Herd Screening Using a Nested RT-PCR Technique to Detect Bovine Viral Diarrhea Virus (BVDV) from Pooled Buffy Coat Samples

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Introduction

Bovine viral diarrhea virus (BVDV) is the leading infectious cause of abortions, stillbirths and weak calves in South Dakota. The ability to detect and identify the BVDV persistently infected animal is imperative for good herd management. There are several methods to detect BVDV in infected herds including virus isolation (VI), reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen capture enzyme-linked immunosorbent assay (AC-ELISA). The microtiter test or the AC-ELISA in a multi-well tissue culture plate have been the most popular for herd screens. The RT-PCR is the most sensitive assay, but due to its high cost has primarily been used as an individual test rather than a herd screening method. Dr. Kenny Brock has designed an RT-PCR to test bulk-milk samples from dairy herds. In our efforts to make RT-PCR more economically feasible as a screening assay for BVDV, we have performed several experiments involving pooled buffy coat (BC) samples.

Materials and Methods

We obtained EDTA-whole blood samples from 54 animals in a BVDV-negative herd and from 2 persistently infected (PI) animals.[1] The whole blood was centrifuged and the buffy coat harvested. RNA was extracted from these samples using a method developed by Chomonski.[2] The RT-PCR method was a modification of the method of Sullivan and Akkina[3] that produces a product in the E^{rns} region. This method not only detects BVDV, but also determines the genotype–type 1 or type 2–of the virus. The RT reaction and the first step of PCR, the outer PCR reaction, were performed. The outer PCR products were used in a second, or nested, PCR reaction. The outer, or single-step, PCR products and the nested, or two-step, PCR products were separated by electrophoresis on a gel.

Of 56 animals tested, only the two PI samples were RT-PCR positive. There were PCR products visible in both the single (outer) step and the nested PCR reaction. Five pools were created by adding 100µl of the following samples; 1) five negative samples, 2) four negative and 1 positive sample, 3) nine negative and 1 positive sample, 4) 19 negative and 1 positive sample, 5) 49 negative and 1 positive sample. An aliquot of 300µl was removed from each of the pools and processed for RNA extraction and BVD RT-PCR as above. The outer reaction did not produce any bands, but the more sensitive nested reaction produced a positive band in the pooled samples in each of the 3 separate runs.

Results and Discussion

These experiments demonstrated the ability to detect one positive sample from a pool of 50 samples. This appears to be an excellent method for herd screening. On initial test of a herd with an unknown BVDV PI status, we feel the pool size should not exceed 20 samples. Any pool that is positive must be retested. There could be several approaches, but the two most likely would be 1) retesting the original pool with RT-PCR using smaller pools, i.e. 5 samples/pool and testing all positive pool samples with microtiter test; or 2) testing all in original pool with microtiter test or AC.

In herds previously screened for PI animals with <1% prevalence of BVDV PI animals, 50 sample/pools are a reasonable approach for an annual BVDV PI monitoring program.

References

1. Kindly provided by Dr. Dan Grooms, Michigan State University.