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Evaluation of reproductive protection against bovine viral diarrhea virus and bovine herpesvirus-1 afforded by annual revaccination with modified-live viral or combination modified-live/killed viral vaccines after primary vaccination with modified-live viral vaccine

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ABSTRACT

The objective of this study was to compare reproductive protection in cattle against bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) provided by annual revaccination with multivalent modified-live viral (MLV) vaccine or multivalent combination viral (CV) vaccine containing temperature-sensitive modified-live BoHV-1 and killed BVDV when MLV vaccines were given pre-breeding to nulliparous heifers. Seventy-five beef heifers were allocated into treatment groups A (n = 30; two MLV doses pre-breeding, annual revaccination with MLV vaccine), B (n = 30; two MLV doses pre-breeding, annual revaccination with CV vaccine) and C (n = 15; saline in lieu of vaccine). Heifers were administered treatments on days 0 (weaning), 183 (pre-breeding), 366 (first gestation), and 738 (second gestation). After first calving, primiparous cows were bred, with pregnancy assessment on day 715. At that time, 24 group A heifers (23 pregnancies), 23 group B heifers (22 pregnancies), and 15 group C heifers (15 pregnancies) were commingled with six persistently infected (PI) cattle for 16 days. Ninety-nine days after PI removal, cows were intravenously inoculated with BoHV-1. All fetuses and live offspring were assessed for BVDV and BoHV-1. Abortions occurred in 3/23 group A cows, 1/22 group B cows, and 11/15 group C cows. Fetal infection with BVDV or BoHV-1 occurred in 4/23 group A offspring, 0/22 group B offspring, and 15/15 group C offspring. This research demonstrates efficacy of administering two pre-breeding doses of MLV vaccine with annual revaccination using CV vaccine to prevent fetal loss due to exposure to BVDV and BoHV-1.

1. Introduction

Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus 1 (BoHV-1) cause reproductive disease in cattle that negatively impacts the economic viability and animal health of many farming operations. Consequences of BVDV and BoHV-1 infection can range from abortion outbreaks affecting large populations of pregnant cattle to more subtle reproductive losses including impaired conception, early embryonic death, premature births, stillbirths, and in the case of BVDV, the birth of persistently infected (PI) offspring [1]. In North America, vaccination provides an important contribution to limiting reproductive losses associated with these viral infections. The majority of vaccines licensed for use against viral reproductive pathogens are multivalent, containing both BVDV and BoHV-1. Vaccines are available as modified-live viral (MLV), killed viral (KV) vaccines, or combination viral (CV) vaccines that contain both MLV and KV components. While multiple doses of MLV vaccine have been demonstrated to provide excellent fetal protection against the negative reproductive effects of BVDV and BoHV-1 [1], maintaining annual revaccination with a MLV vaccine without routinely vaccinating pregnant beef cows is inconvenient. The abortifacient risk associated with administration of multiva-

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lent, MLV vaccines containing BoHV-1 to pregnant cows is the focus of increased scrutiny [2,3].

While cattle producers employ pre-breeding vaccination with MLV vaccine and annual revaccination with KV vaccine due to perceived safety of this protocol, data are unavailable from prospective, randomized, controlled clinical trials that have assessed the efficacy of this protocol for protection against reproductive disease. Assessment of the fetal and abortive protection resulting from pre-breeding vaccination of heifers with an MLV vaccine and annual revaccination with a CV vaccine is warranted. Thus, the objective of this study was to compare fetal and abortive protection against BVDV and BoHV-1 provided by vaccination with two pre-breeding doses of MLV vaccine followed by annual revaccination during pregnancy with either an MLV vaccine or CV vaccine using a maximally rigorous dual-viral challenge involving BVDV PI exposure and intravenous inoculation with BoHV-1.

2. Materials and methods

2.1. Experimental design

The study was designed as a randomized, controlled clinical trial (Fig. 1). All study procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University (PRN # 2011-1899). Prior to study initiation, heifers were assigned to treatment group by randomized block design, resulting in treatment group sizes of n = 30 each for groups A and B and n = 15 for group C. All heifers were administered treatments (vaccines or saline) on days 0, 183, 366, and 738. Following treatments, heifers receiving different vaccines were housed in separate groups for approximately 30 days. Estrus was synchronized in heifers, and all heifers were bred via artificial insemination (AI) on day 214, followed by 60-day exposure to breeding bulls. On day 345, all heifers were examined via ultrasonography to determine pregnancy status and fetal age. Primiparous heifers calved between days 491 and 554. Estrus was synchronized and all cows were bred via AI on day 620, followed by exposure to breeding bulls. On day 715, pregnancy status and fetal age were assessed via ultrasonography and all cows were combined in a single pasture with six cattle PI with BVDV from days 715–731. Pregnancy status was assessed by transrectal palpation and ultrasonography at the conclusion of the BVDV PI exposure (day 731) and again on days 746, 774, 802, and 830. On day 830, all cows were challenged with BoHV-1 by IV inoculation. Following BoHV-1 challenge, pregnancy examination was performed every two weeks (until day 978) until each cow had aborted or calved.

2.2. Animals

Seventy-five, five- to seven-month-old, crossbred beef heifers were enrolled at study onset (Fig. 1). Prior to study initiation, all heifers had negative results when tested for BVDV in serum and white blood cells (WBC) via virus isolation (VI) and ear notches via immunohistochemistry (IHC). In addition, all heifers had negative results when tested for BoHV-1 in serum via VI. Six crossbred beef bulls were used to breed the heifers/cows that did not conceive by AI. Bulls had negative results for BVDV and BoHV-1 in serum by VI and semen by PCR and were confirmed to be satisfactory potential breeders as determined by breeding soundness examination prior to each breeding season. Cattle were maintained on biosecure pastures throughout the study.

Six crossbred steers (n = 2) or cows (n = 4) that were PI with BVDV were commingled with study cows to provide exposure to BVDV. These cattle were PI with BVDV 1a (n = 2), BVDV 1b (n = 2) and BVDV 2 (n = 2) field strains. Each animal was confirmed as PI prior to inclusion in the study based upon positive results obtained via antigen-capture ELISA (ACE) performed on ear notch samples and detection of BVDV by VI on serial serum and nasal swab samples obtained at least three weeks apart.

2.3. Vaccination

All vaccines were USDA-licensed stock material approved for commercial sale. Heifers in group A received 2 mL of multivalent, MLV vaccine (Bovi-Shield Gold FP 5, Zoetis, Florham Park, NJ; Lot No. 1170484) SC in the neck region on days 0, 183, and 366, and MLV vaccine (Bovi-Shield Gold 5, Zoetis, Florham Park, NJ; Lot No. 1291373B) SC on day 738 (Fig. 1). Heifers in group B received 2 mL of multivalent, MLV vaccine (Bovi-Shield Gold FP 5, Zoetis, Florham Park, NJ; Lot No. 1170484) SC in the neck region on days 0 and 183, and received 2 mL of multivalent, CV vaccine (CattleMaster Gold FP5, Zoetis, Florham Park, NJ; Lot No. 1288431) SC on days 366 and 738. Heifers in group C received 2 mL of Dulbecco's phosphate buffered saline solution SC in the neck region on days 0, 183, 366, and 738. All vaccines were administered according to label directions within 2.5 h after reconstitution. These multivalent vaccines contained the following viral fractions: BoHV-1, BVDV 1, BVDV 2, bovine parainfluenza-3, and bovine respiratory syncytial virus. In lieu of multivalent KV vaccine (containing all inactivated viral fractions), the vaccine utilized in this study is a CV vaccine containing temperature-sensitive modified-live BoHV-1 and bovine parainfluenza-3 virus, a modified-live bovine respiratory syncytial virus, and killed BVDV 1 and BVDV 2 fractions. This CV vaccine is labeled for use in pregnant cows.

2.4. BVDV challenge exposure

From days 715–731, six PI cattle were commingled with experimental cows sharing feed and water sources. Serum and WBC samples were collected for BVDV detection by VI as previously described [4] from all study cows on days 715 (immediately prior to BVDV exposure), 721–725, 731, and 738. For each PI animal, the BVDV titer was determined by virus titration as described [5] on serum samples and nasal swab specimens obtained on days 715 and 731.

2.5. BoHV-1 challenge exposure

On day 830, all cows received an IV challenge with 2 mL of BoHV-1 Colorado (Cooper) strain (ATCC VR-864, American Type Culture Collection, Manassas, VA) containing 1 x 10^7 cell culture infective doses at the 50% endpoint ($CCID_{50}$). Sera and deep nasal swab samples were collected for BoHV-1 detection by VI as previously described [5] from all cows on days 830 (immediately prior to IV inoculation), 833–837, 844, and 858.

2.6. Sample collection from aborted fetuses and live born offspring

When intact aborted fetuses were detected, complete necropsies were performed. When available, spleen, thymus, liver, kidney, lung, heart blood/pleural fluid, and placenta were submitted for VI, IHC, and RT-nPCR to detect BVDV, and VI, IHC, and PCR to detect BoHV-1. In addition, skin samples were collected for IHC and ACE to detect BVDV, and placental tissues were collected for VI, IHC, and PCR to detect BoHV-1. When abortion was detected but an intact fetus was unavailable, fetal remnants and/or uterine fluid with placental remnants were assayed for BVDV using VI and RT-nPCR and for BoHV-1 using VI and PCR. Frozen fetal kidney was tested for *Leptospira* species by fluorescent antibody, and fetal abomasal contents were cultured to identify bacterial pathogens.