Field Trial Evaluation of a Reo-Coronavirus Calf Diarrhea Vaccine

E. T. Thurber, E. P. Bass and W. H. Beckenhauer*

ABSTRACT

Field trials were conducted using an experimental, modified live virus, oral vaccine for prevention of reo- and coronavirus calf diarrhea. Prior to the trials, one or both of the specific causative agents were identified from affected calves in each participating herd. In 21 herds, sequential trials were conducted in which results of uninterrupted vaccination were compared with disease rates during a preceding or subsequent control period. In these herds there was a statistically significant reduction in the morbidity and mortality from disease in 1,598 vaccinates compared with the rates in 829 prevaccination control calves. Morbidity and mortality in 206 post-vaccination control calves rose marginally above the rates in the same vaccinates. In 26 other herds, where double blind trials were conducted, rates of morbidity and mortality from disease were virtually the same for 1,080 vaccinated calves and 355 placebo calves. Vaccinates in the sequential trials had the lowest morbidity and mortality rates of any test group in either field trial format.

In a selected dairy herd, both field trial formats were implemented and the results compared. In the double blind trial, vaccinates and placebo calves had comparable rates of morbidity and mortality from disease. When a sequential trial was later implemented, a statistically significant reduction in morbidity and mortality occurred in vaccinates compared with rates in control calves.

---

*Research and Development Division, Norden Laboratories, Lincoln, Nebraska 68501.

Submitted June 18, 1976.
VACCINE

The experimental reo-coronavirus vaccine was prepared by propagating attenuated strains of the viruses in established bovine cell lines. Vaccine was lyophilized in single dose vials and provided with sterile diluent and syringes. Reconstituted vaccine was administered orally in a 4 ml dose as soon as possible and no later than 24 hours following the calf’s birth.

FIELD TRIAL DESIGN

All laboratory diagnoses and field trials were conducted during the 1974-75 calving season under the supervision of cooperating veterinarians who dispensed the experimental vaccine to participating herd owners. Vaccinated and control calves were observed in all cases for a minimum of 21 days following birth for signs of neonatal diarrhea.

In the sequential trials, vaccine was administered to consecutive calves until the specified number of doses had been used. Calves born during a period of comparable length just prior to or following the vaccination interval served as the control group. In the double blind trials, vaccine and placebo were administered interchangeably in a 3:1 ratio. All vials were number coded and otherwise identical in appearance so that neither the veterinarian nor his client could distinguish vaccine from placebo.

All calves involved in the field trials were identified with individual ear tags. Records for each calf were maintained indicating the ear tag number, dose number, date of birth, age of inoculation, occurrence of disease, age of affected calf, type of treatment and response to medication.

COMPARATIVE CASE HISTORY

A direct comparison of the two experimental formats was made by implementing both field trials in a single herd, a 6,500 cow dairy in southern California with a history of severe neonatal calf diarrhea. Just prior to testing in this herd, positive diagnosis of the specific coronavirus in sections of intestine from five diarrheic calves was made by the Veterinary Laboratory Services division of the California Department of Food and Agriculture.

INTRODUCTION

A reovirus and a coronavirus have been incriminated as primary causative agents of neonatal calf diarrhea (2, 4, 5). An experimental modified live reo- and coronavirus vaccine administered orally to newborn calves was developed for prevention of disease and two field trial formats were used to evaluate the vaccine by means of controlled testing in affected herds. In 21 herds, a format was used in which results of uninterrupted vaccination could be compared with disease rates during a preceding or subsequent control period. This format was designated a sequential trial. In 26 other herds, double blind trials were conducted. In a comparative test, both field trial formats were implemented in succession in a large dairy herd with a history of severe neonatal calf diarrhea (1). Results of the two field trial formats were compared to determine if performance of the vaccine was affected by differences in the experimental design. Results of the sequential trials also indicate the expectations from using the vaccine according to recommendations in affected herds.

MATERIALS AND METHODS

HERDS

All trials were conducted in herds with a history of neonatal calf diarrhea. In each herd, positive diagnosis of the specific reo- and/or coronavirus was obtained by fluorescent antibody evaluation prior to testing. The 21 herds where sequential trials were conducted were located in eight states and the 26 herds where double blind trials were conducted were located in 14 states. Both types of field trials included beef and dairy herds.

Management practices specifically designed to reduce the effects of neonatal diarrhea were implemented in the herd prior to the field trials. Under this system of management calves were born in designated areas with concrete floors that were washed down twice daily. Neonatal calves were immediately isolated from their dams and raised in individual pens built to minimize physical stress. Pens were periodically steam cleaned and rotated and waste materials were removed on a daily basis. The feeding program utilized filtered, refrigerated colostrum pooled from fresh cows and pooled fresh cow milk followed by milk replacer, free choice starter ration and water. Routine bacteria counts were made to monitor the quantity of colostrum and milk, which were treated with antibiotics when necessary to avoid excessive contamination. All feeding utensils were cleaned and replaced daily. Calves were observed and health records were updated on a daily basis for the first 30 days following birth. These and other management practices that were in existence prior to testing were maintained throughout the field trials. Thus, the herd was considered suitable for implementing several trials over an extended period with minimal variations in test conditions.

Historical data from the herd was first reviewed to determine the incidence and death loss from neonatal diarrhea prior to the field trials. The initial test was a double-blind trial conducted with 100 calves. In a second test, 100 calves born on even numbered days were vaccinated, with 100 calves born on the intervening odd numbered days serving as a control group. After the alternate day vaccination, three groups of 77 calves each were evaluated in succession in a sequential trial. In the first group all 77 calves were vaccinated, in the second group no vaccination was performed and in the third group all calves were again vaccinated. Following the various controlled tests, 500 consecutive calves were vaccinated and observed.

**RESULTS**

**SEQUENTIAL TRIALS**

Field trial results are summarized in Table I. In the sequential trials, vaccinates had substantially lower morbidity and mortality than the prevaccination control calves, a comparison that is statistically significant (P < 0.01). Although response to treatment was subjectively reported (except in cases of mortality) vaccinates also scored notably better than the prevaccination control calves.

In the postvaccination control group, morbidity and mortality rates rose marginally above the rates in vaccinates but were substantially lower than the rates in the prevaccination control calves. The difference in disease rates for the two control groups in the sequential trials is statistically significant (P < 0.01). Vaccinates had comparable disease rates, however, relative to pre- or postvaccination control groups. Morbidity and mortality for vaccinates with prevaccination controls were 27.6% and 2.8%, respectively, while vaccinates with postvaccination controls had 23.9% morbidity and 3.0% mortality.

Results are reported for the total number of calves in all herds, a procedure whereby large herds influence results more than small herds. Data were also evaluated by averaging the respective rates of morbidity or mortality from each herd. This procedure

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. Herds</th>
<th>Test Group</th>
<th>No. Calves</th>
<th>Incidence of Diarrhea</th>
<th>Response to Treatment(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Morbidity</td>
<td>% Mortality</td>
</tr>
<tr>
<td>Sequential</td>
<td>21</td>
<td>Vaccinates</td>
<td>1,598</td>
<td>25.7</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frevacc. controls</td>
<td>829</td>
<td>52.1</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Postvacc. controls</td>
<td>206</td>
<td>30.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Double blind</td>
<td>26</td>
<td>Vaccinates</td>
<td>1,080</td>
<td>43.2</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebos</td>
<td>355</td>
<td>43.7</td>
<td>8.2</td>
</tr>
</tbody>
</table>

*Key to response to treatment: 1 = excellent, 2 = good, 3 = fair, 4 = poor, 5 = died
gives equal weight to all herds. The differences resulting from the two methods of comparison were minor. When data were transformed using the conventional arc sin method the relative trends in both the total count and equal weight evaluations remained unchanged. In all comparisons, differences in incidence of disease in vaccinates versus prevaccination control calves were statistically significant.

**Double Blind Trials**

Data from the double blind trials (Table I) show no appreciable difference in incidence of disease or response to treatment for vaccinates versus placebo calves. As in the evaluation of data from the sequential trials, results were also analyzed by giving each herd equal weight. Both methods of analysis were repeated using data that had been transformed by the arc sin method. No comparison of morbidity or mortality for vaccinates versus placebo calves showed differences approaching statistical significance.

**Comparative Case History**

Results of the field trials in the dairy herd are shown in Table II. In the double blind trial (test 1), morbidity rates in both vaccinates and placebo calves were similar to the pretrial incidence of disease. There is an indication that death loss decreased from pretrial rates but there was little difference between mortality rates in vaccinates and placebo calves.

Results of the alternate day vaccination (test 2) were similar to those for the double blind trial, with disease occurring at a comparable rate in all calves. Death loss remained below pretrial rates, although mortality data for this particular test tends to favor the vaccinates.

In the sequential trial (test 3) morbidity was markedly reduced and mortality was eliminated when consecutive calves were vaccinated (January-February and March-April). Morbidity in the control group (February-March) was higher than in either vaccinated group, a difference that is statistically significant (chi squares greater than 9.0, P < 0.01). During the second (March-April) vaccination period, incidence of disease dropped to the lowest level recorded for any preceding test group.

In the post trial group of 500 vaccinated calves, morbidity and mortality from disease remained unchanged from the preceding test group. Severity of disease as indicated by response to treatment declined.

### TABLE II. Results of Comparative Field Trials in a Dairy Herd

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Period</th>
<th>Test Group</th>
<th>No. Calves</th>
<th>%Morbidity</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fretrial</td>
<td>Sept 74</td>
<td>Nonvaccinates up to 30 days</td>
<td>300</td>
<td>90+</td>
<td>35</td>
</tr>
<tr>
<td>1. Double blind</td>
<td>Oct-Nov 74</td>
<td>Vaccinates up to 21 days</td>
<td>75</td>
<td>93</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebos up to 21 days</td>
<td>25</td>
<td>84</td>
<td>12</td>
</tr>
<tr>
<td>2. Alternate day......</td>
<td>Nov-Dec 74</td>
<td>Vaccinates up to 21 days</td>
<td>100</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>vaccination</td>
<td></td>
<td>Controls up to 21 days</td>
<td>100</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td>3. Sequential.........</td>
<td>Jan-Feb 75</td>
<td>Vaccinates up to 30 days</td>
<td>77</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Feb-Mar 75</td>
<td>Controls up to 30 days</td>
<td>77</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mar-Apr 75</td>
<td>Vaccinates up to 30 days</td>
<td>77</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Post trial............</td>
<td>Apr-Jun 75</td>
<td>Vaccinates up to 30 days</td>
<td>500</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>
further, however. Affected calves had a relatively mild diarrhea that responded in most cases to one time symptomatic treatment.

DISCUSSION

Results of the sequential trials (Table 1) show that continuous vaccination of newborn calves on a farm with preexisting reo- or coronavirus diarrhea substantially reduced incidence and death loss from the disease. A comparison of results in vaccinates versus prevaccination control calves indicates that vaccination reduced morbidity by almost one half and reduced mortality by a factor of seven. In addition, vaccinates that were treated had a much more favorable response to therapy (63% had an “excellent” or “good” response, while 75% of the prevaccination controls had a “poor” response or died), suggesting they were less severely affected.

The significant difference in the disease rates for the two control groups in the sequential trials indicates that a continuous vaccination program has some residual benefits after discontinuance. Perhaps because vaccination reduced the reservoir of infection into which subsequent calves were born, postvaccination controls had much lower morbidity and mortality rates than prevaccination controls, at least for the 21-day observation period.

In contrast to the sequential trials, results of the double blind trials (Table I) failed to demonstrate any benefit from vaccination. In the double blind format, vaccinates and nonvaccinated placebo calves were intermingled. In effect, this created herds where vaccination was practiced intermittently leaving 25% of the calves unvaccinated. Under these conditions disease affected all calves to a comparable degree. Calves in the double blind trials were not as severely affected, however, as prevaccination control calves in the sequential trials, i.e. a totally susceptible group without prior experience with vaccine.

These comparisons demonstrate that factors inherent in the two field trial formats produced marked differences in test results. In the double blind trials, more than 40% of the nonvaccinated placebo calves were affected by disease. Thus, a substantial reservoir of natural infection was continuously maintained in the presence of the vaccinates. Both the reo- and coronavirus have incubation periods of less than 24 hours (2, 5) so that prevaccination exposure in the face of this heavy infection pressure no doubt occurred.

By contrast, vaccinates and control calves in the sequential trials were managed separately so that no contact existed between the two test groups. This experimental design would allow the vaccine to further reduce the reservoir of infection, thus achieving optimum efficacy and explaining why results in these vaccinates were significantly better than in any other test group.

This interpretation is substantiated by results in the dairy herd case history where both field trial formats were implemented in a large, well managed herd with minimal opportunity for variation in test conditions. This comparison demonstrated that variation in field trial format, not in test herds, is associated with significant differences in performance of the vaccine. The double blind test (test 1) in this herd showed limited benefits from vaccination but the sequential test (test 3) resulted in a significant reduction of morbidity and mortality from neonatal diarrhea. In the sequential test, two groups of vaccinates had substantially lower morbidity than an intervening control group. Given only one such comparison, this result could be considered a possible seasonal variation. However, significantly lower morbidity rates both before and after the control period is a strong indication of the benefits of vaccinating all calves.

The control calves in test 3 also had substantially lower disease rates than nonvaccinated calves in previous tests. This parallels the results of the sequential trials in the 21 other herds where, after continuous vaccination was suspended, disease rates in postvaccination controls remained at levels lower than in the prevaccination controls.

The dairy herd case history also significant because it indicates that by itself, sound management emphasizing sanitation, isolation and good nutrition does little to reduce severe, preexisting viral diarrhea. Only when an experimental vaccination program was implemented did morbidity and/or mortality from disease decrease in this particular herd.

As a test design, the double blind trial can be useful for two reasons. First, use of
a placebo eliminates bias in assessing vaccine performance. Secondly, simultaneous controls offset variation in test conditions. Results of the reo-coronavirus vaccine field trials indicate that the double blind format is not a suitable test design for evaluating a modified live virus oral vaccine, however. Placebo calves that become infected with natural virus may spread disease to vaccinates, precluding or overwhelmed a protective response from vaccination. Certainly, the double blind format does not simulate good farm practice, where a vaccination program is administered to all susceptible animals. Indeed, results of the double blind trials indicate that failure to vaccinate a minority of calves (i.e. 25%, the proportion represented by placebo calves) would militate against a successful program of prevention for neonatal diarrhea.

The sequential trial, on the other hand, approximates good farm practice in that an uninterrupted vaccination of all calves is practiced. A concern for unbiased testing by means of this test format is minimized because mortality is one index of performance and determination of death is not subject to bias. Thus, it is significant that mortality in vaccinates was only one-seventh the rate in prevaccination control calves.

In addition, the sequential trials were characterized by large sample sizes and different geographic locations, factors which tend to reduce the influence of seasonal variation. Thus, by comparing morbidity and mortality in vaccinates with that in controls born before or after the vaccination period, a reasonable assessment of the vaccine’s efficacy can be made. According to this criteria, the reo-coronavirus vaccine makes a significant contribution in controlling morbidity and mortality from neonatal calf diarrhea caused by the reo- and/or coronavirus.

Vaccination of consecutive calves was, in fact, the method used for testing a reo-

**ACKNOWLEDGMENTS**

The authors thank Dr. S. M. Free, Jr. of SmithKline Corporation for his statistical analysis and Mr. R. M. Dana of Norden Laboratories for assistance in preparation of the manuscript.

**REFERENCES**