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ABSTRACT BOOK and FINAL PROGRAMME

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NEW VARIANT RABBIT HEMORRHAGIC DISEASE VIRUS RT-QPCR

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INTRODUCTION:

A new variant of Rabbit Hemorrhagic Disease Virus (RHDVb) has been widely detected in Spain since 2011. Vaccination against classic RHDV strain might be ineffective in terms of protection and fatal consequences are often presented in animals as young as 11 days. Hence, the need of a rapid, specific and sensitive diagnostic technique is evident. RT-qPCR is an updated tool which detects specifically new variant RHDV strain. We proposed to design, develop and validate a RT-qPCR for detecting and quantifying RHDVb.

MATERIALS AND METHODS:

Sixty six VP60 gene sequences of RHDVb facilitated by UniOvi were aligned to select primers and probe which detect specifically this virus. Due to the duplex format of the assay, RHDVb (FAM) and an endogenous control (EC) (HEX) were simultaneously detected in every well. A synthetic ssDNA which contains the target sequence was designed as positive control (uRHDvar).

RNA isolation from liver was carried out in an automatic extraction device following its respective instructions manual. RT-qPCR was set up in 20 µL with 5x102 nM of each primer, 2.5 x102 nM of probe as well as Precision OneStep qRT-PCR MasterMix (2X) from PrimerDesign (United Kingdom). Thermal profile consisted in a reverse transcription step followed by an enzyme activation step and 42 cycles of amplification. Results were analyzed considering as positive samples those whose Cq value was lower than 38.

UniOvi provided a panel of samples determined as RHDVb by sequencing. This validation panel included 103 samples and consisted of 23 samples of classic strain RHDV, 69 samples of RHDVb strains, 2 samples of type A variant RHDV strain and 9 RHDV negative samples.

Specificity test was performed with a group of 22 pathogens including main bacteria, virus and parasites which affect rabbits or are supposed to be genetically related to RHDVb.

Moreover, 61 clinical samples suspected of RHD and submitted from 14 different Spanish provinces and Portugal were analyzed. Liver samples were firstly analyzed by antigen capture double antibody sandwich ELISA INgezim RHDV, which detects either RHDV classic strains or RHDV variant strains. After that, RNA from livers was isolated and analyzed by RHDVb RT-qPCR.

Repeatability intra assay test was performed using ten-fold dilutions of uRHDvar. For that purpose, a standard curve of quantification was set up with 7 dilutions with their respective 3 replicates. Linear range, coefficient of variation range (CV) and lower limit of detection (LOD) were determined.

RESULTS:

Analyzing the validation panel, every primary classified as positive sample resulted RHDVb RT-qPCR positive. 33 samples resulted negative. EC amplification was observed in 100% of samples. RHDV classic strains and type A variant RHDV strains resulted negative by RHDVb RT-qPCR. Kappa value was 0.978. None of the pathogens tested in specificity panel resulted positive.

Statistical parameters were: slope=-3.44, efficiency=95.18% and R2=0.99. Linear range resulted from 1.2x1010 copies/rxn to 1.2x102 copies/rxn. CV values ranged from 0.23% to 12.77%. LOD observed was 1.2x102 copies/rxn.

38 clinical samples resulted positive by ELISA whereas 23 resulted negative. On the contrary, RT-qPCR resulted positive in 42 samples and negative in 19 samples. Samples ranged from 1.12x109 copies/rxn to 4.88x104 copies/rxn. The EC of every clinical sample resulted positive. Four non concordant results were confirmed as positive by sequencing.

DISCUSSION AND CONCLUSIONS:

RT-qPCR was 10% more sensitive than serology. ELISA INgezim RHDV has been extensively used as a screening tool to detect RHDV, however, RT-qPCR improves its sensitivity and specificity since, unlike ELISA, detects merely RHDVb.

No classical strain of RHDV was found during the development of this study. Thus, despite of the restrictions of the sampling, we might suggest a local scenario in which RHDVb tends to displace the classic virus.

The RT-qPCR assay for RHDVb was successfully validated and we suggest its use for qualitative and quantitative diagnosis of new variant RHDV.

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