A Practitioner's Experience with Experimental Reo-Coronavirus Calf Diarrhea Vaccine

Paul E. Blackmer, *D.V.M.*
672 W. 18th Street
Upland, California 91786

During 1974, several of my clients with large dairy calf raising operations suffered exceptionally heavy losses from calf diarrhea. The diarrhea, for the most part, did not pose a problem until the calves were about one week old. Starting usually at the fifth to seventh day of age and continuing until the calves died or recovered 5-10 days later, diarrhea severe enough to require treatment affected close to 90% of the calves. Diarrhea-related mortality at 30 days of age varied from 20-35%. Good sanitation, combined with antibiotic and electrolyte therapy, seemed to have little effect on the course of the disease. The calf management in these herds was considered equivalent to or better than what was working elsewhere within the area.

A majority of the dead calves were autopsied. Most frequently there were no gross lesions other than those associated with dehydration and diarrhea. Also, bacteriology of spleen and liver specimens from these calves usually was negative. This, however, may be attributed to the fact that affected calves received antibiotics during the course of treatment. Some of the calves that were moribund and sick for less than five days were euthanized in order to collect fresh, acceptable specimens to confirm or rule out a diagnosis of reo- or coronavirus diarrhea. From these calves, six-inch ligated sections of small intestine and spiral colon were removed and immediately frozen. Some of these frozen samples were sent to the California State Diagnostic Laboratory and others were sent to Norden Laboratories in Lincoln, Nebraska. The majority of these samples were positive for coronavirus using the fluorescent antibody test. One specimen was positive for both reo- and coronavirus.

Experimental Procedures

Arrangements were made to run field trials in three of these herds using an experimental oral modified reo-coronavirus calf diarrhea vaccine produced by Norden Laboratories. The initial field trial protocol called for a double blind experimental design using 60 to 100 calves per trial. The vaccine-to-placebo ratio was 3:1. Each dose of vaccine was number coded. When a calf was vaccinated, usually within a few hours after birth, the vial number and the calf's ear tag number were recorded. All treatments and responses to treatments were recorded for the first 21 days of each calf's life. Fecal samples were collected and frozen on any calf that scoured as soon as possible after the scouring was observed. All dead calves less than 21 days of age were autopsied and six-inch ligated sections of small intestine and spiral colon were collected and frozen. At the completion of the trials all specimens were sent to Norden Laboratories for evaluation.

Reo-coronavirus vaccine was made available for vaccination of every calf for the remainder of the calving season in those herds that conducted field trials.

One of the herds, Herd 1, elected to use this vaccine without placebo, for further experimentation. An odd-even day vaccination schedule was used. Calves born on the even days were vaccinated, while calves born on odd days were not. One hundred calves were in each group.

Following this odd-even experiment, every calf in Herd 1 was vaccinated for approximately two months. A total of 77 calves were then left unvaccinated, after which vaccination was again resumed. This unvaccinated group within a vaccinated population provided data for a controlled comparison, which was designated an "alternate-interval" test.

A similar alternate-interval experiment was conducted in Herd 3. Following the 90-calf double blind study, 90 consecutive calves were not vaccinated, after which every calf was vaccinated. This procedure provided a group with 75% vaccinates (and 25% placebos), followed by an unvaccinated group, and finally a 100% vaccinated group.

Results

The results of the three double blind field trials are shown in Table 1. In terms of morbidity and mortality no significant differences were noted between vaccinated and placebo groups in any of the three herds.

The results of further experimentation in Herds 1 and 3 appear in Tables 2 and 3. As in the double blind study, the odd-even day vaccination study failed to show any significant differences between vaccinated and unvaccinated control calves.

The alternate-interval studies in Herds 1 and 3 did, however, show a marked reduction in mortality and morbidity in vaccinated versus unvaccinated controls.

Herd 2, after the double blind study, went to an every-calf vaccination policy. Calf morbidity and mortality declined gradually to an all-time low for that herd.

Table 1

Summary of Double Blind Studies in Three Dairy Herds

Herd & Test Group	No. Calves	No. Developing Diarrhea*	% Developing Diarrhea*	No. Died	% Mortality
1 - vaccinates	75	70	93	12	16
placebos	25	21	84	3	12
2 - vaccinates	75	66	88	15	20
placebos	25	24	96	7	28
3 - vaccinates	68	50	73	3	4.5
placebos	22	16	73	0	0

*Before 21 days of age.

Table 2

Comparisons of Experimental Designs in Herd 1

Phase of Study	No. Calves	% Developing Diarrhea*	% Mortality at 21 Days
Historic (Sept. 1974)	300+	90+	35
Double Blind (Oct.-Nov. 1974)			
vaccinates	75	93	16
placebos	25	84	12
Odd-Even (Nov. - Dec. 1974)			
vaccinates	100	90	10
controls	100	90	15
Alternate-Interval			
every calf vaccination (Feb. 1975)**	77	38	0
unvaccinated group (Feb. 1975)	77	51	0
resumption of every calf vaccination (March 1975)	77	25	0

*Before 21 days of age. **Last 77 valves vaccinated before discontinuation.

Table 3

Comparison of Experimental Designs in Herd 3

Phase of Study	No. Calves	% Developing Diarrhea	% Mortality at 21 Days	Ave. No. Treatments
Historic (Feb. 1975)	300	90	15	10
Double Blind (Feb. - March 1975)				
vaccinates	68	73	4	NA
placebos	22	73	0	NA
Alternate-Interval (March 1975)				
unvaccinated	90	69	22	10.2
vaccinates	103	56	3	7.1

Discussion

The failure of the vaccine to show efficacy in the double blind and even-odd day study, while demonstrating efficacy in the alternate-interval study, justifies a discussion of acceptable experimental design in evaluating a live virus vaccine. My experience with the above efficacy studies suggests that there is probably no unbiased way to evaluate live vaccines under field conditions. The alternate-interval method, however, seems to be more valid than the double blind or odd-even day methods.

In retrospect, it appears that the double blind and odd-even day vaccination studies are of limited value in measuring the performances of the vaccine. First, in that we were dealing with a modified live virus vaccine, it is possible for the vaccine virus to spread to the placebo or unvaccinated control group. Apparently, the field virus is easily spread, since it caused such a high incidence of morbidity under "natural" conditions in test herds. If the vaccine is efficacious and its virus can be spread from vaccinates to controls, then the controls no longer function as comparison animals. They, too, have received the protection of the vaccine to some degree. Because 50-75% of the population is vaccinated and in close proximity to the unvaccinated controls, the likelihood that modified vaccine virus is shed to control animals is considerable.

Aside from the possibility of virus shedding in the case of live vaccines, another factor must be considered in field trial evaluations, whether for killed or live vaccines. That is the level of infection on a premise. It is well-accepted that by increasing a challenge dose the likelihood of overwhelming an animal's resistance to a disease is increased. In herd disease epidemics, it is reasonable to assume that disease is occurring because the infective dose, the level of infection on a premise, overwhelms the affected animal's resistance (to the extent that it exists) to that disease. Once a calf is infected with reo- or coronavirus it sheds large amounts of infective fecal material, maintaining the high level of infection in its surroundings. If then, an efficacious vaccine is brought into a herd and administered to some of the animals providing them with resistance to the disease such that they do not become infected, they will not further disseminate or multiply that infection on the premise. Thus, with an efficacious vaccine being used on a premise, the level of infection on that premise will decrease. Unvaccinated animals then will be subjected to a lower natural infective dose. Under this situation the controlled study such as the double blind study loses merit.

Another aspect of experimental design should be considered in assessing the validity of vaccine efficacy data. That is the interrelationship among 1) the degree of immunity provided by a vaccine, 2) the level of challenge (level of infection on a premise), and 3) the length of time the vaccine is used. For example, a vaccine that offers limited protection against coronavirus may appear effective in herds with low levels of infection. In herds with high levels of infection, the natural challenge of the surrounding level of contamination may overwhelm the protection provided by the vaccine to many individual calves. In the latter cases, vaccination of every calf would be required for a prolonged time before the limited efficacy (or ultimate potential) of the vaccine could express itself. The likelihood of any efficacy being demonstrated at all would be lessened if 1) unvaccinated controls continued to contaminate the premise with greater amounts of virus than the vaccinates and 2) the time period during which vaccination occurred was insufficient. I believe this was the case in both the double blind and odd-even day vaccination trials.

The alternate-interval method of evaluating the vaccine does not suffer from the variables inherent in the double blind or odd-even day vaccination trials. The alternate-interval format is affected by changes in day-to-day weather, changes in season, and differences in management from one interval to another.

Historically, in the area where the field trials were implemented, calf morbidity and mortality are greatest during the summer months and lowest during winter and spring. If the test intervals of vaccinating and not vaccinating overlapped these seasons, differences in morbidity and mortality attributed to climate alone could outweigh the influence of the vaccine. By the same token, unusually severe weather over a short period (5-7 days) may be significant enough to unfavorably influence overall morbidity and mortality for the entire test period, while continuous mild weather prevailing for a subsequent trial allows exceptionally good performance in another group.

A remaining problem inherent in the alternate-interval trial method is the introduction of variables in management policies or personnel from one interval to the next. Ideally, the same personnel should handle both vaccinates and controls throughout a given test period.

The bias in assessing alternate-interval test results can be minimized if trials are conducted independently at a number of locations during different periods of time, and under different management systems and personnel. The three herds that I had observed before the introduction of vaccine, during the trials, and later, after every calf vaccination was practiced for a prolonged time, fit this criterion. The fact that all three herds showed significant reductions in morbidity and mortality only after every calf was vaccinated indicates that the reo-coronavirus vaccine is efficacious when used in this manner. The results of the alternate-interval studies in Tables 2 and 3 support this conclusion.