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A panel of complementary tools to go deeper in the characterization of potential viral fish pathogens

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Introduction: One of the main challenges for the coming decades will be to feed 9 billion people in a context of global climate change which, since the Paris agreements in 2017 (COP21), has been recognized as a fundamental threat to the security of the food supply and therefore on sustainable development. Aquaculture occupies a strategic place to produce quality food but also to ensure economically and socially sustainable development. Nevertheless, even though zootechnic improved greatly among the last decades, this market is threatened by epidemic episodes caused by pathogenic agents including a large number of known viruses but also many emerging viruses which potential impact has still to be determined.
In this context, cell culture remains a very efficient and non-specific historical method to detect virus from aquatic organisms showing atypical clinical symptoms, with the advantage to amplify the unknown virus, simplifying subsequent analyses and the demonstration of the Koch's postulate. This research strategy can now be coupled with high-throughput sequencing which allows a fast and deep characterization of viral metagenomes.
Methods: Following abnormal mortality or morbidity events, farmed fish can be delivered to the National Reference Laboratory (NRL) for regulated fish diseases. After inoculation on various derived-fish cell lines, the appearance of cytopathic effect is scrutinized and immunofluorescence assay (IFAT) can be performed to determine if the virus amplified matches or is closed to a known pathogen. In case of negative response, culture supernatant or organ extracts are analysed by next-generation sequencing (NGS) to obtain a consensus full sequence, then compared to any published sequences and integrated in phylogenetic study. Specific real-time PCR can be developed downstream regarding the sequence obtained. Serological assays (i.e. ELISA or seroneutralization tests) can also be performed to assess the global sanitary status of a fish population.
Results: On the last years, several unknown viruses could be identified by the NRL using those tools. For instance, following an unusual mortality among 50-day post-hatching larvae in a seabream hatchery, cytopathic effect was observed on EK-1 cell line. Molecular characterization by NGS gave a viral sequence with no equivalent in the databases but showing a typical picornavirus genome organization. This new virus was assigned to the genus Potamipivirus, and proposed to be named Potamipivirus daurada. More recently, a never described virus apparently belonging to the Totiviridae family was also identified on seabass larvae after cell culture amplification. An ELISA assay was developed to screen genitor status in the fish farm and intend to understand transmission way used by the virus to spread in the hatchery.
Conclusion:
The combination of proven historical tools and modern methods significantly improves viral diagnostics, allowing the rapid development of specific direct and indirect tools and the evaluation of the potential impact of the new agents described in aquaculture. The use of experimental infection remains the best way to check the role of a new virus in the symptomatology but could be difficult to implement on fragile larval stages and/or for cofactorial diseases.