Development of TaqMan-based real-time RT-PCR assay based on N gene for the quantitative detection of feline morbillivirus

ABSTRACT

Background: Morbilliviruses are categorized under the family of Paramyxoviridae and have been associated with severe diseases, such as Peste des petits ruminants, canine distemper and measles with evidence of high morbidity and/or could cause major economic loss in production of livestock animals, such as goats and sheep. Feline morbillivirus (FeMV) is one of the members of Morbilliviruses that has been speculated to cause chronic kidney disease in cats even though a definite relationship is still unclear. To date, FeMV has been detected in several continents, such as Asia (Japan, China, Thailand, Malaysia), Europe (Italy, German, Turkey), Africa (South Africa), and South and North America (Brazil, Unites States). This study aims to develop a TaqMan real-time RT-PCR (qRT-PCR) assay targeting the N gene of FeMV in clinical samples to detect early phase of FeMV infection. Results: A specific assay was developed, since no amplification was observed in viral strains from the same family of Paramyxoviridae, such as canine distemper virus (CDV), Newcastle disease virus (NDV), and measles virus (MeV), and other feline viruses, such as feline coronavirus (FCoV) and feline leukemia virus (FeLV). The lower detection limit of the assay was 1.74×104 copies/μL with Cq value of 34.32 ± 0.5 based on the cRNA copy number. The coefficient of variations (CV) values calculated for both intra- and inter-assay were low, ranging from 0.34–0.53% and 1.38–2.03%, respectively. In addition, the clinical sample evaluation using this assay showed a higher detection rate, with 25 (35.2%) clinical samples being FeMV-positive compared to 11 (15.5%) using conventional RT-PCR, proving a more sensitive assay compared to the conventional RT-PCR. Conclusions: The TaqMan-based real-time RT-PCR assay targeting the N gene described in this study is more sensitive, specific, rapid, and reproducible compared to the conventional RT-PCR assay targeting the N gene, which could be used to detect early infection in cats.

Keyword: Feline morbillivirus; TaqMan-based real-time RT-PCR; N gene