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Bovine viral diarrhea virus fetal persistent infection after immunization with a contaminated modified-live virus vaccine

Roberto A. Palomares [a,*], Shonda M. Marley [b], M. Daniel Givens [b], Rodrigo A. Gallardo [c], Kenny V. Brock [b]

[a] Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
[b] Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA
[c] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California, USA

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ABSTRACT

The objective was to determine whether a multivalent modified-live virus vaccine containing noncytopathic bovine viral diarrhea virus (BVDV) administered off-label to pregnant cattle can result in persistently infected fetuses and to assess whether vaccinal strains can be shed to unvaccinated pregnant cattle commingling with vaccinates. Nineteen BVDV-naïve pregnant heifers were randomly assigned to two groups: cattle vaccinated near Day 77 of gestation with modified-live virus vaccine containing BVDV-1a (WRL strain), bovine herpes virus-1, parainfluenza 3, and bovine respiratory syncytial virus (Vx group; N = 10) or control unvaccinated cattle (N = 9). During the course of the study a voluntary stop-sale/recall was conducted by the manufacturer because of the presence of a BVDV contaminant in the vaccine. At Day 175 of gestation, fetuses were removed by Cesarean section and fetal tissues were submitted for virus isolation, and quantitative reverse transcription polymerase chain reaction using BVDV-1- and BVDV-2-specific probes. Nucleotide sequencing of viral RNA was performed for quantitative reverse transcription polymerase chain reaction-positive samples. Two vaccinated and two control heifers aborted their pregnancies, but their fetuses were unavailable for BVDV testing. Virus was isolated from all eight fetuses in the Vx group heifers and from 2 of 7 fetuses in the control unvaccinated heifers. Only BVDV-2 was detected in fetuses from the Vx group, and only BVDV-1 was detected in the two fetuses from the control group. Both BVDV-1 and BVDV-2 were detected in the vaccine. In conclusion, vaccination of pregnant heifers with a contaminated modified-live BVDV vaccine resulted in development of BVDV-2 persistently infected fetuses in all tested vaccinated animals. Furthermore, BVDV was apparently shed to unvaccinated heifers causing fetal infections from which only BVDV-1 was detected.

1. Introduction

Bovine viral diarrhea virus (BVDV) is a positive-sense, single-stranded RNA virus belonging to the genus *Pestivirus* and the family *Flaviviridae* [1], which affects cattle production worldwide [2,3]. Infection of pregnant cattle with BVDV might result in abortions, stillbirths, congenital defects, and the birth of persistently infected (PI) calves. Persistent infections occur if a susceptible pregnant cow is infected with a noncytopathic (ncp) BVDV strain at 30 to 125 days of gestation [4]. At this time of gestation, the fetal immune system is not completely developed and not able to recognize BVDV as a foreign antigen, accepting the virus as part of the self-antigen repertoire [5,6]; with a resulting negative selection of BVDV-specific B and T lymphocytes [7]. Consequently, BVDV immunotolerance results in absence of

* Corresponding author. Tel.: +1 706 542 6320; fax: +1 706 542 6320.
E-mail address: palomnr@uga.edu (R.A. Palomares).

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humoral and cell-mediated immunity to the virus, failure to clear the infection, and a persistent viremia [8].

Such calves continually shed large amounts of BVDV, representing a risk to susceptible herdmates [9]. Furthermore, although their prevalence is less than 1%, these PI cattle have been recognized as the main source for spread of BVDV within and among cattle herds [10–12].

Strategies to prevent and control BVDV include biosecurity measures to avoid introduction of infected animals in the herd, quarantine of animals to control spread between herds, identification and slaughter of PI animals, and vaccination [13–15]. Inactivated and modified live BVDV vaccines are available. Modified-live virus (MLV) vaccines have the ability to induce high neutralizing antibody levels [16,17] and a strong cell-mediated immunity [18]. The efficacy of modified-live BVDV vaccines to prevent fetal infections and the development of PI animals has been evaluated in several studies, reporting values between 57.9% and 100% [16,19–24]. However, a remarkable disadvantage of MLV vaccines is that these vaccines contain limited antigen mass, requiring viral replication in the host to develop optimal immunity [25].

During the replication cycle, a live virus could recombine or mutate and occasionally revert to virulence and be shed to other susceptible individuals, resulting in severe clinical consequences [26]. Additionally, live BVDV could cross the placental barrier and infect the fetus causing abortion, stillbirth, and developmental defects [27,28]. A major concern of BVDV MLV vaccines is the occurrence of mucosal disease after vaccination [24]. Postvaccinal mucosal disease could result when a PI animal is exposed to the cytopathic (cp) BVDV strain contained in the vaccine [24]. A further disadvantage of MLV BVDV vaccines is their immunosuppressive effect on leukocyte function, resulting in increased susceptibility to other infections [29]. Furthermore, MLV vaccines have the potential risk for contamination with adventitious virulent strains becoming a source of spread of BVDV infections [30].

Most commercial vaccines in the United States contain cp BVDV strains [30,31], however, there are some MLV vaccines containing ncp BVDV isolates, which might represent a risk for the development of PI animals if these vaccines are administered off-label during gestation. Additional risk exists if susceptible pregnant cows are exposed to recently vaccinated animals a few days after immunization. Therefore, the use of ncp BVDV strains in MLV vaccines still generates safety concerns for veterinarians and cattle producers regarding the risk of persistent infections.

The initial aim of this study was to determine whether a multivalent MLV vaccine containing ncp BVDV could result in PI fetuses when administered off-label to seronegative pregnant cattle at approximately 77 days of gestation and to assess whether vaccinal strains could be shed and infect unvaccinated pregnant cattle. However, during the course of this study, a voluntary stop sale/recall was conducted by the manufacturer because of the presence of an extraneous contaminant strain in some vaccine lots (written communication from the vaccine's manufacturer). This unexpected incident redirected the aims of our study toward determination of the effects of the contaminated MLV vaccine on BVDV-naive pregnant heifers.

2. Materials and methods

2.1. Heifers

A total of 25 nonpregnant beef heifers were enrolled in this study. All heifers were clinically normal, free of BVDV based on virus isolation and seronegative to both BVDV-1 and BVDV-2 based on serum virus neutralization assays performed at a serum dilution of 1:2. The bulls for breeding were previously confirmed free of persistent BVDV infection by immunohistochemistry of ear notch samples. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Auburn University.

2.2. Synchronization and breeding

Heifers were synchronized for estrus using 2.0 mL of GnRH (Cystorelin; Merial, Duluth, GA, USA), im. Seven days later, 5.0 mL of $PGF_{2\alpha}$ (dynoprost tromethamine; Lutalyse; Pfizer, Kalamazoo, MI, USA) im was administered to eliminate luteal tissue. On the same day, two healthy bulls of recognized fertility, BVDV seronegative, and virus isolation-negative were placed with the heifers for breeding. The day that the bulls were introduced was considered the start breeding day. Bulls remained with the heifers for 24 days. Day 12 after $PGF_{2\alpha}$ injection was considered the average breeding day (Day 0). Pregnancy diagnosis was performed using transrectal palpation 58 days after Day 0 by an experienced veterinarian (Fig. 1). Nineteen heifers became pregnant with a gestational age between 46 and 70 days. On Day 77, fetal viability (fetal heart beat) was confirmed in 19 pregnant heifers using transrectal ultrasonography.

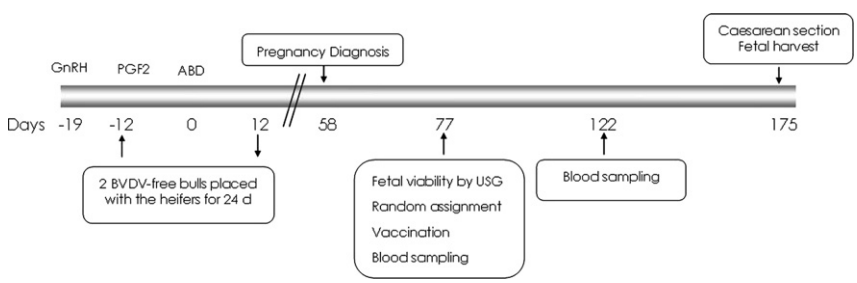

Fig. 1. Experimental protocol for estrus synchronization, breeding, pregnancy diagnosis, USG, blood sampling, Cesarean section, and fetal harvest. ABD, average breeding day; BVDV, bovine viral diarrhea virus; USG, ultrasonography.