DETECTION AND DIFFERENTIATION OF EUROPEAN AND AMERICAN GENOTYPES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) BY MODIFIED ONE TUBE REVERSE TRANSCRIPTASE NESTED POLYMERASE CHAIN REACTION (RT-PCR)

Arūnas Stankevičius, Algirdas Šalomskas, Marija Stankevičienė, Juozas Pieškus

Summary. A single tube nested RT-PCR was developed for rapid detection and identification of PRRSV. The aim of the study was to apply the simplified procedure of reverse transcription and polymerase chain reaction (RT nested PCR) to diagnose European-type and American-type of PRRSV. Total RNA was extracted from virus-containing cell culture supernatant and porcine serum samples. RNA was reversely transcribed and amplified by two methods: the standard three steps and modified closed one-tube. Two sets of PCR primers were used for each method. The first, based on ORF5 European-type PRRSV genome, was specific only for European-type PRRSV strains. The second, designed on American-type PRRSV genome was used for identification of American-type PRRSV strains only. The standard method consisting of three steps was performed in 3 separate reaction tubes: RT, PCR and 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 on the bottom of the tube, while reagents 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 can be concluded that the closed one tube RT-nested PCR method has been very sensitive and less prone to give false positive results compared to standard RT-nested PCR or RT-PCRI, carried out in separate reaction tubes. The method could be an improvement over existing RT-PCR assays for PRRSV genotyping and diagnosis. Furthermore, our studies have indicated that only European-type of PRRSV was prevalent in the Lithuanian swine population.

Keywords: porcine reproductive and respiratory syndrome virus (PRRSV), RT nested PCR, differentiation