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Short communication

A TaqMan-based qRT-PCR assay for *Senecavirus A* detection in tissue samples of neonatal piglets

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ABSTRACT

This study describes a sensitive (1.3×10^1 genomic copies/μL) and specific TaqMan-based qRT-PCR assay able to detect and quantify SVA RNA in porcine biological samples. The technique represents an efficient tool for the virus diagnosis and assessment of SVA load in tissues of infected animals and for epidemiological studies.

1. Introduction

Senecavirus A (SVA) is the single representative species of the *Senecavirus* genus, *Picornaviridae* family [1]. SVA has been associated with vesicular disease in pigs of the United States [2], Brazil [3–6], and China [7]. In 2015, high rates of pig neonatal mortality associated with SVA infection have been reported in these countries; however the pathogenicity of this virus is not completely elucidated.

Different studies used quantitative RT-PCR (qRT-PCR) for the SVA detection and quantification [2,8–13]; however only one describes the technique in details [13]. The development and validation of diagnostic techniques are necessary to identify the virus presence and/circulation, for retrospective and prospective epidemiological studies, and to determine the naive status of experimental animals [14]. In this study a TaqMan-based qRT-PCR assay was designed and validated for SVA RNA detection and quantification in porcine tissue specimens.

The primers and probe were designed and compared among each other and with other SVA representative nucleotide (nt) sequences available in GenBank using the Primer Express Software (Applied Biosystems™, Foster City, CA, USA). The primer and probe positions were defined based on the prototype SVV-01 (GenBank accession number NC_011349). The primers SVV-q2688Fw (5′-CACCGACAACGCCGAGAC-3′) and SVV-q2782Rv (5′-GAGATCGATCAAACAGGAACTTGAC-3′) were designed to amplify a 118 bp length fragment of a conserved genomic region within the VP1 protein of the SVA. The probe was defined as SVV-q2728Pb (5′-FAM-ACTGACACCGATTTC- MGB-3′) (Applied Biosystems™, Foster City, CA, USA). The primer and probe specificities were tested *in silico*, using other picornavirus nt sequences available in GenBank [3].

A fragment of the 118 bp was amplified by conventional RT-PCR using the primers SVV-q2688Fw and SVV-q2782Rv from a fluid vesicular SVA isolate [3]. The RT reaction was performed with SuperScript® III Reverse Transcriptase (Invitrogen™ Life Technologies, Carlsbad, CA, USA), according to the manufacture's instructions and using 40 pmol of SVV-q2782Rv primer. The PCR assay and amplification reaction were performed as described previously [3], using 20 pmol of each primer (forward and reverse) and 55°C/1 min for annealing.

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The amplicon was cloned into the pCR™ 4-TOPO® (Invitrogen™ Life Technologies, Carlsbad, CA, USA) vector, according to the manufacturer's instructions. The clone containing the target sequence was confirmed by sequencing. The quantitation was determined by Qubit® Fluorometer (Invitrogen™ Life Technologies, Eugene, OR, USA). The DNA copy number was calculated using the equation (X (g/μL)/[*clone size in bp* x 649]) x $6.022 \times 10^{23} = n$ copies/μL (Invitrogen™ Life Technologies).

The qRT-PCR was performed using the SuperScript™ III Platinum™ One-Step Quantitative RT-PCR System (Invitrogen™ Life Technologies, Carlsbad, CA, USA) in a 7500 Fast Real-Time PCR System (Applied Biosystems™, Foster City, CA, USA). The reactions were performed in final solutions of 25 μL containing 12.5 μL 2X Reaction Mix, 50 nM of Rox™ Reference Dye, 0.5 μL of the SuperScript™ III RT/Platinum™ Taq Mix, and 5 μL of the genomic template. Gradient solutions were used for the optimization of the primer (300 nM–800 nM) and probe (50 nM–250 nM) concentrations. The final concentrations for each primer and probe were defined as 400 nM and 250 nM, respectively. The cycling conditions were 50°C/15 min, 95°C/2 min, followed by 40 cycles of 95°C/15 s and 60°C/30 s. Sterile ultrapure water was used as negative control in each reaction.

Tenfold serial dilutions of the SVA clone containing 1.3×10^8 to 1.3×10^0 copies/μL were prepared with UltraPure™ diethylpyrocarbonate (DEPC)-treated water (Invitrogen™ Life Technologies, Carlsbad, CA, USA). Each aliquot was used once in each assay for the construction of the standard curve by plotting the quantification cycle (Cq) and the plasmid copy number logarithm.

The limit of detection (LOD) of the qRT-PCR assay was determined using tenfold dilutions of the SVA clone containing 1.3×10^8 to 1.3×10^0 copies/μL. All dilutions were tested in the qRT-PCR in three different times and in duplicate to determine the coefficient of variation (CV). The intra- and inter-assay CVs for Cq values were calculated for each dilution of the standard curve. Conventional RT-PCR assays with primers q2688Fw and q2782Rv were also performed for each SVA clone dilution for comparison purposes. The LOD and sensitivity of the technique was also evaluated by the SVA genome recovery from biological samples that might contain

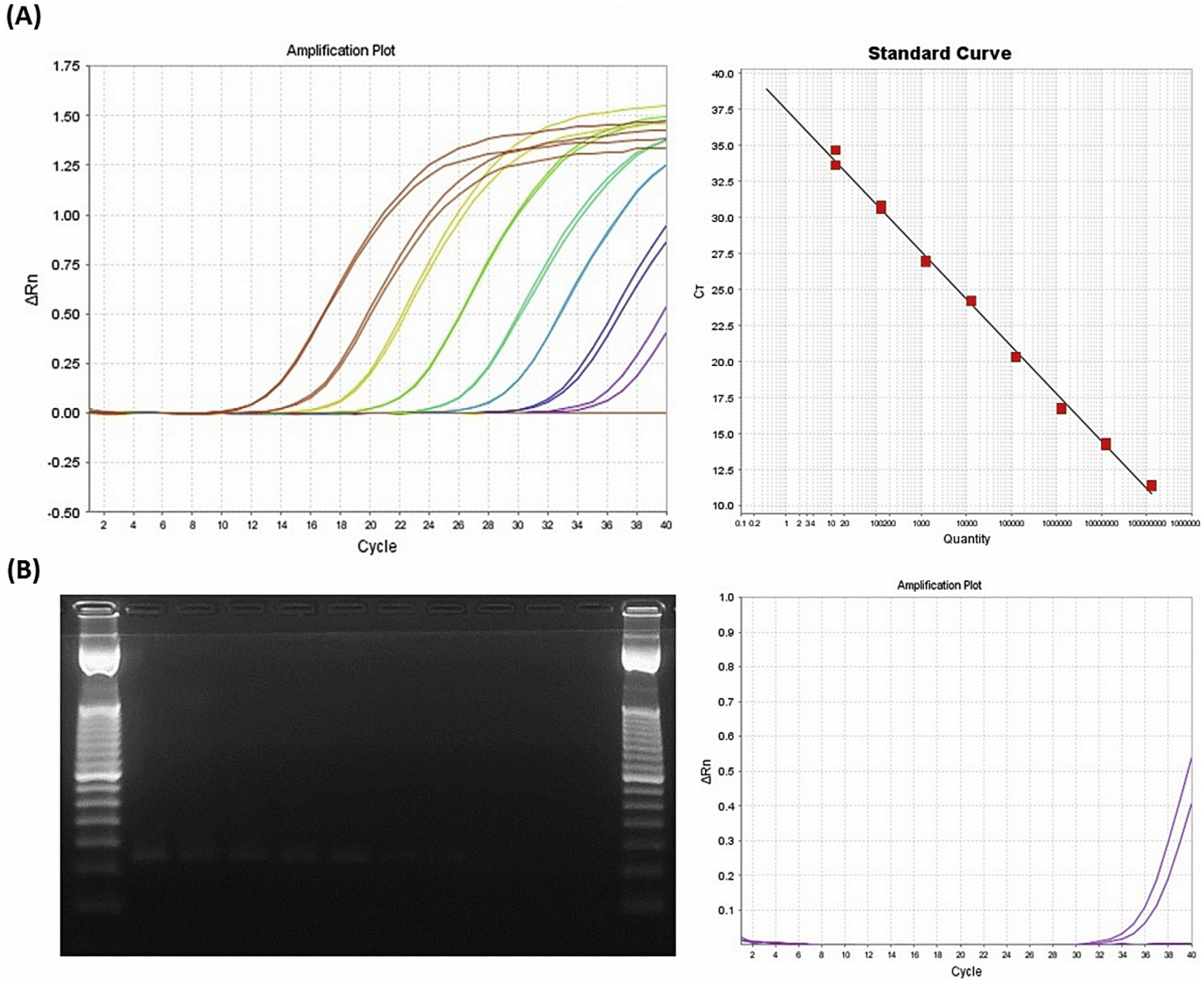

Fig. 1. (A) Amplification curves of TaqMan-based qRT-PCR assay using duplicated tenfold serial dilutions (10^8 to 10^0 copies/μL) of SVA clone genomic template; y = −3.289; $R^2 = 0.997$; E = 101.37%. (B) Limit of detection of the conventional RT-PCR assay. Left: Lanes 1 and 12 = 100 bp DNA ladder, Lanes 2-10 = serial dilutions (1.3×10^8 to 1.3×10^0 copies/μL) of the SVA clone, Lane 11 = negative control; Right: Amplification curve of TaqMan-based qRT-PCR assay from the 10^1 and 10^0 dilutions (13 and 1.3 copies/μL) of the SVA clone.