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DETECTION OF PRRSV IN 218 FIELD SAMPLES USING SIX MOLECULAR METHODS: WHAT WE ARE LOOKING FOR?

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Objectives

The purpose of this study was to determine the sensitivity and the specificity of six molecular methods used for the detection of porcine reproductive and respiratory syndrome virus (PRRSV).

Methods

218 field samples (serum, tissues) were collected between 2009 and 2011 from 50 PRRSV positive and 45 negative pig herds from Slovenia. Total viral RNA was extracted from original samples and stored in aliquots at -70 °C until analysis. RT-PCR and direct sequencing of positive samples was performed as described previously (Toplak et al., 2012). All field samples were analyzed with five commercial real-time RT-PCR kits (named as kit A to E) according to the instructions of producer.

Results

According to determined 258 nucleotides long sequences (ORF7) 102 PRRSV samples belong to Type I (identification of 12 different lineages of EU subtype 1 (a=1, b=8, c=1, d=1, e=61, f=8, g=2, h=4, i=3, j=4, k=1, m=8) with 85.7-93.8 % nucleotide identity between lineages and four samples belong to Type II. In total, 138 PRRSV positive samples were detected with broad range of PRRSV RNA in samples. The highest sensitivity was observed with kit E (96,3%) and with kit B (94,5%), followed by conventional RT-PCR (87,8%) and kit D (82,1%), while the lowest sensitivity was observed with kit A (55,3%) and kit C (53,8%). Reduced sensitivity was directly related to the genetic lineages.

Discussion and conclusion

The study showed that the performance of commercial RT-PCR assays are highly dependent on the genetic make-up of the target viruses and confirm findings of a previous study where we showed some commercial PCR kits failed to detect specific genetic linkages of PRRSV. Thus, these finding emphasise that it is crucial that the manufacturers of diagnostic PCR kits (conventional and real-time) continuously follow the genetic evaluation of especially Type I PRRSV subtype viruses and regularly update their primer sequences..

Keywords: PRRSV, diagnosis, real-time RT-PCR, sensitivity, field samples