One Year Antibody Responses of Four Inactivated Infectious Bovine Rhinotracheitis-Bovine Viral Diarrhea Vaccines

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Introduction

Modified live virus (MLV) vaccines for infectious bovine rhinotracheitis and bovine viral diarrhea have been available and periodically improved over time, but recent reports still indicate questions about their safety.[1-5] On the other hand, some researchers[6,7] and many practitioners with whom the author has talked do not consider inactivated vaccines to be suitable for routine use. Morter[2] and Neaton[3] reviewed the factors involved in choosing between MLV and inactivated vaccines, and the final decision usually is based on the past experience of the veterinarian involved. Unfortunately, their experience with inactivated vaccines may be years in the past and the decision needs to be rethought from time to time. The early experience that inactivated vaccines are less protective than MLV against severe challenge probably will not always hold true.

Serum neutralizing antibody titers have long been recognized as good indicators of protection against naturally occurring disease, and inactivated vaccines that approach the MLV vaccines in ability to protect in all situations likely will be ones that elicit substantial and sustained antibody titers similar to those seen with MLV vaccines. Regulatory personnel recognize a serum neutralizing (SN) antibody titer of 1:4 as protective against challenge infection with infectious bovine rhinotracheitis virus (IBRV), and a 1:8 SN titer as protective against challenge infection with bovine viral diarrhea virus (BVDV). Because resistance to challenge is the primary criterion by which vaccines are approved for release (appropriately so) many inactivated vaccines are marketed even though they maintain the above mentioned titers for only a short period of time. Yet in testing a vaccine it is impossible to duplicate really severe field challenges, some of which involve several concurrent infections.

Two previously used means of enhancing antibody response and presumably increasing challenge resistance to BVDV are the use of "immunomodulating" adjuvants[8] and incorporation of strains of both cytopathic (CP-BVDV) and noncytopathic (NCP-BVDV) biotypes of the virus.[3] When a product that combines these characteristics was introduced to the market about two years ago, a test seemed in order in which the new vaccine would be compared with the two singly "enhanced" vaccines as well as a vaccine typical of the conventional killed BVD-IBR vaccines then available. The results of the year-long study are reported herein.

Methods

Experimental Protocol

Each of four groups of yearling heifers (n = 8 or 9) was vaccinated twice with one of four commercially available vaccines containing, as a minimum, inactivated IBRV and BVDV antigens. A fifth group (n = 7) served as sentinel controls to determine that other exposure to IBRV and BVDV did not occur during the course of the study. Mixed breed yearling heifers were purchased from a South Dakota ranch and moved to a 60 acre pasture near Lincoln, Nebraska where the vaccinations and subsequent bleedings were done. Each of the vaccinates was administered two doses of one of the four test vaccines.

The vaccines used in the study were purchased from regional veterinary supply houses. All were well within expiration dates and were kept refrigerated until time of use. The vaccine names, manufacturers, component antigens and lot numbers are given in Table 1, along with the designation of the group of calves receiving the respective vaccine. The first dose was administered 3 weeks after arrival (day 0) and the second dose 34 days later. The vaccines were injected as directed by the manufacturers with respect to amount and injection site. The cattle were bled at the time of each vaccination and subsequently at approximately 8-week intervals until 1 year after the first vaccination.

Preliminary bleedings on the ranch of origin had indicated that the cattle would probably all be seronegative for IBRV and BVDV. When the SN tests were done, at the end of the study, it was seen that two of the heifers (one each in Groups A and B) had been positive at the highest dilution tested (1:256) at time of the first bleeding and vaccination. Data from those heifers were excluded from the study. Two others were positive at a 1:4 dilution, but

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TABLE 1. Group designations and vaccines administered.

Group/ Vaccine	No. of Heifers	Vaccine	
		Name/Lot Nos.	Antigens (killed)
Control	7	None	–
A	9	Vira Shield-4[A] (Lots 41-003 and 41-005)	IBRV, PI-3, NCP- & CP-BVDV
B	8	Triangle 3[B] 190226B	IBRV, BVDV, & PI-3
C	9	Premier IB & IBPL-5[C] 08-1017 and 135-1004	IBRV, NCP- & CP-BVDV (Plus PI-3 & Lepto, Dose 2)
D	8	Horizon II[D] 9089 and 9149	IBRV & BVDV

[A] Grand Laboratories, Inc.
[B] Ft. Dodge Laboratories, Inc.
[C] Biologics Corporation (TechAmerica)
[D] Diamond Scientific Co.

those heifers (one each in Groups B and C) were included because in both cases they had responded to the vaccine with antibody titers higher than the mean for the group. Sera from all other animals were antibody negative (at 1:2) at the time of first vaccination.

Serum Neutralization Tests

The tests for CP-BVDV and IBRV, which were routine microtitration tests, were done at the Veterinary Diagnostic Center of the University of Nebraska Veterinary Science Department. The endpoints for those tests were the highest dilution at which there was 100 percent neutralization of cytopathic effect. Tests for NCP-BVDV were done by Dr. John Black, American BioResearch Laboratory, Milton, Tennessee. The test endpoints were the highest dilutions at which there was 100 percent neutralization of infection at four days, as measured by immunofluorescence. Laboratory personnel handled samples labelled with calf numbers only, without knowledge of which vaccine any animal had received. The target viral $TCID_{50}$ was 300 per serum dilution for all tests. In order to minimize variations from cell or virus differences, all of the tests were run at one time (NCP-BVDV), or in two consecutive settings one week apart (IBRV and CP-BVDV). Doubling dilutions were made from 1:2 through 1:256. Although some of the sera that tested positive at 1:256 doubtless were not at endpoint, they were treated statistically as having a titer of 1:256, and those that were negative at 1:2 were treated as having a titer of one. Geometric mean titers (GMT) were calculated for each group at each bleeding time and used in the tabulation of data.

Results

The results, in the form of SN titer GMT for each group of heifers at each bleeding date, are presented graphically in Figures 1–3. The numerical values (as GMT reciprocals) are shown in Tables 2–4.

Discussion

In this study in mostly seronegative cattle, the vaccine given to Group A calves stimulated much higher SN titers than did the other three vaccines. The BVDV titer in the Group A calves peaked at a GMT of just over 1:94 at both the 89 and 146 day bleedings. The peak GMT for IBRV was just under 1:12 at 89 days. At the end of a year, the calves in Group A still had very substantial GMT values of 1:87, 1:17 and 1:6 for CP-BVDV, NC-BVDV and IBRV viruses, respectively. The GMT peaks for the heifers receiving the other 3 vaccines were much lower, and apparently there was little sustained production of antibodies.

Evidence of the heterogeneity of BVDV virus isolates[9-11] indicates that any method of broadcasting the antigenicity in BVDV vaccines is desirable. The vaccines given to Group A was the only one of the vaccines tested here to elicit more than a minimal level of antibody to the test strain of NCP-BVDV. The fact that it contains a strain of NCP-BVDV as well as CP-BVDV is probably important. However, the other test vaccine (Group C) that contains both biotypes of BVDV virus elicited comparatively low BVDV antibody responses.

The manufacturer attributes the relatively high and long lasting antibody titers obtained with Vaccine A to the use of an immunomodulating adjuvant. The vaccine given to Group D also contains an immunomodulating adjuvant.[8] In previous reports, vaccines used in Groups B and D both were reported to elicit SN titers that were still reasonably high one year after vaccination.[8,12] The author can only speculate about the discrepancy between those

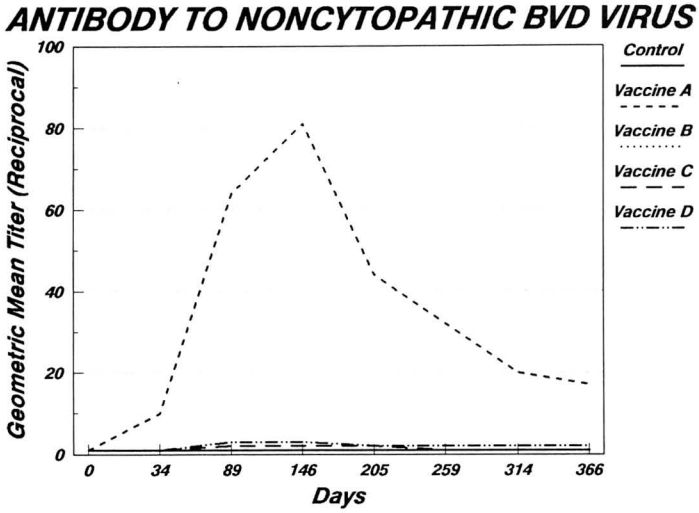

FIG. 1. Geometric mean antibody titers to cytopathic BVD virus at indicated intervals in non-vaccinated control calves (n=7) and in four groups of calves (n=8 or 9) given respectively, 1 of 4 different killed vaccines (all received 2 doses, at 0 and 34 days).

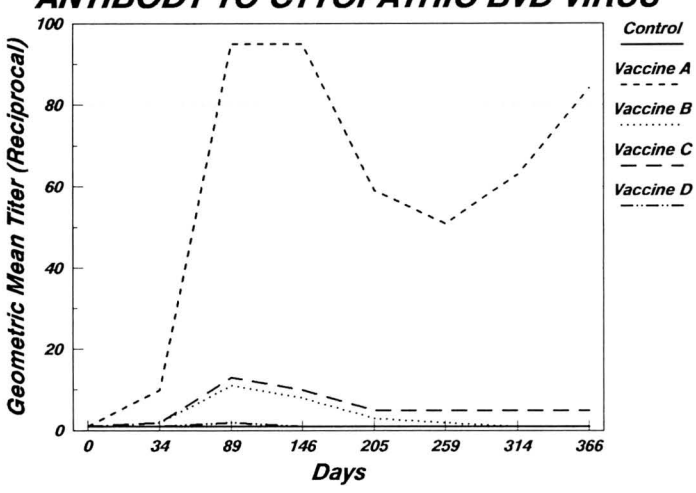

FIG. 2. Geometric mean antibody titers to cytopathic BVD virus at indicated intervals in non-vaccinated control calves (n=7) and in four groups of calves (n=8 or 9) given respectively, 1 of 4 different killed vaccines (all received 2 doses, at 0 and 34 days).

results and the ones obtained in this study, guessing that it is due to use of a more sensitive serological test in the earlier work, or to lot-to-lot variation in the amount of antigen in the products in question.

Objections commonly voiced in the past about inactivated IBRV-BVDV vaccines are that they produce a less rapid protection, that they are less capable of eliciting immunity when they are given in the presence of more than minimal maternal antibody titers, and that they produce shorter-lived protection.[3,4,7] Those parameters were

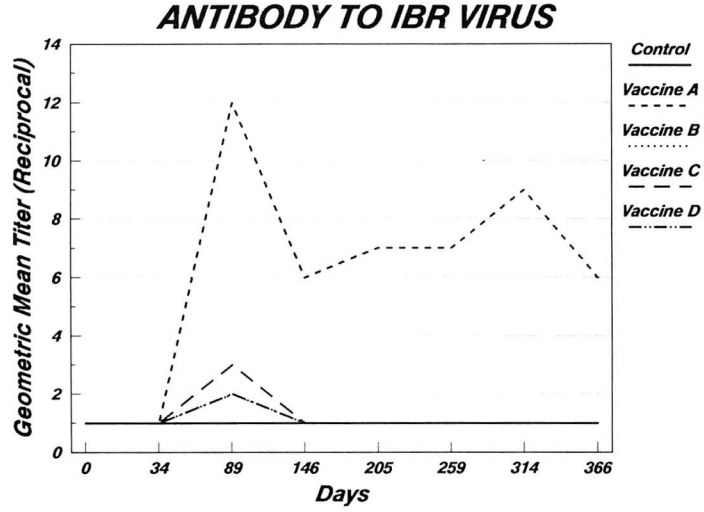

FIG. 3. Geometric means titers to IBR virus at indicated intervals in non-vaccinated control calves (n=7) and in four groups of calves (n=8 or 9) given respectively, 1 of 4 different killed vaccines (all received 2 doses, at 0 and 34 days).

not directly investigated in this study and they should be addressed.

Obviously, the more definitive measures of vaccine efficacy are resistance to challenge with a virulent strain of the virus and possibly determination of the rapidity of the anamnestic response after challenge infection. The fairest comparison of long-term efficacy of vaccines would be based on those criteria as well as antibody titers, plus an assessment of cell-mediated immunity. However, the easiest and most economical (and therefore most widely used) test for predicting protection against viral disease is in vitro determination of the level of neutralizing antibodies in the serum. From experience, this is known to be highly correlative to immunity for the great majority of viruses. To find an inactivated vaccine that is equally as immunogenic as the MLV vaccines, then, it would seem that the first step would be to find one that at least elicits and

TABLE 2. Geometric mean antibody titers to cytopathic BVDV.

Group	Days after first vaccination 0	34	89	146	205	256	314	366
Control	<2	<2	<2	<2	<2	<2	<2	<2
Vaccine A	<2	10	94	94	59	51	64	87
Vaccine B	<2	3	11	7	4	3	2	3
Vaccine C	<2	3	13	9	5	5	5	6
Vaccine D	<2	<2	3	2	2	2	3	2

TABLE 3. Geometric mean antibody titers to noncytopathic BVDV

Group	Days after first vaccination 0	34	89	146	205	256	314	366
Controls	<2	<2	<2	<2	<2	<2	<2	<2
Vaccine A	<2	10	64	81	44	32	20	17
Vaccine B	<2	<2	<2	<2	<2	<2	<2	2
Vaccine C	<2	<2	2	2	2	<2	<2	<2
Vaccine D	<2	<2	3	3	2	2	2	2

TABLE 4. Geometric mean antibody titers to IBRV.

Group	Days after first vaccination 0	34	89	146	05	256	314	366
Controls	<2	<2	<2	<2	<2	<2	<2	<2
Vaccine A	<2	<2	12	6	7	7	9	6
Vaccine B	<2	<2	2	<2	<2	<2	<2	<2
Vaccine C	<2	<2	3	<2	<2	<2	<2	<2
Vaccine D	<2	<2	2	<2	<2	<2	<2	<2

maintains high SN titers in calves kept under comparatively optimal conditions. Relative to that criterion, the vaccine given to the calves in Group A did very well.

Conclusions

Because of their safety, the use of inactivated vaccines will almost certainly continue to increase. Four federally licensed, inactivated BVDV-IBRV vaccines were compared in this trial. Only one of the four was effective in eliciting high SN titers that persisted over a one-year period. No determination was made, however, of the relative effectiveness of the four vaccines in protecting against challenge infection with virulent virus.

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