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Classical Swine Fever Virus: Discrimination Between Vaccine Strains and Korean Field Viruses by Real-time RT-PCR

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Introduction

Classical swine fever (CSF) is a contagious disease of swine with serious economic losses in pig industry [1]. The disease is caused by CSFV which belongs to the viruses of bovine viral diarrhea (BVDV) and border disease virus (BDV) make up the *Pestivirus* genus within the family *Flaviviridae* [2]. Attenuated Korean LOM strains were used in Korea. For these reasons a practical approach for discrimination between vaccine and field strains is needed. Here, we described the deveopment of real-time RT-PCR to discriminate between vaccine strains and Korean field viruses of CSFV.

Materials and Methods

Viral RNA was extracted from K-lom and field isolated CSFV using TRI reagent (MRC, USA). Viral RNA was reverse transcripted using reverse priming and MMLV reverse transcriptase (RT). PCR primers and fluorogenic probes were designed for all target genes according to the published sequences using Primer Express software (Applied Biosystems, USA). The fluorogenic probes contained a reporter dye (FAM, 6-carboxy-fluorescein) covalently linked at the 5' end and a quencher dye (TAMRA, 6-carboxy-tetramethyl-rhodamine) covalently attached at the 3' end. Extension from the 3' end was blocked by attachment of a 3'-phosphate group. Reagents from ABI Biosystems were used to prepare master mixture recipes according to the guidelines of the manufacturer. For quantification, an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) was used.

Results and Discussion

All CSFVs were detected with cycle threshold values ranging from 25 to 30. Positive fluorogenic signals were observed from K-LOM strain and field isolated CSFV respectively. The aim of this study was to develop a multiplex real-time RT-PCR assay for rapid and semi-automated detection of K-LOM strain and field isolated CSFV over a wider detection range by using one set of primers and two fluorogenic probes enabling amplification and immediate detection in a single test tube. The real-time RT-PCR assay described in this study is sensitive and accurate method for specific detection.

References

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