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Setting out zebrafish (Danio rerio) as a model to study nervous necrosis virus-host interaction

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Introduction: Viral nervous necrosis is responsible for important economic losses in aquaculture facilities. The causative agent is the nervous necrosis virus (NNV), with an RNA-bipartite genome. Four NNV species have been described, although only RGNNV and SJNNV have been detected in the Mediterranean area. Moreover, RGNNV-SJNNV reassortants have also been isolated from several fish species. In order to design strategies to improve fish resistance to NNV, in vivo studies in commercial and model species are required to study the mechanisms underlying fish susceptibility to viral isolates or species. Zebrafish is a model to study viral infections, as its small size, optical transparency and genome editing tools constitute important advantages. The aim of this work was to set up zebrafish as model of NNV infection. To fulfil this aim, zebrafish susceptibility to three different NNV isolates was determined, and viral replication and innate immune response were characterized.

Material and methods: Three days post-fertilisation zebrafish larvae were infected by intracerebral injection with 107 TCID50/mL of SJ93Nag (SJNNV), Dl956 (RGNNV from seabass), and RG/SJ (from seabream). Larvae were daily monitored for 4 days to record clinical signs and mortality. At 1 and 4 days post-infection (dpi), 3 pools of 6 larvae were sampled for viral genome quantification. Innate immune response was also assessed, focusing on genes related to IFN- I, apoptosis and inflammation signaling pathways. Transcriptional analyses were completed by in vivo 3D imaging approaches on a zebrafish transgenic line expressing GFP in neutrophils (Tg (mpx:GFP) to monitor neutrophils recruitment in brain at 1, 2, and 4 dpi.

Results: RGNNV was the most virulent isolate compared to SJNNV and RG/SJ. These observations were consistent with viral genome replication, as the highest number of viral genome copies was in RGNNV-infected larvae. The immune response, assessed by the induction of immune-related genes and the recruitment of neutrophils in brain, was also higher in RGNNV-infected larvae. Therefore, further experiments can be designed in this successfully established model to better understand the mechanisms underlying NNV virulence in its hosts.

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