Detection of infectious bursal disease virus using SYBR Green I based real-time polymerase chain reaction

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The current available method to differentiate very virulent and vaccine strains of infectious bursal disease virus (IBDV) is by restriction fragment length polymorphism of VP2 gene. However, this method is time consuming, prone to error and less sensitive. The newly developed TaqMan real-time PCR is very sensitive but not suitable as routine test since the test is expensive. In addition, the application of the assay in detecting very virulent and vaccine strains of IBDV has not been reported. In this study the performances of SYBR Green I real-time, ELISA and conventional agarose detection methods in detecting nested PCR products were compared. We found the real-time PCR was at least 100 more sensitive than ELISA detection method with a detection limit of 250 pg/µl. The developed assay detects both very virulent and vaccine strains of IBDV but not other RNA viruses such as Newcastle disease virus and infectious bronchitis virus. However, the assay was unable to differentiate different strains of IBDV. In the subsequent study, we use strain-specific primer (match primer) combinations for the detection and differentiation of IBDV strains using two steps SYBR Green I based real-time PCR. The primers and PCR condition were optimized and validated using both very virulent and vaccine strains. By using the strain-specific primer combinations specific amplification based on measurement of Cₚ and Tm were detected. In an optimized PCR condition, specific amplification associated with early amplification with Cₚ value between 19 to 28 and Tm between 86 to 88°C meanwhile nonspecific amplification from mismatch primer associated with late amplification with Cₚ value > 29 and Tm < 82°C or no amplification (Cₚ value 0 and Tm < 82°C). These characteristic Cₚ and Tm values were consistently detected following amplification with 4000 ng/ul of cDNA. Hence, the differentiation of IBDV strains were based on detection of Cₚ values whilst detection of Tm was for confirmation of the specific amplification. The detection of Tm value alone was not sufficient to differentiate IBDV strains. Even though, the detection limit of the real-time PCR to detect IBDV strains was between 6.6 to 7.7 ng/µl, it is recommended that for testing of clinical samples, the cDNA concentrations been maintained between 4000 ng/µl to 66 ng/µl for PCR amplification since amplification from insufficient primer-template concentration promote amplification of mismatch PCR product. In this study, we showed for the first time application of SYBR Green I based real-time PCR for the detection and differentiation of very virulent and vaccine strains of IBDV. The assay was found to be sensitive, specific, less expensive and less turn around time compared to the current available diagnostic methods.