et al. (1998) vaccinated crossbred beef steers with clostridial vaccines at the base of the ear s.c. and reported no reduction in feedlot performance.

Mean titers for *Cl. chauvoei*, *Cl. sordellii*, and *Cl. perfringens* type D did not differ on d 0 between those with and without lesions (Table 1). Lesioned heifers had elevated antibody titer levels for *Cl. chauvoei* on d 28 ($P < 0.08$) and 84 ($P < 0.07$) compared with the non-lesioned heifers. There were no differences between lesioned and non-lesioned heifers on d 56 and 112. *Clostridium chauvoei* is a soil-borne infection that causes sudden death and is more common with pastured cattle (Radostits et al., 1994). Although not as common, it also has been reported in feedlot cattle (Galyean and Eng, 1998; Glock and DeGroot, 1998). *Clostridium sordellii* titers in the lesioned heifers were higher on d 28 ($P < 0.07$) and 56 ($P < 0.02$) than those in non-lesioned heifers, but no differences were detected on d 84 and 112. *Clostridium sordellii* can cause a fatal myositis and be identified as *Cl. chauvoei* or malignant edema (Radostits et al., 1994). Titers for *Cl. perfringens* type D were enhanced in the lesioned heifers on d 28 ($P < 0.02$), 56 ($P < 0.04$), and 84 ($P < 0.07$), compared with the non-lesioned heifers, but not on d 112. *Clostridium per-*

Table 1. Mean titers for *Cl. chauvoei*, *Cl. sordellii*, and *Cl. perfringens* Type D in serum of calves with (L) or without (NL) injection-site lesions (Exp. 1)^a

Experimental period, d	<i>Cl. chauvoei</i>			<i>Cl. sordellii</i>			<i>Cl. perfringens</i> type D		
	L	NL	Significance level	L	NL	Significance level	L	NL	Significance level
0	5.0 ^b	5.7	NS ^d	0.05 ^c	0.05	NS	0.05 ^c	0.05	NS
28	46.7	19.9	$P < 0.08$	0.32	0.16	$P < 0.07$	0.17	0.08	$P < 0.02$
56	30.1	20.1	NS	0.20	0.10	$P < 0.02$	0.21	0.10	$P < 0.04$
84	38.1	15.7	$P < 0.07$	0.15	0.10	NS	0.30	0.12	$P < 0.07$
112	19.1	16.4	NS	0.08	0.08	NS	0.30	0.16	NS

^aWeaned crossbred heifers (8 mo of age) that did (L; n = 24) or did not (NL; n = 13) develop lesions to Alpha-7 vaccination.

^bUnits of measure = microagglutination titers.

^cUnits of measure = antitoxin units.

^dNS = not significant ($P > 0.10$).

fringens type D, or pulpy kidney, can also cause sudden death, especially in calves between 1 and 4 mo of age. It is a short-term inhabitant that does not usually persist in the soil for more than 1 yr (Radostits et al., 1994). Bovine enterotoxemia in the feedlot has been identified as the result of enteric infection with *Cl. perfringens* types C and D (Barker et al., 1993).

The *Cl. chauvoei*, *Cl. sordellii*, and *Cl. perfringens* type D antibody response between lesioned and non-lesioned heifers followed the same basic pattern. Serum antibody titer levels for all three clostridial diseases started at the same level, but over time (d 0 to d 84) heifers that developed injection-site lesions showed an enhanced antibody response. Although the clostridium antibody response for the non-lesioned heifers was not as high as that for the lesioned heifers, the antibody response seemed to be adequate to protect them from a natural clostridium exposure. No heifers died during the experimental period.

There was a location effect for *Cl. chauvoei* on d 56 ($P < 0.05$) and 112 ($P < 0.06$) and a group \times location interaction on d 112 ($P < 0.04$). In data not reported here in tabular form, *Cl. chauvoei* titers were higher for heifers at the Greenbrier location on d 56 and 112 than for heifers at the Fayetteville location (39.0 vs 15.6 and 25.8 vs 12.1, respectively). The group \times location interaction on d 112 occurred because the Greenbrier lesioned heifers had higher titers than the Fayetteville

lesioned heifers (36.6 vs 19.9, respectively). One possible explanation for these location effects is that the heifers from the Greenbrier location were vaccinated for the clostridium diseases twice before the study. Therefore, their immune systems were already prepared to respond to additional vaccinations.

There was a group \times location interaction for *Cl. sordellii* on d 28 ($P < 0.005$), 56 ($P < 0.02$), and 84 ($P < 0.003$, Table 2). On d 28, Greenbrier non-lesioned heifers had enhanced titers similar to those of the Fayetteville lesioned heifers. The Fayetteville lesioned heifers had enhanced titer levels, but it seemed that the Fayetteville non-lesioned heifers did not respond. On d 56, the Fayetteville lesioned heifers' titers were still elevated compared with those of the other three groups. It seemed that the Fayetteville lesioned heifers were the only group to respond with elevated *Cl. sordellii* titers and that the Greenbrier heifers (lesioned and non-lesioned) responded similarly to the vaccine. On d 84, the interaction ($P < 0.003$) occurred because the Fayetteville lesioned heifers and the Greenbrier non-lesioned heifers had elevated titers compared with the Fayetteville non-lesioned and the Greenbrier lesioned heifers. The cause of this response is not known.

Experiment 2. Blood samples were collected and analyzed for *Cl. chauvoei*, *Cl. sordellii*, *Cl. perfringens* type D, *Cl. novyi*, *Cl. septicum*, and *Cl. perfringens* type C on d 0, 28, 56, 84, 112, 140, and 180. After d 112, titers

Table 2. Mean titers for *Cl. sordellii* in serum of calves with (L) or without (NL) injection-site lesions from the Fayetteville and Greenbrier locations (Exp. 1)^a

Experimental location	Day 28		Day 56		Day 84		Day 112	
	L	NL	L	NL	L	NL	L	NL
Fayetteville	0.37 ^b	0.07	0.39	0.07	0.24	0.07	0.09	0.09
Greenbrier	0.24	0.37	0.16	0.16	0.09	0.16	0.08	0.09
Lesion \times group interaction	$P < 0.005$		$P < 0.02$		$P < 0.003$		NS ^c	

^aWeaned crossbred heifers (8 mo of age) that did (L; n = 24) or did not (NL; n = 13) develop lesions to Alpha-7 vaccination.

^bUnits of measure = antitoxin units.

^cNS = not significant ($P > 0.10$).

Table 3. Mean titers for *Cl. chauvoei*, *Cl. sordellii*, and *Cl. perfringens* Type D in serum of calves vaccinated with either Alpha-7 (A7) or Ultrabac 7 (UB7) (Exp. 2)^a

Experimental period, d	<i>C. chauvoei</i>			<i>C. sordellii</i>			<i>C. perfringens</i> type D		
	A7	UB7	Significance level	A7	UB7	Significance level	A7	UB7	Significance level
0	6.7 ^b	5.7	NS ^d	0.05 ^c	0.05	NS	0.05 ^c	0.05	NS
28	61.4	13.6	$P < 0.05$	0.40	0.30	NS	0.30	0.10	$P < 0.06$
56	27.7	156.1	$P < 0.02$	0.40	1.80	$P < 0.01$	0.30	0.30	NS
84	42.5	37.3	NS	0.30	0.80	$P < 0.01$	0.30	0.20	NS
112	17.7	20.0	NS	0.10	0.10	NS	0.50	0.10	$P < 0.01$

^aStocker heifers (194 ± 28.5 kg) were vaccinated with either Alpha-7 (A7; n = 15) or Ultrabac 7 (UB7; n = 15).

^bUnits of measure = microagglutination titers.

^cUnits of measure = antitoxin units.

^dNS = not significant ($P > 0.10$).

for all clostridial disease units were below detectable levels and therefore are not reported. Titers for *Cl. chauvoei*, *Cl. sordellii*, *Cl. perfringens* type D, *Cl. novyi*, *Cl. septicum*, or *Cl. perfringens* type C did not differ ($P > 0.10$) between Alpha-7 or Ultrabac 7 groups before vaccination on d 0. *Clostridium chauvoei* ($P < 0.05$), *Cl. perfringens* type D ($P < 0.06$), and *Cl. novyi* ($P < 0.06$) titers from the Alpha-7 heifers were higher than those from the Ultrabac 7 heifers on d 28 (Tables 3 and 4). No differences were detected for *Cl. sordellii*, *Cl. septicum*, or *Cl. perfringens* type C. On d 56, *Cl. chauvoei* ($P < 0.02$), *Cl. sordellii* ($P < 0.01$), *Cl. novyi* ($P < 0.01$), and *Cl. perfringens* type C ($P < 0.01$) titers were higher in Ultrabac 7 heifers than in Alpha-7 heifers. This increased immune response was due to the second injection of Ultrabac 7 on d 28. Titers did not differ between treatments on d 56 for *Cl. perfringens* type D or *Cl. septicum*. Day-84 *Cl. sordellii* and *Cl. perfringens* type C titers remained higher ($P < 0.01$) for Ultrabac 7 heifers. By d 112, differences between treatments were only detectable for *Cl. perfringens* type D; Alpha-7 heifers had higher ($P < 0.01$) levels than Ultrabac 7 heifers. By d 140, all titers were below detectable levels.

Clostridium novyi is an acute, infectious disease of sheep, sometimes of cattle, and rarely of pigs and horses (Merck, 1998). Sudden feedlot deaths occur as a result of infections with *Cl. novyi*, which proliferate in parenchymal tissues with subsequent fatal toxemia (Glock

and DeGroot, 1998). This organism multiplies in areas of liver necrosis resulting from the migration of liver flukes, and it produces a powerful necrotizing toxin (Merck, 1998). *Clostridium septicum* is generally fatal in cattle, horses, sheep, goats, and swine. It is found in the soil and intestinal contents of animals (including humans) throughout the world. Infection ordinarily occurs through contamination of wounds (castration, docking, insanitary vaccination, etc.). *Clostridium perfringens* type C is an extremely fatal disease caused by two toxins, alpha and beta, which are produced as metabolic by-products of the bacteria. This disease shows itself as severe enteritis usually demonstrated by a purple or blackish section of intestine. It occurs in calves from 1 to 10 wk of age; the most common age is between 3 and 6 wk. It also causes severe enteritis with diarrhea and dysentery in young lambs, pigs, and foals. In very acute cases, death occurs in a few hours, sometimes without diarrhea being evident (Radostits et al., 1994). *Clostridium perfringens* type C can also affect calves in the feedlot (Barker et al., 1993).

Following labeled instructions for all vaccines is a critical component for vaccination success. Beef calves are often vaccinated for clostridial diseases with one injection even though the vaccination label states that two injections should be given 4 to 6 wk apart. Troxel et al. (1997) demonstrated that vaccinating beef calves at 50 d of age with one injection of Ultrabac 7 and not

Table 4. Mean titers *Cl. novyi*, *Cl. septicum*, and *Cl. perfringens* Type C in serum of calves vaccinated with either Alpha-7 (A7) or Ultrabac 7 (UB7) (Exp. 2)^a

Experimental period, d	<i>Cl. novyi</i>			<i>Cl. septicum</i>			<i>Cl. perfringens</i> type C		
	A7	UB7	Significance level	A7	UB7	Significance level	A7	UB7	Significance level
0	0.05 ^b	0.05	NS ^c	0.05 ^b	0.05	NS	0.08 ^b	0.09	NS
28	0.90	0.08	$P < 0.06$	0.70	0.90	NS	4.00	1.90	NS
56	0.60	2.10	$P < 0.01$	0.60	0.90	NS	3.50	8.40	$P < 0.01$
84	0.50	0.60	NS	0.50	0.60	NS	0.80	2.80	$P < 0.01$
112	0.25	0.10	NS	0.50	0.60	NS	0.60	1.40	NS

^aStocker heifers (194 ± 28.5 kg) were vaccinated with either Alpha-7 (A7; n = 15) or Ultrabac 7 (UB7; n = 15).

^bUnits of measure = antitoxin units.

^cNS = not significant ($P > 0.10$).

again until d 170 may not provide adequate protection against clostridial diseases. In the current study, Alpha-7 seemed to cause an enhanced antibody response compared with the first Ultrabac 7 injection, but the second Ultrabac 7 injection on d 28 enhanced the titers for *Cl. chauvoei*, *Cl. sordellii*, *Cl. novyi*, and *Cl. perfringens* type C on d 56. Even with the enhanced antibody response resulting from the second Ultrabac 7 injection, long-term antibody response through d 112 was not improved. Therefore, one injection of Alpha-7 seemed to provide the same long-term protection as two injections (2 to 4 wk apart) of Ultrabac 7.

Experiment 3. Titers for *Cl. chauvoei*, *Cl. perfringens* type C, and *Cl. perfringens* type D were elevated in the serum of newborn beef calves 3 ± 3 d of age (d 0) and decreased throughout the experimental period ($P < 0.01$). There were no titer differences ($P > 0.10$) detected between calves treated with Alpha-7 and calves that remained as untreated controls for *Cl. chauvoei*, *Cl. perfringens* type C, and *Cl. perfringens* type D on any of the days sampled. Therefore, titer means for *Cl. chauvoei*, *Cl. perfringens* type C, and *Cl. perfringens* type D were pooled across treatments and reported as one mean per sampling period. The mean titers for *Cl. chauvoei* on d 0, 28, 56, 84, and 112 were 699, 227, 258, 156, and 141, respectively. These levels are much higher than *Cl. chauvoei* levels reported by Troxel et al. (1997). In that study, *Cl. chauvoei* serum titers for d 50 ranged between 22 and 46, compared with 258 for d 56 in this study. The mean titers for *Cl. perfringens* type C on d 0, 28, 56, 84, and 112 were 6.8, 5.3, 2.6, 2.4, and 0.6, respectively, and for *Cl. perfringens* type D were 3.3, 1.2, 0.5, 0.5, and 0.3, respectively. Because all dams received Alpha-7 vaccination before calving, it is not known whether Alpha-7 vaccination enhanced the passive immunity for clostridial diseases to the suckling calf.

Maternal antibody, or the antibody acquired by the calf from absorption of colostrum antibody during the first 24 to 48 h after birth, has a profound suppressive effect on active immunization to most vaccines. Maternal antibody prevents the vaccine from immunizing for variable periods after birth. The period during which the colostrum-acquired antibody interferes with active immunization depends on the amount of antibody present in the dam and the amount of colostrum absorbed (Schultz, 1994). Kaeberle et al. (1997) vaccinated calves between the ages of 28 and 69 d (d 0) with commercially available infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza₃, and bovine respiratory syncytial virus inactivated vaccines. Almost all calves had detectable antibodies against each of the viruses at the time of initial vaccination (d 0). Thirty days following primary vaccination, mean antibody titers to all viruses had declined in all groups of calves indicating minimal, if any, humoral response to the vaccines. It was not until the second vaccination on d 32 that vaccination effects were detectable. In the current study, pregnant cows were vaccinated to enhance colostrum passive pro-

tection against clostridial diseases, and attention was given to ensure colostrum consumption by each calf. Although passively acquired antibody is essential for calf survival, the maternal antibody levels must decline before vaccines can actively immunize (Schultz, 1994).

Research conducted by Boehringer Ingelheim Vetmedica (P. W. Widel, personal communications) demonstrated that Alpha-7 enhanced a *Cl. perfringens* type C and *Cl. chauvoei* immune response in dairy calves vaccinated at less than 1 wk of age. At 60 d of age, titer levels for *Cl. perfringens* type C and *Cl. chauvoei* were enhanced, and the *Cl. chauvoei* titers continued to increase up to 92 d of age. This suggested that these calves were mounting an active antibody response to Alpha-7. Although titer levels for *Cl. perfringens* type C did not increase from 60 to 92 d of age, the titer levels were similar. This suggested that no loss of antibody production occurred during this time. This study suggests that an oil adjuvant vaccine may enhance an antibody response in very young dairy calves.

Implication

These results indicate that titers against clostridial diseases are enhanced when injection-site lesions develop. Lesions associated with an injection should not be a discounting factor when pricing cattle, but rather a sign that the cattle were properly immunized. Many cow-calf and stocker cattle producers are not interested in gathering calves to administer a second clostridial vaccination as indicated by label. One injection of Alpha-7 seemed to provide the same length of protection as two injections of Ultrabac 7 given 4 to 6 wk apart. These data also emphasize that vaccinating newborn beef calves will not enhance their antibody protection against clostridial diseases. The success of a vaccination program depend on management, proper timing of vaccination, and using the product correctly.

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