Establishment of TaqMan probe qRT-PCR for detecting bovine viral diarrhea virus and clinical applications

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Objective: The present study aimed to establish a novel TaqMan quantitative real-time PCR (qRT-PCR) for detecting and typing Bovine Viral Diarrhea Virus (BVDV), also to develop a diagnostic protocol which simplifies sample collection and processing.

Method: Universal primers and TaqMan-MGB probes were designed from the known sequences of conserved 5' - and 3'-untranslated regions (5'UTR, 3'UTR) of the NADL strain of BVDV. Prior to optimizing the assay, cDNAs were transcribed in vitro to make standard curves. The sensitivity, specificity and stability (reproducibility) were evaluated, respectively. The qRT-PCR was tested on the feces specimens collected from persistently infected (PI) calves.

Results: The optimum conditions for qRT-PCR were 17.0 μmol/L primer, 7.5 μmol/L probe and 51.4 annealing temperature. The established qRT-PCR assay could only specially detect BVDV without detecting any other viruses; its detection limit was 1.55×100 copies/μL for viral RNA. It was 100000-fold higher than conventional PCR with excellent specificity and reproducibility. 312 samples of feces were tested using this method and universal PCR from six dairy farms, respectively. Positive detections were found in 49 and 44 feces samples for both assays. The occurrence rate was 89.80%.

Conclusion: The established qRT-PCR could rapidly detect BVDV and effectively identify PI cattle. The detection limit of TaqMan qRT-PCR was 1.55 copies/μL. It will be beneficial for enhancing diagnosis and therapy efficacy and reduce losses of cattle farms.

Keywords: Bovine viral diarrhea virus; Quantitative real time PCR; TaqMan probe.

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