Comparison of Three Reverse Transcription-Polymerase Chain Reaction Assays for the Detection and Genotyping of Bovine Viral Diarrhea Virus Isolates

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Introduction

Bovine viral diarrhea virus (BVDV) is an important infectious disease in cattle. Isolates of BVDV are classified into type I or type II based upon the genomic sequences. Several reverse transcription-polymerase chain reaction (RT-PCR) techniques utilizing different genomic regions, such as 5'UTR, E0, and NS5B, have been developed for detecting and genotyping BVDV isolates. The purpose of this study was to compare the agreement among RT-PCR assays based upon these three different BVDV genomic regions in detecting and genotyping BVDV field isolates.

Materials and Methods

Forty BVDV field isolates were obtained from the Kansas Animal Diagnostic Laboratory and four reference isolates were included for study. All isolates were propagated in embryonic bovine kidney cells. Detection of BVDV was performed using virus isolation-fluorescent antibody (FA) testing and RT-PCR assays utilizing the 5'UTR, E0, and NS5B genomic regions. Genotyping of BVDV was performed using PCR on the 5'UTR, E0, and NS5B region-based first-round RT-PCR product. One primer set was designed from genomic sequence information, and remaining primer sets were obtained from previous descriptions.[1-3] Conditions were optimized using reference BVDV isolates.

Results and Conclusions

The RT-PCR assays correctly identified and genotyped all four BVDV reference isolates. The FA test and the RT-PCR assay based upon the 5'UTR region of the genome identified all 40 BVDV field isolates. In contrast, the RT-PCR assays based upon the NS5B and E0 regions only identified 36 and 30 BVDV field isolates, respectively. Of the 40 BVDV field isolates, only 28 isolates were detected by all three RT-PCR assays.

Of the 28 isolates detected by all three RT-PCR assays, there was agreement in genotyping of 24 isolates, with 14 isolates identified as type I and 10 isolates as type II. In the remaining four isolates, two isolates could not be genotyped by the 5'UTR or E0 PCR assays, respectively, and two isolates were genotyped as type II by 5'UTR and NS5B, and as type I and type II together by E0.

Development and application of RT-PCR assays for detection of BVDV has increased to the point where many diagnostic laboratories routinely perform them instead of virus isolation. Consideration should be given as to their specificity, due to nucleotide sequence diversity among BVDV isolates.

References

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