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Introduction

Betanodaviruses are genetically very diverse with at least five genogroups described and a large range of variants within each group, including reassortants carrying components of two groups (Panzarin *et al.*, 2011; Toffolo *et al.*, 2007). Their genome is composed of two strands of RNA: RNA1 and RNA2. Till now, only one universal real-time RT-PCR method has been published which detects all variants of RNA2 (Panzarin *et al.*, 2010) in clinical cases. An additional method based on detection of RNA1 would be useful to confirm results obtained from RNA2. Another advantage of detecting RNA1 is to bring valuable genetic information on the second genome component of a given isolate. ANSES has developed new DNA probes targeting RNA1 with the goal of detecting all genotypes of nodaviruses. The aim of the project is thus:

- To organize, conduct and report an inter-laboratory proficiency test for detection of aquatic nodaviruses by real time RT-PCR targeting RNA1 and RNA2, respectively.
- To test a newly developed real-time RT-PCR targeting RNA1: sensitivity, specificity, range of detection and genetic information provided by sequencing the PCR product.

Materials & methods

Primers and probes specific for RNA 1 and RNA2 were ordered by ANSES and aliquots from the same batch were provided to all partners. IZSVe produced for all partners 10 samples (1-11) of inactivated isolates produced in cell culture and covering 4 genogroups. The samples were forwarded by ANSES and RNA had to be extracted by each partner. In the meantime, ANSES produced and distributed RNA extracted from healthy or infected fish (2 genogroups), or cell culture; one sample from cell culture had to be serially diluted to test the sensitivity of each method in partners' hands. Samples were tested in duplicates and the mean Ct values reported.

Results

A total of 192 virus-containing samples were tested (negative controls excluded), implicating 5 partners performing each two or four methods (16 tubes with virus * 12 methods). For each genetic component (RNA1 or RNA2), the same ratio of detection (80 / 96) was found, indicating that both RNA1 and RNA2 probes are globally equivalent for detection of all genotypes. When the samples sent were considered, the rate of false positives was 5,5% (2/36) and the rate of false negative 13,5% (31/228). Both RNA1 and RNA2 proved to be valuable targets for viral detection, with advantages and disadvantages depending on the method and the viral isolates.

Conclusions

Both sets of the nodavirus probes proved to be useful for detection of betanodaviruses in each lab's usual conditions. Ideally, both RNA should be targeted for samples exhibiting high Ct values (for instance in healthy carriers) to confirm the presence of low loads of virus.

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