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DETECTION AND TYPING OF INFECTIOUS SALMON ANEMIA VIRUS, AN ORTHOMYXOVIRUS BY REAL-TIME RT-PCR

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Dr. Khalid Munir

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ABSTRACT

Infectious salmon anemia (ISA) virus (ISAV), a member of the family *Orthomyxoviridae* and the new genus *Isavirus*, is an economically significant fish pathogen in the Northern Hemisphere. The virus causes natural clinical disease in farmed Atlantic salmon and subclinical infections in both farmed and wild fish. It is generally believed that novel virulent ISAV strains emerge from these so-called background asymptomatic infections in wild fish and cause clinical disease in farmed Atlantic salmon. ISAV strains vary genetically and in their antigenicity and virulence. The molecular bases of antigenic variation and virulence in ISAV are not yet known. The highly polymorphic region (HPR) and/ or presence of amino acid motifs such as ^{333}NIT^{335}, ^{349}NQT^{351} or ^{331}FNTN^{334} in or close to the HPR of the hemagglutinin-esterase (HE) protein have been hypothesized to affect several biological properties including antigenicity and virulence of ISAV. The HPR is considered an important genotyping tool and epidemiological marker of ISAV strains. The limitations associated with the current ISAV detection procedures call for the development of rapid and sensitive methods that can detect, quantify and type ISAV isolates preferably in the context of ISAV antigenicity and virulence as well as assist in hypothesis-based investigations on host-virus interactions. The main aim of this study was to develop improved ISAV detection/typing methods and apply these methods to characterize ISAV isolates and investigate the connection, if any, between the HE/HPR genotypes and phenotypes of ISAV as a way to better understand the genetic determinants of ISAV virulence and antigenicity. For this purpose, a rapid, highly reproducible and sensitive one-tube real-time RT-PCR using SYBR Green chemistry and primers targeting the ISAV genomic segment 8 was developed that allowed quantitative detection of ISAV in biological samples; the assay was highly flexible in detecting ISAV isolates of different geographic origins and was found to be more sensitive than the conventional one-tube RT-PCR assay when compared on the same samples. The ability of ISAV isolates to cause CPE in the CHSE-214 cell line, viral titration of the infected CHSE-cell harvests, and temporal analysis of viral RNA levels in CHSE-214 cells at different post-infection times by SYBR Green real-time RT-PCR allowed the characterization of three CHSE-phenotypes of ISAV: replicating cytopathic, replicating non-cytopathic, and non-replicating non-cytopathic; there does not appear to be any correlation between the HPR groups or sizes and the CHSE-phenotypes of ISAV. A genotyping approach consisting of conventional and the SYBR Green and probe-based real-time formats of single-tube RT-PCR was also developed that utilized genotype-specific primers and probes targeting the ISAV RNA segment 6. Phylogenetic analysis of nucleotide sequences of the HE gene of ISAV isolates confirmed the existence of two ISAV HE genotypes. The conventional and SYBR Green real-time RT-PCR assays allowed concurrent detection and differentiation of ISAV isolates into North American and European genotypes while three-hydrolysis probe(s)-real-time assays permitted not only rapid and specific classification of ISAV isolates into two genotypes but also simultaneously recognized ISAV isolates with amino acid motifs ^{333}NIT^{335}, ^{349}NQT^{351}, and ^{331}FNTN^{334} suggested to be functionally important. The melting curve analysis of SYBR Green real-time RT-PCR products allowed further differentiation of North American HPR20 and HPR21 groups with the potential to discriminate other HPRs

that may differ significantly in their melting temperatures. Hemagglutination (HA)/ elution tests were tailored to analyze the elution patterns of ISAV and the usefulness of hemagglutination inhibition (HI) tests was also explored for ISAV serotyping;ISAV isolates could be separated into two groups based on rabbit erythrocyte elution patterns, elution-positive and elution-negative group. The exact biological significance of elution patterns of ISAV is not known. A meaningful association between the elution pattern and HPR groups and/or presence of amino acid motifs ^{333}NIT^{335}, ^{349}NQT^{351} and ^{331}FNTN^{334} in the HE of ISAV could not be inferred. Further work involving a greater number of ISAV strains and RBCs of other species, particularly Atlantic salmon, is required to substantiate the usefulness of HA, elution and HI tests for the analysis of ISAV strains. The ISAV genotyping SYBR Green real-time RT-PCR assay may be used as a screening method on infected materials from fish farms where the presence of both European and North American genotypes and multiple HPR groups of ISAV is suspected. The probe-based real-rime RT-PCR assays may assist in establishing the correlation, if any, between the presence or absence of amino acid motifs ^{333}NIT^{335}, ^{349}NQT^{351} and/or ^{331}FNTN^{334} and biological phenotypes of ISAV. The identification of three CHSE- phenotypes and/or elution patterns of ISAV may have important implications from a diagnostic and biological point of view.