

## Senegalese sole transcriptomic profiles in response to different betanodavirus RGNNV/SJNNV reassortant strains

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Betanodaviruses are the causative agents of viral nervous necrosis (VNN), a disease that has been reported in more than 40 marine and freshwater fish species worldwide, including Senegalese sole (*Solea senegalensis*). Based on the variable region T4 (RNA2), betanodaviruses have been classified into 4 genotypes: striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), redspotted grouper nervous necrosis virus (RGNNV), and barfin flounder nervous necrosis virus (BFNNV). Reassortant isolates combining genomic segments from the SJNNV and RGNNV genotypes have been obtained from farmed fish species. The reassortant SpSs-IAusc160.03 (wild type reassortant), with a genome consisting of RGNNV-type RNA1 and SJNNV-type RNA2 segments, is more suited for infecting sole than the parental genotypes, causing 100% mortality by bath challenges. Furthermore, compared with the parental SJNNV genotype, this reassortant strain presents two aminoacidic substitutions (positions 247 and 270) at the extreme C-terminal of the capsid protein, which are involved in host specificity. In the current study, the RNA-Seq technology has been used to determine changes in Senegalese sole transcriptome after infection with the wild type and a less virulent recombinant (rSs160.03<sub>247+270</sub>) with mutations at aminoacids 247 (serine to alanine) and 270 (serine to asparagine), provoking a 40% decreased mortality. Animals (5 g weight) were distributed into two groups to be intramuscular injected with the above described viral strains ( $2 \times 10^5$  TCID<sub>50</sub>/fish). A negative control group (L15-injected) was also established. Head kidney and nervous tissues (eye+brain) were sampled at 48 post-inoculation (p.i.). A total of 633 genes were differentially expressed (DEGs) in animals infected with the wild type isolate (358 up-regulated and 49 down-regulated in head kidney; 206 up-regulated and 20 down-regulated in eye+brain), whereas only 393 genes were differentially expressed in animals infected with the mutated isolate (129 up-regulated and 10 down-regulated in head-kidney; 28 up-regulated and 226 down-regulated in eye+brain). The results obtained indicate a 37.9% decrease in the number of DEGs after infection with the mutated reassortant, as well as an inversion in the proportion of genes up/down-regulated in nervous tissue of these animals. In addition, the expression patterns of genes coding for proteins involved in the IFN type I pathway were different in both group of animals. Thus, genes coding for proteins acting as mediators of IFN type I expression (MDA5, LGP2, IRF3, IRF7) and IFN-stimulated genes (ISG15, Mx, PKR, IFI6, IFI35, IFI44, IFIT-1, among others) were up-regulated in animals infected with the wild type reassortant, whereas no-differential expression of these genes was observed in animals infected with the mutated isolate. The different transcriptomic profiles obtained could help to better understand NNV pathogenesis in Senegalese sole, setting up the importance as virulence determinants of aminoacids at positions 247 and 270 within the RNA2 segment. Furthermore, the results obtained permit to identify DEGs that could be used to develop new strategies to control this infectious disease, which has reached high relevance in the aquaculture sector.

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