Results of the 2nd VER Inter-laboratory Proficiency Test

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Abstract

Betanodaviruses, the causative agents of VER, are classified into four different species, based on the phylogenetic analysis of the RNA1 and RNA2 segments: the striped jack nervous necrosis virus (SJNNV), the tiger puffer nervous necrosis virus (TPNNV), the barfin flounder nervous necrosis virus (BFNNV) and the red-spotted grouper nervous necrosis virus (RGNNV). Additionally, due to the segmented nature of their genome, reassortment events amongst different betanodaviruses have been described. In the Mediterranean basin, the presence of the RGNNV and the SJNNV genotypes, as well as of the RGNNV/SJNNV and RGNNV/SJNNV reassortants has been extensively documented. Different betanodaviruses show diverse pathogenicity, host and temperature tropism. Therefore, the capability of detecting all viral species, as well as their correct identification is of utmost importance to provide accurate and reliable laboratory results.

In 2016, the first inter laboratory proficiency test (IPT) for VER molecular diagnostics was organized, with the purpose of assessing the capability of the laboratories working with this pathogen to detect a single genotype (RGNNV) by Real-Time PCR (rPCR) methods. The results of this first exercise were published in the EAFP bulletin (Toffan et al., Bull. Eur. Ass. Fish Pathol., 70, 37(2) 2017).

In 2017-2018 the 2nd VER IPT was organized within the framework of the MedAID European project. The panel comprised 10 vials containing different viral species, including reassortant strains, and different molecular methods (conventional and/or rPCR) could be used for viral detection. Viral species identification was also requested, although it was neither mandatory nor subjected to scoring. The participants were asked to fill a spreadsheet reporting the results, to provide the details related to the diagnostic protocol used to outline the lab's activities connected to VER diagnosis.

The panel was shipped to 32 laboratories located in 18 different countries, most of which European. However, only 29 laboratories out of 32 provided results within the deadline.

Of the 29 respondent laboratories, 15 obtained the maximum score. Ten laboratories produced a percentage of correct answers ranging between 70-90 %, and the remaining 4 laboratories produced less than 50% of correct results. Therefore, the average percentage of correct answers turned out to be 85.5% while the overall agreement (k) was 0.5387 (p = 0.0000).

Only 13 laboratories out of 29 (44,8%) were able to perform the complete and/or partial molecular characterization of the positive samples, and only 2 out of 13 obtained the maximum score.

Meaningful differences were observed among laboratories located in different geographic regions in their capacity of detecting betanodaviruses. Reassortant strains appeared to be the most challenging viruses to detect.

This work has received funding from the European Union's Horizon 2020 research and innovation programme MedAID under grant agreement No 727315