NOTE

Genomic relationships of the North American isolate of infectious salmon anemia virus (ISAV) to the Norwegian strain of ISAV

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ABSTRACT: Nucleotide and deduced amino acid sequences were determined for a 436 bp reverse transcriptase-polymerase chain reaction (RT-PCR) cDNA fragment from genome segment 8 and a 1151 bp RT-PCR cDNA fragment from genome segment 2 of the North American isolate of infectious salmon anemia virus (ISAV) and compared to the published sequences of Norwegian isolates of ISAV. The North American ISAV isolate exhibited 82.9% identity with the Sotra 92/93 ISAV isolate from Norway in the partial cDNA sequence of genome segment 2, which encodes a polymerase component protein (PB1). The North American ISAV exhibited 88 and 89% identity with 2 partial cDNA sequences of genome segment 8 (nonstructural, NS, gene) reported for the Glesvaer/2/90 isolate from Norway. The North American ISAV exhibited 96.6% similarity with the Sotra 92/93 ISAV isolate from Norway in the deduced amino acid sequences of the PB1 protein. The deduced amino acid sequence of the protein encoded in the partial cDNA fragment of open reading frame (ORF) 1 of genome segment 8 of the North American ISAV exhibited only 71.2 and 66.7% similarity with the 2 sequences of the Norwegian Glesvaer/2/90 isolate. However, the North American ISAV isolate exhibited 96.2 and 87.2% similarity with the 2 sequences of the Norwegian Glesvaer/2/90 isolate in the deduced amino acid sequences of the protein encoded in the partial cDNA of ORF 2. Comparison of these partial cDNA nucleotide and deduced amino acid sequences confirmed that the North American isolate is ISAV. However, the differences observed in these genomic sequences suggest that the North American isolate may represent a distinct genomic variant from the previously described Norwegian strains.

KEY WORDS: Infectious salmon anemia virus · Nucleotide sequence

Infectious salmon anemia virus (ISAV) is the etiological agent of a serious disease in Atlantic salmon *Salmo salar* L. which has been observed in Norway for the last 15 yr. ISAV virus has been shown to be an enveloped

virus, 100 to 130 nm in diameter, which replicates by budding from the membrane of infected cells (Hovland et al. 1994, Dannevig et al. 1995, Nylund et al. 1995). Recently the genome of ISAV has been characterized by Mjaaland et al. (1997). The genome consists of 8 segments ranging in size from 1.0 to 2.3 kb, with a total molecular size of approximately 14.5 kb. Based on these morphological and replication data, these investigators concluded that ISAV resembles members of the viral family *Orthomyxoviridae*. The nucleotide sequence has been reported for a partial cDNA clone of the second largest genome segment (segment 2) of ISAV isolate Sotra 92/93 from Norway (Krossoey et al. unpubl., GenBank accession no. AJ002475). Genome segment 2 encodes the polymerase component protein PB1 of orthomyxoviruses. Two partially overlapping cDNA sequences have been reported for the smallest genome segment (segment 8) for the Glesvaer/2/90 ISAV isolate from Norway (Devold et al. unpubl., GenBank accession no. AJ012285; Mjaaland et al. 1997, GenBank accession no. Y10404). Segment 8 encodes 2 nonstructural (NS) proteins. No significant homology was found between the sequences of these Norwegian ISAV isolates and other orthomyxoviruses.

Recently, a new disease, originally called hemorrhagic kidney syndrome (HKS), has appeared in Atlantic salmon cultured in the Bay of Fundy, New Brunswick, Canada. HKS is associated with high levels of mortality in affected fish farms. Although not identical, some of the clinical signs characteristic of HKS were similar to those previously described for ISA. As described by Bouchard et al. (1999—this issue), we isolated a virus from moribund Atlantic salmon exhibiting HKS which we identified as ISAV on the basis of morphology and a reverse-transcriptase polymerase chain reaction (RT-PCR) assay using ISAV-specific primers.

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Table 1. Primers used in RT-PCR of North American ISAV isolate

Primers	Partial cDNA RT-PCR target	
	Polymerase gene (PB1)	NS gene
Upstream	5'-CTGCTGACTGGGGAAGATTC-3'	5'-GGCTATCTACCATGAACGAATC-3' ^a
Downstream	5'-CGTAGATGCCGTGTAAAAAGC-3'	5'-GCCAAGTGTAAGTAGCACTCC-3'

^aPreviously described by Mjaaland et al. (1997)

In this investigation, we compared the genomic relationship of this North American isolate of ISAV with the Norwegian strains of ISAV using partial cDNA sequences of the NS proteins encoded in genome segment 8 and the PB1 encoded by genome segment 2.

Materials and methods. Virus: The North American strain of ISAV was propagated in the chinook salmon embryo (CHSE-214) cell line (Lannan et al. 1984) at 15°C as described by Bouchard et al. (1999).

RT-PCR: RNA extraction and reverse transcription were performed as described previously (Blake et al. 1995). ISAV-specific primer sets (Table 1) were developed using the Oligo Primer software program (National Biosciences, Inc. [NBI], Plymouth, MN, USA) based on published sequences of the Norwegian ISAV strain (Mjaaland et al. 1997, GenBank accession no. Y10404, Krossoey et al. unpubl., GenBank accession no. AJ002475). These primers were used to amplify a 493 bp cDNA fragment from genome segment 8 and a 1209 bp fragment from genome segment 2 of the North American isolate of ISAV. A 10 µl aliquot of the reverse transcriptase reaction mixture was added to the PCR Master Mix containing 20 mM Tris-NaCl, 50 mM KCl, 1.5 mM MgCl₂ and 0.2 mM each of dNTPs. Primers were then added to a final concentration of 50 pmol. After addition of 2 drops of mineral oil, 0.5 µl of Ampli-Taq DNA Polymerase (Perkin-Elmer, Norwalk, CT, USA) was added with gentle mixing and the samples placed in a programmable thermal cycler (MJ Research). After an initial incubation at 95°C for 4 min, the samples were subjected to 35 cycles of PCR (94°C for 45 s, 59°C for 45 s, and 72°C for 1 min 45 s) followed by incubation at 72°C for 7 min. The samples then were electrophoresed in a Sea Plaque agarose gel containing ethidium bromide and examined under UV light. PCR amplification products were cut from the gel and processed for DNA sequencing.

Sequencing: The nucleotide sequences of the RT-PCR products were determined by the dye-terminator dideoxy system using an Applied Biosystems 373A automated DNA sequencer. A 1151 bp sequence was determined for the polymerase protein gene PB1 and a 436 bp sequence was determined for the NS gene of the North American ISAV isolate. Nucleotide and deduced amino acid sequences of the North American

ISAV isolate were aligned and compared to the corresponding sequence previously published for the Norwegian strain using the Lasergene DNASTAR software package.

Results. RT-PCR was used to amplify a 1209 bp fragment from genome segment 2 and a 493 bp fragment from genome segment 8 of the North American isolate of ISAV. Genome segment 2 encodes a protein (PB1) and genome segment 8 encodes the NS protein(s) of orthomyxoviruses. Agarose gels of these RT-PCR amplification products are shown in Fig. 1. Sequences were determined for 1151 bp of the cDNA from the polymerase gene and 434 bp of the NS gene.

Comparison of these partial cDNA nucleotide sequences (Figs. 2 & 3) with published sequences of ISAV from Norway confirmed that the North American virus isolate is ISAV. No areas of extensive sequence variation were observed between the 2 viruses. Rather, variations appeared as differences in 1 or 2 nucleotides and were more or less evenly distributed throughout the entire genomic region sequenced. The North American ISAV isolate exhibited 82.9% identity with the Sotra 92/93 ISAV isolate from Norway in the polymerase protein PB1 gene. The 2 previously reported cDNA sequences of the NS gene of the same Norwegian ISAV isolate (Glesvaer/2/90) had an identity of 98.5% in the region examined. The major difference in

Fig. 1 Agarose gel of RT-PCR amplified cDNAs of North American isolate of ISAV. Lane A: negative control, uninfected CHSE cells; Lane B: polymerase PB1 gene; Lane C: NS gene; Lane D: molecular size markers

Nor:	CAGTATGTCGAAATGATGTGCTTGTCTTTACSTTTATGATGGACCAATGAGGTTTTTCACAAACGCA:	70
NA:	CAGTATGTCGAAATGATGTGCTTGTCTTTACSTTTATGATGGACCAATGAGGTTTTTCACAAACGCA:	70
Nor:	CTAATGCCACGTGCAAAGTGTAAGGGAAGTGACCAGTTCTCAAAGGGGAAAACAAAGAGATTATTA:	140
NA:	TTAATGCCAAGCTGCAAAGTGTAAGGGAAGTGACCAGTTCTCAAAGGGGAAAACAAAGAGATTATTA:	140
Nor:	TAGATTGCTTTGGAATGAAAGGATGTGGGACATAGGGAACAACACCTTGAAGATGAAATTTGAGCGA:	210
NA:	TTGACCTGTTTGGGAATGAAAGGATGTGGGACATAGGGAACAACACCTTGAAGATGAAATTTGAGCGA:	210
Nor:	AACGTGTTGCTTTGCAAGCTTATGGCTTTGTGAAATACCTGATAACAACAAGTACGATGAGGAGAGAA:	280
NA:	AACGTGTTGCTTTGCAAGCTTATGGCTTTGTGAAATACCTGATAACAACAAGTACGATGAGGAGAGAA:	280
Nor:	ACTAGCTTAAGGAAGCAATGGAAGAAGCTTCGAGGAAGTCTATGAACGAAGAATTTGCTTACTGAGCA:	350
NA:	ACAAGTTTAAGGAAGCAATGGAAGAAGCTTCGAGGAAGTCTATGAACGAAGAATTTGCTTACTGAGCA:	350
Nor:	AAGGGAATTCACCCATGACATATCTCCGACACAAACCTCCGTGTGTAATTTCTGCTTAAAACTGGAT:	420
NA:	AAGGGAATTCACCCATGACATATCTCCGACACAAACCTCCGTGTGTAATTTCTGCTTAAAACTGGAT:	420
Nor:	AGTASCAACAGGTTTCAGAGGAAGAAGCATGTGACAGCTGATTGAACACCATTTCAGGTGATGCAAGGG:	490
NA:	AGTASCAACAGGTTTCAGAGGAAGAAGCATGTGACAGCTGATTGAACACCATTTCAGGTGATGCAAGGG:	490
Nor:	AAACAAGAGTGAAAGGATACATATGGAAACATAATACAACGAGGACTTAAAGAGAAAACAATAAGCA:	560
NA:	AAACAAGAGTGAAAGGATACATATGGAAACATAATACAACGAGGACTTAAAGAGAAAACAATAAGCA:	560
Nor:	AAGAAGAAGTTAAGTTGACAGAGAGAAATACACCTCGAGAAGCTTCAGACTACTTCTCTCTTGAGGAA:	630
NA:	AAGAAGAAGTTAAGTTGACAGAGAGAAATACACCTCGAGAAGCTTCAGACTACTTCTCTCTTGAGGAA:	630
Nor:	CAGCGAAGGAACTTAACCTGAGCCGAGAGCAGTGTTCACAGCTGGAGTACCTTGGAGGGCTTTCATCTTC:	700
NA:	CAGCGAAGGAACTTAACCTGAGCCGAGAGCAGTGTTCACAGCTGGAGTACCTTGGAGGGCTTTCATCTTC:	700
Nor:	GTCTAGAACAGACAATGCTCTCTGTAACAAGTTGGACCCAAATCAGTCACTCTGGATGGGAAGCGATG:	770
NA:	GTCTAGAACAGACAATGCTCTCTGTAACAAGTTGGACCCAAATCAGTCACTCTGGATGGGAAGCGATG:	770
Nor:	CCAAATATAACACGCAAACTCCAGGATAAAGGAATCGGAATGAAGAAACAAGGCACAAACACTTGTAAAC:	840
NA:	CCAAATATAACACGCAAACTCCAGGATAAAGGAATCGGAATGAAGAAACAAGGCACAAACACTTGTAAAC:	840
Nor:	GCTCAGTGGAGCAACTCTAAATACAATGAGAGCATGTGCCAGAGGTGATGATGCTTTTCCTAAGAGAA:	910
NA:	ACTCAGTGGAGCAACTCTAAATACAATGAGAGCATGTGCCAGAGGTGATGATGCTTTTCCTAAGAGAA:	910
Nor:	CTTGGGATAAAGGACCAATCTGGAAGTACTCSACTATGCTTTGTGGCAGTTTTCACAAAAGTGTCA:	980
NA:	CTTGGGATAAAGGACCAATCTGGAAGTACTCSACTATGCTTTGTGGCAGTTTTCACAAAAGTGTCA:	980
Nor:	AACCAATAGCACCTATAAAGAAAGAAACAGAGCTTACCTGTGTAGTCAAGGCGACCTGTTAAAGTA:	1050
NA:	AACCTTTGACACCTATAAAGAAAGAAACAGAGCTTACCTGTGTAGTCAAGGCGACCTGTTAAAGTA:	1050
Nor:	ATCCAGAGACCTTTCAATGAAAAGGAGCTGGAACTCTATCAAGGAGTASATGGATGGACATGGATT:	1120
NA:	GTCCAGAGACCTTTCAATGAAAAGGAGCTGGAACTCTATCAAGGAGTASATGGATGGACATGGATT:	1120
Nor:	ATCAGAGTGAGAGAGGAATGTTGATCGGAA:	1151
NA:	GTCCAGAGTGAGAGAGGAATGTTGATCGGAA:	1151

Fig. 2. Comparison of partial cDNA sequence of polymerase PB1 gene of North American isolate of ISAV (NA) with Norwegian ISAV strain Sotra 92/93 (Nor: sequence from Krossoey et al. unpubl., GenBank accession no. AJ002475)

NA-NS:	CCATGAACGAATCACAATGGATACAAAACATCTA	20	40	60	66
Nor-NS-A:	CCATGAACGAATCACAATGGATACAAAACATCTA				31
Nor-NS-B:	CCATGAACGAATCACAATGGATACAAAACATCTA				66
NA-NS:	GGGAGCTGATCAGACATGCCTTGAAGAA	80	100	120	132
Nor-NS-A:	GGGAGCTGATCAGACATGCCTTGAAGAA				97
Nor-NS-B:	GGGAGCTGATCAGACATGCCTTGAAGAA				132
NA-NS:	TTTTCACACTTGGCGGAAAGCGGATTGTGT	140	160	180	198
Nor-NS-A:	TTTTCACACTTGGCGGAAAGCGGATTGTGT				163
Nor-NS-B:	TTTTCACACTTGGCGGAAAGCGGATTGTGT				198
NA-NS:	AGTCAAAACCTTGAACCAATTGGTGAACGGAATGATCAGTCGAGCGACGATGAC	200	220	240	264
Nor-NS-A:	AGTCAAAACCTTGAACCAATTGGTGAACGGAATGATCAGTCGAGCGACGATGAC				229
Nor-NS-B:	AGTCAAAACCTTGAACCAATTGGTGAACGGAATGATCAGTCGAGCGACGATGAC				264
NA-NS:	TGATGAAGATCCACCACTCTGGAAGCATGGGAC	280	300	320	330
Nor-NS-A:	TGATGAAGATCCACCACTCTGGAAGCATGGGAC				295
Nor-NS-B:	TGATGAAGATCCACCACTCTGGAAGCATGGGAC				330
NA-NS:	AGGAGTAATCAGGATGCCAGTACCGGATGGTGGAGAGAAAA	340	360	380	396
Nor-NS-A:	AGGAGTAATCAGGATGCCAGTACCGGATGGTGGAGAGAAAA				361
Nor-NS-B:	AGGAGTAATCAGGATGCCAGTACCGGATGGTGGAGAGAAAA				396
NA-NS:	TTTACCCACATGCTGAGGAGAAGACGATGTGAAGG	400	420		435
Nor-NS-A:	TTTACCCACATGCTGAGGAGAAGACGATGTGAAGG				400
Nor-NS-B:	TTTACCCACATGCTGAGGAGAAGACGATGTGAAGG				434

Fig. 3. Comparison of partial cDNA sequence of NS gene of North American isolate of ISAV (NA-NS) with 2 reported sequences of the Norwegian ISAV strain Glesvaer/2/90 (Nor-NS-A: sequence from Devold et al. unpubl., GenBank accession no. AJ012285; Nor-NS-B: sequence from Mjaaland et al. 1997, GenBank accession no. Y10404)

these 2 sequences of the same Norwegian isolate was the deletion of a single thymine at position 399 (Fig. 3) in the sequence reported by Mjaaland et al. (1997). Comparison of the cDNA sequence of the NS gene of the North American ISAV isolate with the Norwegian isolate NS gene revealed 88% identity with the sequence reported by Krossoy et al. (unpubl., GenBank accession no. AJ002475) and 89% identity with the sequence reported by Mjaaland et al. (1997).

The deduced amino acid sequences of the partial cDNA fragments of the North American and Nor-

wegian ISAV isolates also were compared. The North American ISAV exhibited 96.6% similarity with the Sotra 92/93 ISAV isolate from Norway in the deduced amino acid sequences of the PB1 protein encoded by the partial cDNA of genome segment 2 (Fig. 4). The cDNA sequence of genome segment 8 contains 2 putative open reading frames (ORF) (Mjaaland et al. 1997, GenBank accession no. Y10404; Devold et al. unpubl., GenBank accession no. AJ012285). The North American ISAV isolate exhibited only 71.2% (GenBank accession no. AJ012285) and 66.7% (GenBank acces-

Nor:	SMSNDVSLVYVDGPMRVFSQNALHPTLQSVKRSQDFSGKGTGRFIIIDLFMGRMWDIGNKHELEDENLDE	20	40	60	71
NA:	SMSNDVSLVYVDGPMRVFSQNALHPTLQSVKRSQDFSGKGTGRFIIIDLFMGRMWDIGNKHELEDENLDE				71
Nor:	VGVADLGLVKYLINNKYDEAEKTSLRKSEEEAFKSMNEEFVVLNKGKSANDIISDTNAVCKFCVKNWIVA	80	100	120	142
NA:	VGVADLGLVKYLINNKYDEAEKTSLRKSEEEAFKSMNEEFVVLNKGKSANDIISDTNAVCKFCVKNWIVA				142
Nor:	IGFRGRTHSDLIHHFRCHQGGQEVKGYIWKHKYNERLKRKQLSKKEEVKFDREYTSRSFRLLSFLKNSER	160	180	200	213
NA:	IGFRGRTHSDLIHHFRCHQGGQEVKGYIWKHKYNERLKRKQLSKKEEVKFDREYTSRSFRLLSFLKNSER				213
Nor:	EKLEPRAVFTAGVPWRAFIIVLEQTHLVNKLDPNSVIMHGSQAKNTTNSRKEIGMKNGQGTIVTLTG	220	240	260	284
NA:	EKLEPRAVFTAGVPWRAFIIVLEQTHLVNKLDPNSVIMHGSQAKNTTNSRKEIGMKNGQGTIVTLTG				284
Nor:	NSKYNESMCPEVNNIFLRELGIKGPWLEVLDAVHQSQSKSVKVPVPIKKTSTTVVVKADARECRDAF	300	320	340	355
NA:	NSKYNESMCPEVNNIFLRELGIKGPWLEVLDAVHQSQSKSVKVPVPIKKTSTTVVVKADARECRDAF				355
Nor:	DEKELEDLQGVNMDDEFFVRRGHLME	360	380		383
NA:	DEKELEDLQGVNMDDEFFVRRGHLME				383

Fig. 4. Comparison of partial deduced amino acid sequence of PB1 protein of North American isolate of ISAV (NA) with Norwegian ISAV strain Sotra 92/93 (Nor: sequence from Krossoy et al. unpubl., GenBank accession no. AJ002475)

Fig. 5. Comparison of partial deduced amino acid sequence of NS protein encoded in putative ORF 1 of North American isolate of ISAV (NA-NS-P1) with 2 reported sequences of the Norwegian ISAV strain Glesvaer/2/90 (Nor-NS-AP1: sequence from Devold et al. unpubl., GenBank accession no. AJ012285; Nor-NS-BP1: sequence from Mjaaland et al. 1997, GenBank accession no. Y10404)

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      *           20           *           40           *           60
NA-NS-P1: HERITMDTKTST MHERSKPNTTGA TCTCLEB EKETRGGI RRGSSSD TGGRE RTORG SCGRFKKOT : 66
Nor-NS-AP1: ----- MHERSKPNTTGV DCTCTES EEAASSL RRGSAFYWRGR RIVCR PGSRFEEGE : 54
Nor-NS-BP1: HERITMDTKTST MHERSKPNTTGV DCTCTES EEAASSL RRGSAFYWRGR RIVCR PGSRFEEGE : 66

      *           80           *           100          *           120          *
NA-NS-P1: GQNLPIGERNDQSSDDP LLLCDERSTIGKHGN AERPHQZ LAEGG IAMPGRGWRRGKRGHGVYD : 132
Nor-NS-AP1: GQNLPIGERNDQSSDDP LLLCDERSTVWKHG TDPEGSH CKLAEGRVMPRR GGWRRGKRGHGVYD : 120
Nor-NS-BP1: GQNLPIGERNDQSSDDP LLLCDERSTVWKHG TDPEGSH CKLAEGRVMPRR GGWRRGKRGHGVYD : 132

      140
NA-NS-P1: FTWHRGEGDDAE : 144
Nor-NS-AP1: FTWHRGGGDDVE : 132
Nor-NS-BP1: SPQWPEEKTMLK : 144

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Fig. 6. Comparison of partial deduced amino acid sequence of NS protein encoded in putative ORF 2 of North American isolate of ISAV (NA-NS-P2) with 2 reported sequences of the Norwegian ISAV strain Glesvaer/2/90 (Nor-NS-AP2: sequence from Devold et al. unpubl., GenBank accession no. AJ012285; Nor-NS-BP2: sequence from Mjaaland et al. 1997, GenBank accession no. Y10404)

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      *           20           *           40           *           60
NA-NS-P2: MNESQNIQKHL ECHREANFKPRELIRHAL EKKRPEVVYAMGVLLTLGGESGLT TFPVFEECTK : 66
Nor-NS-AP2: ----- ECHREANFKPRELIRHAL EKKRPEVVYAMGVLLTLGGESGLT TFPVFEECTK : 55
Nor-NS-BP2: MNESQNIQKHL ECHREANFKPRELIRHAL EKKRPEVVYAMGVLLTLGGESGLT TFPVFEECTK : 66

      *           80           *           100          *           120          *
NA-NS-P2: VKTLNQLVNGMISRATMTLYCVMKDP PGGSMATLMRDHIRNWLKEESGCQADGGEEKWAVVYGMT : 132
Nor-NS-AP2: VKTLNQLVNGMISRATMTLYCVMKDP PGGSMATLMRDHIRNWLKEESGCQADGGEEKWAVVYGMT : 121
Nor-NS-BP2: VKTLNQLVNGMISRATMTLYCVMKDP PGGSMATLMRDHIRNWLKEESGCQADGGEEKWAVVYGMT : 132

      140
NA-NS-P2: SPD----MAE EKTMLK : 148
Nor-NS-AP2: SPD----MAE EKTMLK : 133
Nor-NS-BP2: HQTCTRRRRRCPEEGL : 148

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sion no. Y10404) similarity with the Norwegian isolate in the deduced amino acid sequence of the protein encoded in of the partial cDNA fragment of ORF 1 of genome segment 8 (Fig. 5). However, the North American ISAV isolate exhibited 96.2% similarity in the deduced amino acid sequences of the protein encoded in the partial cDNA of ORF 2 reported by Devold et al. (unpubl., GenBank accession no. AJ012285) and 87.2% similarity with the sequence reported by Mjaaland et al. (1997, GenBank accession no. Y10404) (Fig. 6).

Discussion. The results of this investigation confirm that the virus isolated from cultured Atlantic salmon in the Bay of Fundy, New Brunswick, Canada, which exhibit a new disease syndrome, is similar to ISAV, an orthomyxo-like virus previously reported only in Norway. The North American ISAV isolate exhibited 82.9% identity with the Sotra 92/93 ISAV isolate from Norway in the polymerase protein PB1 gene encoded in genome segment 2. Similarly, the North American ISAV isolate exhibited 88 and 89% identity in the partial nucleotide sequence of the NS gene (segment 8) with the 2 published sequences of the Glesvaer/2/90 ISAV isolate from Norway. The major difference between the 2 reported partial sequences of the NS gene of the same Norwegian isolate was the deletion of a single thymine at position 399 (Fig. 3) in the sequence reported by Mjaaland et al. (1997). The absence of this

thymine deletion in the sequence of the North American ISAV isolate reported here suggests that this deletion is likely an error in the sequence reported by Mjaaland et al. (1997).

The deduced amino acid sequences of the partial PB1 protein of the North American ISAV and the Sotra 92/93 Norwegian isolate were 96.6% similar. In addition, there was a 96.2% similarity between the partial NS protein encoded in ORF 2 of the North American ISAV and the sequence reported for the Norwegian Glesvaer/2/90 isolate by Devold et al. (unpubl., GenBank accession no. AJ012285). There was significantly lower similarity (71.2%) between the deduced amino acid sequences of the partial NS protein encoded in ORF 1 of genome segment 8 of the North American ISAV and the Norwegian ISAV described by Devold et al. (unpubl., GenBank accession no. AJ012285).

Comparison of the deduced amino acid sequences of the PB1 protein of both ISAV isolates with PB1 of a variety of other orthomyxoviruses (data not shown) failed to reveal any regions of significant similarity and revealed an overall similarity of only approximately 16% with other orthomyxoviruses. No similarity was found between the NS proteins of ISAV and NS proteins of other orthomyxoviruses. Therefore, ISAV apparently is not closely related to other orthomyxoviruses in genomic sequences. More extensive studies

of the genomic sequences of ISAV are required to permit a definitive determination of the phylogenetic relationships (e.g. phylogenetic tree) between ISAV and other orthomyxoviruses.

Based on virion morphology and the number and size of genome segments, ISAV appears to resemble members of the *Orthomyxoviridae*. The nucleotide and deduced amino acid sequence comparisons of partial cDNA RT-PCR amplification products from 2 of the 8 genome segments of the North American virus isolate with the published sequences of the Norwegian isolates of ISAV confirm that the North American virus clearly is ISAV. However, the genomic sequence differences between the North American and Norwegian ISAV isolates revealed in this investigation suggest the possibility that the North American ISAV isolate may represent a distinct genomic variant, and the sudden appearance of ISAV in North America may not be the result of a recent importation of infected fish from Norway or a recent introduction of a Norwegian strain by naturally infected wild populations. However, little is known about possible reservoirs of ISAV in natural fish populations in Norway or in North America. Furthermore, these are the only isolates of ISAV for which genomic sequence information is available. Sequences of these and other genome segments should be determined for a variety of ISAV isolates both in Norway and in North America to determine the level of genomic variation among ISAV isolates in a given geographic area.

Nucleotide sequence accession number. The GenBank accession no. for the partial nucleotide sequence of the polymerase gene PB1 of the North American

isolate of ISAV described in this study is AF095254. The GenBank accession no. for the partial nucleotide sequence of the NS gene of the North American isolate of ISAV described in this study is AF095255.

Acknowledgements. This study was supported in part by the Maine Agricultural and Forestry Experiment Station (MAFES). MAFES publication 2298.

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Editorial responsibility: Jo-Ann Leong, Corvallis, Oregon, USA

*Submitted: July 21, 1998; Accepted: December 2, 1998
Proofs received from author(s): January 21, 1999*

Erratum

Isolation of infectious salmon anemia virus (ISAV) from Atlantic salmon in New Brunswick, Canada

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Diseases of Aquatic Organisms 35:131–137, 1999

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Diseases of Aquatic Organisms 35:139–144, 1999

In both articles, an incorrect primer sequence appeared. The error does not change any results or conclusions.

- Page 132, under 'Materials and Methods; RT-PCR', lines 6–7, the sentence should begin: 'The downstream primer (5'TAGGGGCATACATCTGCATC3') was designed...'. That is, the correct primer sequence is TAGGGGCATACATCTGCATC, and not the sequence published.
- Page 140, in Table 1, the downstream primer for NS gene should likewise be 5'TAGGGGCATACATCTGCATC3' and not the sequence published.