Bovine Viral Diarrhea Viruses (BVDV) in the Feedlot: Prevalence of Persistent Infections, Utilization of Diagnostic Tests, and Distribution of BVDV Subtypes 1a, 1b, 2a

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Introduction

Bovine viral diarrhea viruses (BVDV) represent significant pathogens for cattle. The BVDV affect several organ systems; however, the respiratory tract and fetal infections receive critical attention based on disease impact and potential for reservoirs/transmission. BVDV may cause primary infection of the respiratory tract and also serve to predispose the infected bovine to bacterial pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Archanobacterium pyogenes* and *Mycoplasma* spp. Calves infected *in utero* and born persistently infected (PI) are important reservoirs of infection as they are life-long shedders of virus to susceptible cattle. Respiratory diseases in the feedlot are significant based on morbidity mortality and economic losses. Detection and removal of PI cattle could potentially lessen the economic effect of BVDV in the feedlot. Also, current BVDV control programs utilize vaccines in the US, primarily those containing BVDV1a and BVDV2a strains. Research at our institution has shown that the BVDV1b subgenotype is the major BVDV strain in our diagnostic laboratory accessions. The purpose of this study was three-fold: (1) determine prevalence of PI cattle entering a feedlot; (2) to utilize and compare various diagnostic tests to identify PI cattle; and (3) to determine the distribution of the BVDV1a, BVDV1b and BVDV2a subgenotypes in PI cattle entering the feedlot.

Materials and Methods

From July 2004 to December 2004, cattle entering the feedlot were routinely processed and an ear notch (fresh) collected in PBS. The PBS ear notches were then tested for BVDV antigen in the fluids of the sample held overnight at refrigerator temperature. The initial testing was performed at the veterinary clinic near the feedlot. The test kit was a commercial antigen capture ELISA (ACE) kit. There were 21,743 cattle tested with 88 ACE positive cattle on the initial test. The study was also designed to evaluate other tests such as immunohistochemistry (IHC) and viral isolation on samples collected from these 88 ACE positive on the initial test. In over 90% of the positive ACE positive cases, additional samples were collected within 48 hours of the first collection. These included sera for virus isolation, a second fresh ear notch in PBS and a formalin-fixed ear notch in 10% neutral buffered formalin. These samples were sent to Oklahoma State University Center for Veterinary Health Sciences for additional testing. Serums samples for virus isolation were inoculated onto bovine cell cultures. The cultures were examined for presence/absence of cytopathology, and BVDV positive samples were submitted to the USDA ARS NADC, Ames, IA for BVDV subtyping and phylogenetic analysis.

Results

Testing of the 21,743 revealed 88 AC ELISA positives on the initial test. Subsequent confirmatory testing on the second set of samples detected 86 animals positive the second time using the ACE test, and 86 were also positive by the BVDV IHC test. There was 100% agreement on the two tests from samples collected at the same time. There was also 100% agreement with these 86 samples (ACE test and IHC positives) by the viral isolation in cell culture. Based on these confirmatory tests, there was a prevalence rate of 0.4% for PI status (86/21,743). There were two animals, originally positive by AC ELISA on ear notch at entry, that were negative on subsequent samples by IHC ear notch, a second AC ELISA ear notch and cell culture viral isolation. These two animals were considered to be acute/transiently infected cattle. Based on the initial test, the positive ACE results were 97.7% (86/88) predictive of PI status. The viruses isolated from the serum samples were all noncytopathic (NCP) BVDV strains. Based on

differential PCR and sequencing of a 5'-UTR with phylogenetic analysis, BVDV1a, BVDV1b and BVDV2a strains were identified. Controls included reference BVDV strains, including vaccinal strains in the US plus BVDV2b. A BVDV2b had been isolated from a feedlot in Oklahoma pneumonia case, thus surveillance for that BVDV subgenotype continues. There were 67/86 (77.9%) BVDV1b; 10/86 (11.6%) BVDV1a; and 9/86 (10.5%) BVDV2a. The BVDV1b was more common than BVDV1a or BVDV2a ($P<0.05$). None of the 86 isolates were genetically identical to the BVDV subgenotypes in US vaccines.

Significance

These results indicate that an antigen capture ELISA test on fresh PBS notches identifies a very high percentage (97.7%) of cattle defined as PI. While a small number in this study (2/88) were considered only acute/transiently infected, the feedlot veterinarian is presented extremely important information for BVDV control/management based on positive or negative results. At least all the positive animals could be segregated and/or tested again to confirm PI status. By testing the animals individually, as in the initial ACE, solid evidence is obtained for each animal rather than retesting individual animals a second time as expected with a positive-pooled number of samples (PCR). The distribution of the BVDV in cattle entering the feedlot into subgenotypes confirms our prior findings of BVDV positive diagnostic laboratory accessions, with BVDV1b being the predominant BVDV strain. Equally important is the issue that effective vaccines must be developed/used to control this predominant BVDV strain in the US.

Characterization of Bovine Viral Diarrhea Virus (BVDV) Genetics, Antibody Response and Viremia from a Group of BVDV Persistently Infected Calves

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Introduction

Bovine viral diarrhea virus (BVDV) infections cause major problems in the US cattle industry. BVDV persistent infections are the result of fetal infections in the first trimester of pregnancy. Persistently infected animals are a major reservoir of the virus in nature and are extremely efficient at spreading the virus among cattle populations. Understanding the nature of persistent infections and developing diagnostics and surveillance schemes that eliminate PI animals is vital to the control of BVDV. In this study we look at variations in clinical presentation, viral spread, immune response and viral stability in a large group of calves infected with the same BVDV strain.

Materials and Methods

One hundred twenty-eight bred cows were obtained from a private ranch and moved to a university field station. The vaccination history of the herd indicated that a BVDV type 1 vaccine had been used. Following weaning the calves were screened in September 2004 and 44 were found to be immunohistochemistry (IHC) positive for BVDV antigen. Polymerase chain reaction (PCR) analysis indicated that the animals were infected with a BVDV type 2a isolate. Five of these animals died prior to being moved to a university research facility. Three months later in December 2004, the remaining 39 calves were tested via PCR and IHC, and 36 of the 39 were positive for BVDV by both tests.

Results

Sequence comparison of the 5'UTR of the 36 isolates showed a > 99% sequence homology. Comparison of the highly variable region coding for the E2 polypeptide showed a greater than 96% sequence conservation among strains. Three of the PI animals had titers against BVDV. Studies are ongoing to see if the presence of titers will affect viral sequence over time. Viral titers are being assessed. The animals are being tested monthly for viral and antibody titers. Additional lymphocyte marker and neutrophil studies are also ongoing.