APPLICATION OF ONE TUBE REVERSE TRANSCRIPTION AND NESTED POLYMERASE CHAIN REACTION (RT-NESTED PCR) FOR RAPID DETECTION AND DIFFERENTIATION OF PESTIVIRUSES

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Summary. A single tube nested RT-PCR was developed for rapid detection and identification bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV). The aim of the study was to apply the simplified procedure of reverse transcription and polymerase chain reaction (RT nested PCR) for diagnosis BVDV and CSFV. Total RNA was extracted from virus-containing cell culture supernatant and whole EDTA blood. Five microliters of RNA were reversely transcribed and amplified by two methods: standard, three steps and modified closed one-tube. Two sets of PCR primers were used for each method. The first, based on 5'UTR of pestivirus genome, was specific for all pestiviruses. The second, designed on E2 protein-coding region was used for specifically differentiate CSFV from BVDV. The standard method consisting of 3 steps was performed in 3 separate reaction tubes: RT, PCR, nested PCR. In the single-tube method all three steps were performed in a single closed tube. In this method reagents for RT-PCR step were deposited in tube bottom, while re-agents for nested PCR were immobilized in a tube cap using carbohydrate trehalose. After the RT-PCR step was completed the tube was vortexed, centrifuged and the nested PCR was performed. It was concluded that the closed one tube RT-nested PCR method was very sensitive and less prone to giving false positive results compared to standard RT-nested PCR or RT-PCRI, carried out in separate reaction tubes. This method could be an improvement over existing RT-PCR assays for BVDV and CSFV.

Keywords: pestivirus, RT-nested PCR, BVDV, CSFV.