Development of a consensus RT-nested PCR to detect genotypic variants of yellow head virus identified in geographically isolated populations of *Penaeus monodon*

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Abstract

Yellowhead virus (YHV) is the causative agent of the yellowhead disease in *Penaeus monodon*. Presently, at least 5 genotypes distinct from the pathogenic YHV isolate originally identified in Thailand have been detected in disparate populations of *P. monodon* from various geographic locations. In the present, a reverse transcription polymerase chain reaction (RT-PCR) was developed for consensus detection of all known genotypic variants is the YHV complex. To assess performance of the RT-PCR, representative isolates of Genotype 1 (YHV 1) and Genotype 2 (Australian gill-associated virus genotype) as well as Genotypes 3 to 6 originating from Vietnam, India, Thailand, Malaysia and Mozambique was tested. Degenerate primer pairs were designed to conserved sequences in a 671 nt region 3'-proximal to the helicase domain in the ORF1b gene for which sequence data had been generated for 57 field isolates comprising representative of the 6 genotypes. Two primers were designed to each site used in the primary and nested steps of the PCR to kept degeneracy of any single primer at ≤8-fold. One primer of each pair accommodated sequence variations amongst different Genotype 1 isolates, whilst the other accommodated variations amongst isolates of the other 5 genotypes. Amplicon lengths of the PCR (358 bp) and nested PCR (146 bp) steps were also kept short to accommodate RNA degradation encountered in clinical specimens. Using a GAV synthetic RNA, the sensitivity limits of the PCR and nested PCR were in the order of 1250 and 1.25 RNA copies, respectively. Comparisons using RNA from 17 YHV infected *P. monodon* demonstrated the robustness of the test and highlighted limitations of 2 other diagnostic RT-PCR tests recommended for detection of YHV by Office des Epizootics (OIE). Phylogenetic analysis using the 95 nt sequence between the nested PCR primers identified intra-genotypic relationships consistent with an extended 671 nt sequence amplified using another RT-nested PCR test. The consensus RT-nested PCR test should find application in detecting and classifying YHV genotypic variants, in tracking movements of infected *P. monodon* and identifying yet unknown YHV variants.

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