Detection and differentiation of velogenic and lentogenic Newcastle disease viruses using SYBR Green I real-time PCR with nucleocapsid gene-specific primers

ABSTRACT

SYBR Green I real-time PCR was developed for detection and differentiation of Newcastle disease virus (NDV). Primers based on the nucleocapsid (NP) gene were designed to detect specific sequence of velogenic strains and lentogenic/vaccine strains, respectively. The assay was developed and tested with NDV strains which were characterized previously. The velogenic strains were detected only by using velogenic-specific primers with a threshold cycle (Ct) 18.19 ± 3.63 and a melting temperature (Tm) 86.0 ± 0.28 °C. All the lentogenic/vaccine strains, in contrast, were detected only when lentogenic-specific primers were used, with the Ct value 14.70 ± 2.32 and Tm 87.4 ± 0.21 °C. The assay had a dynamic detection range which spans over a 5 log10 concentration range, 1096105 copies of DNA plasmid/reaction. The velogenic and lentogenic amplifications showed high PCR efficiency of 100% and 104%, respectively. The velogenic and lentogenic amplifications were highly reproducible with assay variability 0.45 ± 0.31% and 1.30 ± 0.65%, respectively. The SYBR Green I real-time PCR assay detected successfully the virus from tissue samples and oral swabs collected from the velogenic and lentogenic NDV experimental infection, respectively. In addition, the assay detected and differentiated accurately NDV pathotypes from suspected field samples where the results were in good agreement with both virus isolation and analysis of the fusion (F) cleavage site sequence. The assay offers an attractive alternative method for the diagnosis of NDV.

Keyword: Newcastle disease virus; Pathotypes; Detection and differentiation; SYBR Green I real-time PCR; Nucleocapsid gene-specific primer