REAL TIME RT-PCR BASED ASSAYS FOR THE SPECIFIC DETECTION OF *PESTE DES PETITS RUMINANTS* VIRUS

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DEPARTMENT OF VETERINARY MICROBIOLOGY

MADRAS VETERINARY COLLEGE

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

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CERTIFICATE

This is to certify that the thesis entitled "**REAL TIME RT-PCR BASED ASSAYS FOR THE SPECIFIC DETECTION OF *PESTE DES PETITS RUMINANTS* VIRUS**" submitted in partialfulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Microbiology to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, is a record of a bonafide research work carried out by TSEGALEM ABERA WOLDEMEDHIN, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, fellowship or other similar titles or prizes. However as per TANUVAS regulation, a portion of the thesis has been sent for publication in a peer reviewed journal and a copy of the manuscript is enclosed.

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ABSTRACT

Title	:	**REAL TIME RT-PCR ASSAYS FOR THE SPECIFIC DETECTION OF *PESTE DES PETITS RUMINANTS* VIRUS**
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Peste des petits ruminants (PPR) is an economically significant disease of sheep and goats. Specific and sensitive RT-PCR methods are needed for the diagnosis of this economically important viral disease. In this study, two-step SYBR Green I based real time RT-PCR assays targeting the M and N gene for the specific detection and quantification of PPRV were developed. Two primer sets each for the M and N gene were designed and reaction conditions were optimized. PPRV CBE field isolate was propagated on Vero cells and virus titre of $10^5 TCID_{50}$/ml was used to standardize the assays and to test the sensitivity of the assays based on infectivity titre. Two plasmid DNAs carrying fragments of 348

and 488 bp of M and N gene respectively containing the real time RT-PCR primers binding sites were used to construct standard curves and test reproducibility and analytical sensitivity of the assays.

Quantitation was performed against the standard curves based on the plasmid DNAs. The lower detection limit achieved for M gene based real time RT-PCR assay was 2.88 copies/µl with corresponding Ct value of 35.93. For N gene based real time, RT-PCR the lower detection limit was 5.11 copies/µl with a corresponding Ct value of 33.67±0.5.

The lower detection limits were $0.0001TCID_{50}$/ml and $1TCID_{50}$/ml for M gene based real time RT-PCR and conventional RT-PCR, respectively with respect to tissue culture infectivity titre. For N gene based real time RT-PCR and conventional RT-PCR, the lower detection limit was found to be 0.001 and $1TCID_{50}$/ml, respectively.

The developed assays had dynamic detection limit which spans over 7 log10 concentration range for both M and N gene targets. The correlation coefficient for amplification of M and N gene target was $R^2 = 0.9947$ (Slope = -3.0763) and $R^2 = 0.995$ (Slope = -3.1926), respectively. Both M and N gene target amplifications showed high PCR efficiency of 111 % and 105 %, respectively.

The calculated coefficient of variations (CV) values for intra-assay variability for both M and N based real time RT-PCR assays were low, ranging from 0.21 % - 1.83 % and 0.32 % - 2.31 %, respectively. Whereas, the inter-assay CV values were in the range of 0.44 % - 1.97 % and 0.71 % - 5.32 % for M and N gene target assays, respectively. This showed that both assays had excellent intra- and inter-assay reproducibility.

The specificity of the assays was assessed against viral nucleic acid extracted from a range of animal viruses including CDV, MV, BTV and NDV. But none of the viruses and no template control (NTC) showed an amplification signal for both M and N gene based real time RT-PCR assays.

Swab materials spiked with known titre of Vero cell propagated PPRV were equally well detected in both assays.

The newly developed SYBR Green I based real time RT-PCR assay targeting the M gene was found to be 10,000 times more sensitive than conventional RT-PCR with respect to infectivity titre. Whereas, the N gene based real time RT-PCR assay was 1000 times more sensitive than conventional RT-PCR.

To evaluate the performance of newly developed assays, a total of 36 clinical samples suspected of PPR were screened for the presence of PPRV in parallel with conventional RT-PCR. The M gene specific real time assay detected PPRV in 32 (88.8%) of clinical samples compared to 19 (52.7%) by conventional RT-PCR. The N gene specific real time assay detected PPRV 30 (83.3%) compared to 16 (44.4%) by standard RT-PCR.

Thus, the two-step SYBR Green I based real time RT-PCR assays targeting the M and N gene of PPRV reported here were highly sensitive, specific, reproducible and rapid for detection and quantification of PPRV nucleic acids.

Key words: SYBR Green I, Real time RT-PCR, PPRV, M gene, N gene, Virus detection, Quantitation