Study of the efficiency of ORF5 PCR depending on the CT values of ORF7 PCR in PRRS virus

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INTRODUCTION

Porcine reproductive and respiratory syndrome virus continues to serve as one of the economically most important pathogens in the global swine industry. A good way to monitor the isolate coming in a farm company or area is the sequencing of ORF5. The main problem of this technique is the lower sensibility that the ORF5 PCR (that has to be done previous to the sequencing) usually has compare to ORF7 PCR chosen for that to diagnose the disease.

In this work we analyze the performance of two in house methods for the real time RT-PCR for ORF7 and ORF5 genes respectively of field serum samples belonging to animals potentially infected with PRRS virus.

MATERIALS AND METHODS

2309 pools of 5 serum samples were obtained from 74 different PRRS positive farms across Spain during a year. ORF 7 and ORF5 in house real time RT-PCR was evaluated as a previous step to sequencing process. Commercial primers were used in the ORF7 PCR. To generated the ORF5 pair of primers for the PCRs more than 68 divergent isolates of the Genbank sequences were used.

The positive ORF 7 PCR samples were divided in 5 different groups regarding their threshold Cycles (CT) values obtained. Group 1: <24 CT, group 2: 24-29, group 3: 30-32 CT, group 4: 33-35 CT, group 5: >36 CT. Each of these groups was correlated with the % of positive in ORF5 PCR.

RESULTS

Pooled sera yield a positive result in RT-PCR for ORF7 in the 32 % of the samples. In average 81 % of ORF7 positive samples gave a positive result for ORF5 but these results are quite variable depending of the cts values results of the ORF7 PCR.Group 1 and 2 reached 100 % and 95,1 % of positive results coming from a positive ORF 7 PCR sample.Group 3 and 4 obtained 74,9 % and 51,5 % of positive results respectively, been 0 % the result in case of group 6.

Table 1: Summary of the % of positive results in ORF5 PCR in the different groups of ORF 7 PCR CT values.

ORF7 CT	ORF7 PCR+	ORF5 PCR-	ORF5 PCR-	% Positive
< 24	114	0	114	100 %
24 – 29	265	13	252	95,10 %
30 – 32	219	55	164	74,90 %
33 – 35	130	63	67	51,50 %
36 – 37	5	5	0	0 %

DISCUSSION AND CONCLUSION

This study shows again, that the preferred ORF to diagnose is ORF7 otherwise according to our results 19 % of positive samples been positive they would have been considered negative using ORF5 PCR. The ability to have a positive result in ORF 5 PCR from a positive ORF7 PCR sample is inversely proportional to the CT value of the ORF7 PCR result. Since the RNA used in both amplifications is the same, and the PCR conditions are quite similar, the factor influencing the different results are independent of the template and probably are related with the size of the amplicon, the efficiency of the primers or both.