

Risk factors associated with viral nervous necrosis in hybrid groupers in Malaysia and the high similarity of its causative agent nervous necrosis virus to reassortant redspotted grouper nervous necrosis virus/striped jack nervous necrosis virus strains

ABSTRACT

Background and aim: Viral nervous necrosis (VNN) is a serious disease of several marine fish species. VNN causes 100% mortality in the larval stages, while lower losses have been reported in juvenile and adult fish. This study aimed to detect the occurrence of VNN while identifying its associated risk factors and the genotypes of its causative agent in a hybrid grouper hatchery in Malaysia. Materials and methods: A batch of newly hatched hybrid grouper fry (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) were followed from the larval stage to market size. Samples of the hybrid groupers, water, live feed, and artificial fish pellets were collected periodically from day 0 to 180 in the hybrid grouper hatchery. Reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR amplifications were carried out on VNN-related sequences. The phylogenetic tree including the sampled causative agent of VNN was inferred from the coat protein genes from all known Betanodavirus species using Molecular Evolutionary Genetics Analysis (MEGA). Pearson's correlation coefficient values were calculated to determine the strength of the correlation between the presence of VNN in hybrid grouper samples and its associated risk factors. Results: A total of 113 out of 146 pooled and individual samples, including hybrid grouper, water, and artificial fish pellet samples, demonstrated positive results in tests for the presence of VNN-associated viruses. The clinical signs of infection observed in the samples included darkened skin, deformation of the backbone, abdominal distension, skin lesions, and fin erosion. VNN was present throughout the life stages of the hybrid groupers, with the first detection occurring at day 10. VNN-associated risk factors included water temperature, dissolved oxygen content, salinity, ammonia level, fish size (adults more at risk than younger stages), and life stage (age). Detection of VNN-associated viruses in water samples demonstrated evidence of horizontal transmission of the disease. All the nucleotide sequences found in this study had high nucleotide identities of 88% to 100% to each other, striped jack nervous necrosis virus (SJNNV), and the reassortant strain red-spotted grouper NNV/SJNNV (RGNNV/SJNNV) isolate 430.2004 (GenBank accession number JN189932.1) (n=26). The phylogenetic analysis showed that quasispecies was present in each VNN-causing virus-positive sample, which differed based on the type of sample and life stage. Conclusion: This study was the first to confirm the existence of a reassortant strain (RGNNV/SJNNV) in hybrid groupers from Malaysia and Southeast Asia. However, the association between the mode of transmission and the risk factors of this virus needs to be investigated further to understand the evolution and potential new host species of the reassortant strain.

Keyword: Epidemiology; Quasispecies; Reassortant; Red-spotted grouper nervous necrosis virus-striped jack nervous necrosis virus; Viral nervous necrosis