Title: RGNNV capsid protein amino acids 247 and 270 are involved in betanodavirus virulence to European sea bass (*Dicentrarchus labrax*)

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Introduction: European sea bass is severely affected by nervous necrosis disease, caused by nervous necrosis virus (NNV, *Betanodavirus* genus). The genome of this virus is composed of two single-stranded, positive-sense segments: RNA1 (viral polymerase) and RNA2 (capsid protein, CP). Two out of the four betanodavirus genotypes (RGNNV and SJNNV) have been detected in sea bass, although showing different levels of virulence to this fish species. Thus, sea bass is highly susceptible to RGNNV, whereas outbreaks caused by SJNNV have not been reported in this fish species. In the present work, we evaluate the implication of CP amino acids 247 and/or 270 in the viral replication and virulence, as well as in host immune response.

Methodology: Recombinant RGNNV viruses harbouring SJNNV-type amino acids at positions 247 and/or 270 (Mut247Dl965, Mut270Dl965, Mut247+270Dl965) were generated by reverse genetics. The effect of these modifications on viral replication was evaluated in cell culture and in infected fish. Experimental infections were also performed to analyse viral virulence and fish immune response.

Results: Differences regarding the replication of the mutated viruses on E-11 cells were reported. In particular, Mut247+270Dl965 showed the most important differences with titres significantly lower than those obtained for the wild type. *In vivo*, fish mortality caused by mutated viruses was 60% lower and viral replication in sea bass brain was reduced in comparison with the non-mutated virus. In addition, mutated viruses triggered lower induction of IFN I system- and inflammatory response-related genes. Furthermore, mutations caused changes in viral serological properties, inducing higher seroconversion and changing antigen recognition.

Conclusions: Amino acids 247 and 270 of the RGNNV CP sequence are important virulence determinants to sea bass. Differences in viral replication *in vitro* and *in vivo* suggest that the mutations considered can affect cell recognition and entry. In addition, the immunological analysis points out the importance of IFN I system and inflammatory process in response to betanodavirus infection. Finally, the double mutated virus induced the highest seroconversion, and antibodies in sera from infected animals recognized both, RGNNV and SJNNV antigens, suggesting its potential use in vaccination assays.

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