Safety of a Modified-Live Combination Vaccine Against Respiratory and Reproductive Diseases in Pregnant Cows*

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ABSTRACT
A combination vaccine (Bovi-Shield FP4 + L5, Pfizer Animal Health) containing modified-live virus (MLV) components against bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), parainfluenza virus-3 (PI3), bovine respiratory syncytial virus (BRSV), and inactivated cultures of Leptospira canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona was evaluated for safety in pregnant beef and dairy animals. Heifers vaccinated pre-breeding with the minimum immunizing dose (lowest antigen level initiating immunizing effects) of the vaccine’s MLV BHV-1 or BVDV components and during pregnancy (approximately 200 days of gestation) with vaccine containing 10× doses of the same BHV-1 and BVDV components delivered live, healthy calves that were determined to be serologically negative (titer less than 1:2) for neutralizing antibodies to BHV-1 and BVDV prior to nursing. Additionally, in three field safety studies, previously vaccinated cows and heifers that received a field dose (vaccine containing antigen levels required for commercial sale of the MLV combination vaccine during either the first, second, or third trimester of pregnancy had abortion rates similar to those of pregnant cows and heifers vaccinated during the same stage of pregnancy with sterile water diluent.

INTRODUCTION
Modified-live virus (MLV) bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), parainfluenza virus-3 (PI3), and bovine respiratory syncytial virus (BRSV) combination vaccines have been proven safe in non-pregnant animals in controlled studies and years of field use. However, their use has been contraindicated in pregnant cattle because of con-
cerns that abortions and developmental disorders are likely to occur when BHV-1 and BVDV cross the placenta of seronegative cows and infect the fetus. The purpose of the four studies reported here was to evaluate the safety of administering a combination vaccine containing MLV BHV-1/BVDV/PI3/BR SV to pregnant cattle that had been vaccinated prior to breeding with the same MLV vaccine components.

The first study was an intensive safety evaluation in which nonvaccinated pregnant cattle and pregnant cattle previously vaccinated (pre-breeding) with a minimum immunizing dose (MID) of the MLV components received a vaccine formulation containing 10× doses of the MLV components. MID levels are established prior to vaccine licensing and reflect a lower volume of antigenic virus than is present in the commercial product. MID levels were used in the present study to demonstrate a worst-case scenario (i.e., vaccination at the lowest levels of antigen to initiate an immune response), followed by subsequent challenge with a 10× dose level of viruses. Determination of the MID helps ensure that a product used at release levels will consistently stimulate adequate protection against disease.

The other three studies were field safety evaluations in which all study cattle were initially vaccinated prior to breeding with field dose levels of a combination vaccine containing the MLV BHV-1, BVDV, and PI3 components or a combination vaccine containing these three components plus MLV BR SV. Cattle were then vaccinated during the first, second, or third trimester of pregnancy with sterile water diluent or a vaccine containing field dose levels of vaccinal BHV-1, BVDV, PI3, and BR SV.

### MATERIALS AND METHODS

#### Intensive Safety Study

Pregnant crossbred beef heifers (n = 79) in approximately 160 to 220 days of gestation were selected from a commercial herd for enrollment in the study. All animals were identified by uniquely numbered ear tags and were main-

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<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Animals</th>
<th>Prebreeding Vaccination History</th>
<th>MID Component</th>
<th>Day 0 Vaccination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>None</td>
<td>None</td>
<td>10× MLV vaccine†</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>14</td>
<td>None</td>
<td>MLV vaccine†</td>
<td>BHV-1</td>
</tr>
<tr>
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<td>15</td>
<td>MLV vaccine†</td>
<td>MLV vaccine†</td>
<td>BHV-1</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>15</td>
<td>None</td>
<td>MLV vaccine†</td>
<td>BVDV</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>15</td>
<td>MLV vaccine†</td>
<td>MLV vaccine†</td>
<td>BVDV</td>
</tr>
</tbody>
</table>

*Day 0 = approximately 100 to 220 days of gestation.
†MLV vaccine = vaccine containing bovine herpesvirus-1, bovine respiratory syncytial virus, bovine viral diarrhea virus, and parainfluenza virus-3 administered in combination with *Leptospira* spp bacterin.
††MLV vaccine (same components as listed above) administered in combination with *Campylobacter fetus–Leptospira* spp bacterin. MID = minimum immunizing dose.
tained together on pasture throughout the study. Animals had free access to well or surface water and grass pasture supplemented with a balanced feed ration. From this group, 20 pregnant heifers with no history of vaccination against BHV-1 or BVDV that were negative (titer less than 1:2) for serum-neutralizing antibodies to BHV-1 and/or type-1 and -2 BVDV (BVDV-1 and BVDV-2) prior to vaccination were allocated to an untreated control group (Table 1). Thirty of the 59 pregnant heifers had been vaccinated approximately 150 days prior to breeding with a combination vaccine formulated to contain the MID of either BHV-1 (n = 15) or BVDV (n = 15) and were assigned to the vaccine group (Table 1). These 30 heifers and the remaining 29 heifers assigned to that group were then vaccinated approximately 30 days before breeding with a combination vaccine formulated to contain the MID of either BHV-1 or BVDV components of the BHV-1/BVDV/PI3/BR SV test vaccine. All animals were observed daily beginning on Day –1 for general health status and abortions through parturition.

The experimental design for the intensive safety study is summarized in Table 1. On Day 0, the pregnancy status of each animal was confirmed, and all 79 pregnant heifers were vaccinated (IM) with a vaccine formulated to contain 10 × the dose levels of the MLV BHV-1, BVDV, PI3, and BRSV components of the test vaccine/Leptospira spp bacterin. Duplicate blood samples were collected from each animal in serum separator tubes on Day 0 and again on Day 28. Serum samples were assayed at BenchMark BioLabs, Lincoln, NE, for neutralizing antibodies to BHV-1, BVDV-1, and BVDV-2.

Heifers that aborted between the time of vaccination and parturition were identified, and duplicate blood samples were collected from each of these animals on the day of abortion and again 2 weeks later. Serum was extracted, and samples were labeled with the identity of the animal and date of collection, and were stored frozen. Aborted fetuses were shipped to the Kansas State University Veterinary Diagnostic Laboratory in Manhattan, Kansas to determine possible causes of the abortions.

The health and nursing status of each calf born during the study was determined on the day of calving. Duplicate blood samples were collected in serum separator tubes from each calf on the day of birth and at 3 days of age. Serum samples collected from the calves were assayed at BenchMark BioLabs for neutralizing antibodies to BHV-1, BVDV-1, and BVDV-2. All animal caregivers and laboratory personnel were unaware of treatment group assignments throughout the study.

**Field Safety Studies**

Pregnant crossbred beef cattle (n = 1,422) from commercial herds were enrolled in three field safety evaluations. In the first field study, 599 beef cows were randomly allocated to two groups. The cattle were identified by numbered ear tags, freeze brand, or both. All animals were in the first trimester of pregnancy and had been vaccinated prior to breeding with a combination vaccine containing the same MLV BHV-1, BVDV, and PI3 components as the test vaccine. Cows in one group (n = 302) were not vaccinated during pregnancy and served as an untreated control group; the remaining 297 cows were assigned to the vaccine group and were given a single IM injection of the commercial vaccine containing MLV BHV-1, BVDV, PI3, and BRSV plus Leptospira spp bacterin on Day 0 (Table 2). At the time of vaccination (Day 0), one control cow was eliminated because the ear tag number was a duplicate of another animal in the study and three vaccinates were removed when their pregnancy status was determined to be problematic (i.e.,
uterine adhesions, questionable viability of calf, previous abortion). Three other vaccinates were removed when it was discovered their ear tag numbers were duplicates of other animals in the group. The remaining 297 cows in the vaccinate group were given a single IM injection of the commercial vaccine containing MLV BHV-1, BVDV, PI3, and BRSV plus Leptospira spp bacterin on Day 0. Cattle were managed in accordance with the standard procedures of the study site, with access to grass pasture and potable water. Cattle were observed weekly for general health status and for abortions through parturition. A second pregnancy examination was performed on Day 77, and any animal that was not pregnant at that time was recorded as aborted.

In the second field study, 475 pregnant Holstein heifers in their second trimester from a commercial dairy herd were enrolled. All heifers had been vaccinated prior to breeding with a combination vaccine containing the same MLV BHV-1, BVDV, PI3, and BRSV plus Leptospira spp bacterin on Day 0. Cattle were managed in accordance with the standard procedures of the study site, with access to grass pasture and potable water. Cattle were observed weekly for general health status and for abortions through parturition. A second pregnancy examination was performed on Day 77, and any animal that was not pregnant at that time was recorded as aborted.

Cattle were housed together in an outdoor drylot pen and were moved to maternity pens as they approached their estimated calving dates. Animals were fed an appropriate custom-blended ration and had free access to well water. In this study, 238 animals were initially designated to serve as controls and the remaining 237 animals received the test vaccine (Table 2). All animals were evaluated daily for general health status and abortions from Day –1 through parturition.

Crossbred beef cows (n = 348) in their third trimester of pregnancy from a commercial herd were enrolled in field Study 3. Animals were identified by uniquely numbered ear tags and were maintained on pasture throughout the study. The diet was supplemented according to standard procedures on the site, and animals had free access to well or surface water. All animals had a prebreeding vaccination with a combination vaccine containing the same MLV BHV-1, BVDV, and PI3 components as the test vaccine. One hundred fifty cows were initially assigned to the control group and 198 to the test vaccine group (Table 2). However, on Day 0 (day of vaccination during the third trimester of preg-
nancy), one control was removed from the study because of an injury, and on Day 12, a vaccinated cow was removed from the study because it had been injured. All study animals were observed weekly for health status and abortions through parturition.

**Evaluations**

The experimental design of the three field safety studies is shown in Table 2. Duplicate prevaccination (either Day –1 or Day 0) blood samples were collected in serum separator tubes from each animal in each study, and examinations were performed for all animals to confirm pregnancy status. The health condition (normal or abnormal) of each neonatal calf was determined in all three studies.

Aborting animals in the studies were identified, and duplicate blood samples were collected from these cows at the time an abortion was diagnosed. Two additional blood samples were collected from these animals approximately 2 weeks after an abortion. The aborted fetus and placenta (if available and acceptable for diagnostic purposes) were shipped to veterinary diagnostic facilities in close proximity to the test sites (Department of Veterinary Diagnostic Services, North Dakota State University for Study 1; California Animal Health and Food Safety Laboratory System for Study 2; and Kansas State University Veterinary Diagnostic Laboratory for Study 3). Serum samples were shipped to the respective diagnostic laboratories for serologic evaluations. Calf deaths that were attributed to fetotomy, dystocia, poor mothering, or adverse weather conditions were not considered for diagnostic tests. All animal caregivers and laboratory personnel were unaware of treatment group assignments throughout the studies.

**Analysis**

Data were analyzed using SAS (SAS/STAT Release 6.12, SAS Institute). Descriptive statistics were calculated as appropriate for all four studies. For the field safety studies, abortion and calving rates were compared between treatment groups using Fisher’s exact test.

**RESULTS**

**Intensive Safety Study**

Six of 11 (55%) control heifers that were negative (titer less than 1:2) for serum-neutralizing antibodies to BHV-1 on Day 0 aborted their calves due to BHV-1 infection between Days 25 and 43 (Table 3). Four of the remaining five calves delivered by control heifers were negative for prenursing antibodies to BHV-1. A prenursing blood sample was not obtained from one calf that nursed before a sample could be collected. In comparison, no abortions occurred in heifers vaccinated with the BHV-1, BVDV, PI3, and BRSV components of the combination test vaccine prior to breeding. All 59 (100%) of these heifers delivered live, healthy calves, and all 58 calves sampled prior to nursing were negative for serum neutralizing antibodies to BHV-1 (Table 3). The one calf that was antibody-positive had nursed prior to sample collection.

All 13 control heifers that were negative for BVDV-1 and -2 neutralizing antibodies on Day 0 and that did not abort due to BHV-1 infection delivered live, healthy calves (Table 3). However, prenursing sera from nine of 12 calves sampled from control heifers had neutralizing antibodies for BVDV-1 (titer 1:23 or higher) and BVDV-2 (titer 1:19 or higher). A prenursing sample was not obtained from one calf born to a control heifer. None of the 58 calves from the heifers vaccinated prebreeding had neutralizing antibodies for BVDV-1 and -2 (Table 3). A prenursing sample was not obtained from one calf.

**First Trimester Study**

At the examination on Day 77, one of 302 control cows had aborted compared with no
abortion detected from 297 cows vaccinated during the first trimester of pregnancy (Table 4). Following that examination, 72 animals (39 controls and 33 vaccinates) were removed from the commercial breeding herd for various conditions unrelated to the vaccine study (i.e., low body condition score, poor production, disposition, cancerous eye, lameness, or udder or teat defects) in accordance with standard management practices. At the end of the study (Day 252), one additional control animal and one vaccinated animal had aborted. Overall, the difference in abortion rates between these groups (0.8% for controls versus 0.4% for vaccinated cows) was not significant. The cause of the abortions was not identified by the diagnostic laboratory and the presence of BHV-1 or BVDV was not demonstrated for any of these abortions.

Seven sets (4 controls, 3 vaccinates) of normal, healthy twins were delivered to cows in this study.

**Second Trimester Study**

Two control animals and two vaccinates were removed from the study before the pregnancy examination. These included one control and one vaccinate that had sustained injuries and one control and one vaccinate that were found to have incorrect breeding dates recorded. Abortions were recorded for 11 of the remaining 236 (4.7%) control heifers and 14 of the remaining 235 (6.0%) heifers vaccinated during the second trimester of pregnancy (Table 4). The difference between groups was not significant. One abortion in the vaccinated group was attributed to BHV-1 infection and the remaining 24 abortions in the study were from *Neospora* infection (3 controls, 6 vaccinates), congenital defects (1 control), and undiagnosed causes (7 controls and 7 vaccinates). The historical record showed that the participating dairy typically experienced abortion rates up to 10% in first-calf heifers during the time of the year when this study was conducted.

The calving rate (percentage of confirmed pregnant animals delivering normal calves) was not statistically different than the rate for

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**TABLE 3. Effects of a Modified-Live Virus Combination Bovine Vaccine Given to Cows Before Breeding and During Pregnancy (10× Dose) on Pregnancy and Status of Calves Born to These Cattle**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>BHV-1</th>
<th>BVDV-1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Abortions</td>
<td>No. of Calves with Prenursing Antibodies</td>
</tr>
<tr>
<td>Control</td>
<td>6/11*</td>
<td>0/4†</td>
</tr>
<tr>
<td>Vaccinates</td>
<td>0/59</td>
<td>0/58†</td>
</tr>
</tbody>
</table>

*Abortions occurred between Day 25 and Day 43.
†Prenursing blood samples were not obtained from one calf in each group.
‡Control calves with prenursing antibodies had titers of 1:23 or higher for BVDV-1 and 1:19 or higher for BVDV-2.
BHV = bovine herpesvirus; BVDV = bovine viral diarrhea virus.
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heifers treated with sterile water diluent (95.3%) and heifers receiving the test vaccine (93.6%). Of the total number of calves delivered by control group heifers, 79.7% were alive and healthy and 20.3% were normal but died during parturition. In comparison, 78.4% of the calves delivered by vaccination group heifers were alive and healthy and 21.2% died during parturition. The rate of dystocia mortality for first-parity heifers typically ranges between 20% and 25% at this dairy.

**Third Trimester Study**

One of the 149 (0.7%) control cows and one of 197 (0.5%) vaccinated cows aborted during the study (Table 4). The calf for one vaccinated cow could not be located and was presumed to have been lost to predator attack; therefore, data for this cow were not included in the analysis. The cause of the abortions was not identified by the diagnostic laboratory and the presence of BHV-1 or BVDV was not demonstrated.

Five calves died during parturition (2 controls, 3 vaccinates) and one calf from a vaccinated cow died from exposure to adverse weather conditions. All of the remaining control group and vaccinate group calves were delivered alive and healthy.

**DISCUSSION**

Approval for a new label indication for the use of the MLV components of Bovi-Shield FP (BHV-1/BVDV/PI3/BRSV/Leptospira spp bacterin) and the companion vaccine PregGuard FP (BHV-1/BVDV/PI3/BRSV/Campylobacter fetus/Leptospira spp bacterin) in pregnant cattle that have received a pre-breeding vaccination was granted based on the studies reported here, with the provision that the vaccines must be used in accordance with label directions. The products were also given approval for a label claim of safety and efficacy in calves nursing pregnant cattle based on these studies. Additional testing was not required for the approval to use in nursing calves because administration of the vaccine directly to pregnant cows was determined to be a more stringent test of vaccine safety, circumventing the variability and inefficiency of test models in which pregnant cattle are exposed to recently vaccinated calves. The intensive study model assured exposure to multiple standard doses of the vaccinal viruses and circumvented the variability and inefficiency of test models in which pregnant cattle are exposed to recently vaccinated calves.

In the initial safety study, administration of a test vaccine formulated to contain 10× field doses of the MLV BHV-1, BVDV, PI3, and BRSV components of a commercial vaccine did not cause any untoward clinical effects or adversely affect the pregnancies of heifers vaccinated prior to breeding with vaccine containing MID levels of

**TABLE 4. Summary of Abortions for Nonvaccinated Cattle and Cows or Heifers Vaccinated with Modified-Live Virus Combination Vaccine Prebreeding and During Different Trimesters of Pregnancy**

<table>
<thead>
<tr>
<th>Time of Vaccination</th>
<th>Percentage of Group Having Abortion (Number Aborting/Total Examined*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>First Trimester</td>
<td>0.8% (2/263)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>4.7% (11/236)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>0.7% (1/149)</td>
</tr>
<tr>
<td>Total</td>
<td>2.2% (14/648)</td>
</tr>
</tbody>
</table>

*The total number of cattle examined excludes animals that were missing or eliminated for various reasons unrelated to vaccinations on the day of vaccination or at other times during the study. There were no significant differences between controls and vaccinates for abortion rates (or corresponding calving rates) within any of the trimesters.
the BHV-1 or BVDV components. All pregnant heifers vaccinated according to this protocol delivered healthy calves with no developmental disorders or prenursing neutralizing antibodies to BHV-1 and BVDV-1 and -2.

Results of the three field safety studies demonstrated that vaccination of pregnant cows and heifers with the MLV BHV-1, BVDV, P11, and BRSV components of the test vaccine during all three trimesters of pregnancy was safe provided the animals had been vaccinated prior to breeding with the same MLV components. Pregnant animals vaccinated pre-breeding and during pregnancy with field dose levels of the same MLV components had abortion and normal calving rates similar to those of cows and heifers vaccinated during pregnancy with sterile water diluent.

It was concluded that the results of the intensive safety study and the field safety studies supported an exception to the restriction against use of the MLV BHV-1 and BVDV vaccine components in pregnant cattle provided the pregnant animals were previously vaccinated before breeding with the same MLV components.

REFERENCES