Vol. 44: 1–6, 2001 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published January 26

Phylogenetic analysis of infectious salmon anaemia virus isolates from Norway, Canada and Scotland

B. Krossøy[1,4,*], F. Nilsen[2], K. Falk[3], C. Endresen[1], A. Nylund[1]

[1]Department of Fisheries and Marine Biology, University of Bergen, 5020 Bergen, Norway
[2]Department of Aquaculture, Institute of Marine Research, PO Box 1870 Nordnes, 5024 Bergen, Norway
[3]National Veterinary Institute, PO Box 8156 Dept, 0033 Oslo, Norway
[4]Intervet Norbio, Thormohlensgt. 58, 5008 Bergen, Norway

ABSTRACT: The sequences of gene segments 2 and 8 from 10 different isolates of infectious salmon anaemia virus (ISAV) sampled in Norway, Canada and Scotland between 1987 and 1999 were determined and compared. Pairwise comparisons revealed a high degree of homology between the European isolates, with identities of 98 to 100% for both genes examined. The Canadian isolate showed identities of 84 and 87 to 88% with the European isolates for the nucleotide sequence of segments 2 and 8, respectively. Phylogenetic analyses were performed to establish the interrelationship between the European virus isolates. The evolutionary rate based on 4 Norwegian isolates clustered together in the analysis of segment 2 was calculated to be 0.96×10^{-3} nucleotides site^{-1} yr^{-1}. On the basis of this mutation rate it was estimated that the Norwegian Glesvaer 90 and Canadian Bay of Fundy 97 isolates diverged around 1900, which coincides with transportation of salmonids between Europe and North America starting in the late nineteenth century.

KEY WORDS: ISAV · Virus isolates · Phylogenetic analysis · Mutation rate

INTRODUCTION

Infectious salmon anaemia (ISA) is a severe disease affecting farmed Atlantic salmon with typical pathological changes being characterized by anaemia, leukopenia, ascites, haemorrhagic liver necrosis and petechia of the viscera (Thorud & Djupvik 1988, Evensen et al. 1991, Thorud 1991). The disease was first reported in a hatchery on the southwestern coast of Norway in 1984 and has now been diagnosed in salmon farms in all the counties along the Norwegian coast from Rogaland to Finnmark. Until 1997 there was no record of ISA outside Norway, but the disease has now been detected in Canada and Scotland as well (Mullins et al. 1998, Rodger et al. 1998). Infectious salmon anaemia virus (ISAV), the causative agent of ISA, has been suggested to represent a new genus in the family *Orthomyxoviridae* (Krossøy et al. 1999). The genome consists of single-stranded RNA with 8 segments of negative polarity (Mjaaland et al. 1997).

When this study was performed, the sequences of 2 segments were published. The cDNA sequence of segment 8 is reported to be 930 nucleotides long and to contain 2 putative open reading frames (Mjaaland et al. 1997). The function of these 2 putative proteins is unknown. The cDNA sequence of segment 2 is reported to be 2245 nucleotides long, encoding a putative 708 amino acid protein predicted to be the RNA-dependent RNA polymerase and corresponding to the PB1 of the influenza viruses (Krossøy et al. 1999). Recently, the sequence of the predicted segment 7 was also published (Rimstad, Mjaaland & Sandvik unpubl. Genebank accession no. AF 220607).

Differences between North American and European ISAV isolates have been reported in 2 recent publications (Blake et al. 1999, Kibenge et al. 2000). Minor variation between a Scottish isolate and a Norwegian

*E-mail: bjorn.krossoy@ifm.uib.no

isolate, demonstrating that these isolates are not identical, has also been shown (Cunningham & Snow 2000). However, as pointed out by these authors, the significance of the nucleotide variation between the Norwegian and Scottish isolates cannot be determined until the degree of variation among Norwegian isolates sampled from different sites and different years has been determined.

This study was initiated to clarify the relationship between Norwegian isolates that are geographically and temporally separated, and compare these sequences with isolates from North America and Scotland.

MATERIALS AND METHODS

RT-PCR. Viral RNA was isolated from infected fish or from infected cell culture after 1 or 2 passages using the Trizol reagent according to standard protocols (Gibco). The virus isolates shown in Table 1 were grown in Atlantic salmon kidney cells (ASK) (Devold et al. 2000) using the method described by Dannevig et al. (1995). Reverse transcription (RT) was performed using Moloney murine leukemia virus reverse transcriptase according to standard protocols with random hexamers as primers (Promega). Subsequently, the single stranded cDNA served as template in PCR using Taq DNA polymerase according to recommended conditions (Pharmacia) and primers given in Table 2. Segment 8 from all isolates was amplified with the primer pair FA4/RA4 generating a 776 bp fragment spanning positions 66 to 840 in the full length sequence of Mjaaland et al. (1997). Segment 2 was amplified using primer pairs PB1-1F/PB1-5R and PB1-6F/PB1-9R giving 2 overlapping fragments spanning positions 74 to 2198 in the full length sequence of Krossøy et al. (1999). The Canadian sequence of segment 2 was amplified using 4 partly overlapping fragments using primer pairs PB1-1F/PB1-2R, PB1-4F/PB1-5R, PB1-6F/PB1-7R and PB1-9F/PB1-9R. The PCR fragments were then cloned into TOPO TA vectors according to the manufacturer's instructions (Invitrogen).

Sequence analysis. The PCR products were gel purified and sequenced using reagents for automatic sequencing (BigDye Sequencing Kit, Amersham). All sequences were determined on both strands. At least 3 parallel clones were sequenced when plasmid vectors were used. The sequences were aligned using CLUSTAL X (Thompson et al. 1994), and the multiple sequence alignment editor GeneDoc (developed and distributed by Nicholas & Nicholas 1997) was used to perform pairwise comparisons between the different sequences from the 10 ISA isolates. To perform pairwise codon-by-codon comparison of aligned protein coding sequences the programme Diverge in the University of Wisconsin sequence analysis package (Genetics Computer Group, Madison, Wisconsin) was used. This programme estimates the number of synonymous and nonsynonymous substitutions. Phylogenetic analyses of the data sets were performed using the PAUP* 4.0 version (Swofford 1998). The following analyses were used and compared: quartet puzzling analysis from the maximum likelihood model, the distance method (LogDet/Paralinear) with neighbor-joining tree building, the maximum parsimony method with heuristic search and the maximum likelihood method using heuristic search settings. The first 3 methods used 10 000 puzzling steps or bootstrap replicates. Trees were drawn using TreeView (Page 1996). The rate of nucleotide substitutions based on all nucleotide mutations in segment 2 for the 4 isolates clustered together in the phylogenetic tree was calculated using the Kimura 2- and 6-parameter test (Gojobori et al. 1990). The estimated average mutation rate (assumed to represent a constant rate of nucleotide substitution) was then used to calculate a divergence date between a Norwegian and Canadian isolate.

Table 1. Accession numbers of ISAV sequence data determined in this study and abbreviations of the virus isolates

Country	County	Location	Year of isolation	Abbreviation	Sequence accession no.	
					Segment 2	Segment 8
Norway	Hordaland	Eikelandsosen	1987	Ei 87	AF262394	AF262386
Norway	Hordaland	Glesvaer	1990	Gl 90	AF262398	AF262382
Norway	Troms	Gullesfjord	1994	Gu 94	AF262396	AF262384
Norway	Hordaland	Varaldsøy	1996	Va 96	AF262397	AF262388
Norway	Nordland	Svolvaer	1996	Sv 96	AF262393	AF262381
Norway	Hordaland	Bremnes	1998	Bm 98	AF262391	AF262385
Norway	Sogn og Fjordane	Brekke	1998	Bk 98	AF262390	AF262380
Norway	Sør-Trøndelag	Hitra	1999	Hi 99	AF262395	AF262383
Canada	New Brunswick	Bay of Fundy	1997	BF 97	AF262399	AF262389
Scotland	Highland	Loch Nevis	1998	LN 98	AF262392	AF262387

Table 2. Sequence of the primers used in PCR and sequencing of segment 2 and 8 of ISAV isolates

Primer	Sequence (5′-3′ polarity)	Segment
FA4	GCAAAGATTGGCTATCTACCATGAAC	8
RA4	TCAAGTACACAACCCAAATATCCC	8
PB1-1F	AGCAAAGAACGCTCTTTAATAACCATG	2
PB1-4F	CAACAGGTTTCAGAGGAAGAACCA	2
PB1-6F	CAGGTCTACTGTTGTAGTGAAGGC	2
PB1-9F	GGGAACAGAAAATACCAAGAACTGAAGA	2
PB1-2R	TCGAGGTGTATTCCTCTCTGTCGAA	2
PB1-5R	TCCATGTTCCATCCACTCTACTCC	2
PB1-7R	AACTCTGAGGAAGTTCCCAAGGTTT	2
PB1-9R	TCGTCAATTCCGTTATTACACACA	2

RESULTS

Pairwise comparisons

The results show a high degree of similarity between the 9 European isolates, with identity values between 98 and 100% for both segment 2 and 8 at the nucleotide level (Table 3). The nucleotide sequences of the same 2 segments from the Canadian isolate are 84 and 87 to 88% identical to the European isolates, respectively. The Canadian isolate shows a high degree of similarity with 97 to 98% identities at the amino acid level to the European isolates. In a pairwise comparison between the Norwegian Gl 90 isolate and the North American BF 97 isolate, the ratio between synonymous substitutions per synonymous site and nonsynonymous substitutions per nonsynonymous site was calculated to be 82.5, which shows the conserved nature of this protein. For the 695 amino acids residues of the partial PB1 sequences, amino acid substitutions were observed at 23 positions when compared to the Ei 87 isolate (Table 4). Pairwise comparisons of the 2 deduced amino acid sequences of segment 8 reveal that one of the predicted proteins is more conserved than the other, with the Canadian sequences showing 76 and 95% identities to the European isolates (Table 3b).

Phylogenetic analysis

Phylogenetic analysis based on segment 8 was inconclusive, as the resulting trees were almost totally unresolved (results not presented). However, the phylogenetic trees representing segment 2, although partly unresolved, clustered together 4 isolates sampled from 1990 to 1999 with high bootstrap support (Fig. 1). It also clustered together the 2 isolates, Bm 98 and Bk 98, sampled the same year at different locations. The branch lengths of this tree clearly demonstrate the close relationship between the European isolates as opposed to the single North American strain used in this study.

Table 3. Identity between genome sequences from ISAV isolates. Nucleic acid identities (%) for (a) segment 2 and (b) segment 8 are given in the upper right portion of the table, while amino acid identities (%) are given in the lower left portion of the table. In (b) the 2 amino acid values are for open reading frame (ORF) 1 and ORF 2, respectively. Abbreviations are as given in Table 1

	Ei 87	Gl 90	Gu 94	Sv 96	Va 96	Bm 98	Bk 98	Hi 99	BF 97	LN 98
(a) Segment 2										
Ei 87	–	99	98	99	98	99	99	98	84	99
Gl 90	99	–	98	99	98	99	99	98	84	99
Gu 94	99	99	–	98	99	98	98	99	84	98
Sv 96	99	99	100	–	98	99	99	98	84	99
Va 96	99	99	99	99	–	98	98	99	84	98
Bm 98	99	99	99	99	99	–	99	98	84	99
Bk 98	99	99	99	99	99	100	–	98	84	99
Hi 99	99	99	100	100	99	99	99	–	84	98
BF 97	97	97	98	98	97	98	98	98	–	84
LN 98	99	99	99	99	99	99	99	99	97	–
(b) Segment 8										
Ei 87	–	99	99	99	99	100	99	99	88	99
Gl 90	99/100	–	99	99	99	99	99	99	88	99
Gu 94	99/100	99/100	–	100	99	99	99	99	88	99
Sv 96	99/100	99/100	100/100	–	99	99	99	99	88	99
Va 96	99/99	99/99	99/99	99/99	–	99	99	99	88	99
Bm 98	100/100	99/100	99/100	99/100	99/99	–	99	99	88	99
Bk 98	99/99	98/99	99/99	99/99	98/98	99/99	–	99	88	99
Hi 99	98/100	98/100	99/100	99/100	98/99	98/100	98/99	–	87	99
BF 97	76/95	76/95	76/95	76/95	77/95	76/95	76/95	76/95	–	88
LN 98	99/100	99/100	100/100	100/100	99/99	99/100	99/99	99/100	76/95	–

Table 4. Predicted amino acid differences in the polymerase sequence of different ISAV isolates. Only those residues that differ from that shown for Ei 87 are given. Amino acid positions are as in the full length polymerase sequence of Krossøy et al. (1999)

	16	52	61	78	101	277	315	353	354	359	363	365	368	386	390	496	579	638	646	659	697	702	705
Ei 87	V	Q	F	H	N	T	I	S	R	V	A	N	K	E	I	I	K	T	K	M	D	I	K
Gl 90					D								R						N				
Gu 94													R										
Sv 96													R										
Va 96						A							R								N		
Bk 98		K					V						R										
Bm 98		K					V						R										
Hi 99													R										
BF 97	I			Q			V	G	K	I	S	K	R	D	V	M	R					V	R
LN 98			L										R					I		V			

Substitution rate

A recommended method of estimating the rate of nucleotide substitutions is to examine the evolutionary relationship of genes obtained from different isolates first and then use only isolates that are closely related by descent (Saitou & Nei 1986). Calculations of the mutation rate for the 4 isolates clustered together in the phylogenetic analysis indicated an average substitution rate of 0.96×10^{-3} nucleotides site^{-1} yr^{-1} for both the 2- and 6-parameter test, which is to be expected when the data set contains a small numbers of substitutions (Gojobori et al. 1990). When this mutation rate is used to calculate the divergence date between the Norwegian Gl 90 and Canadian BF 97 isolates, indications are that this divergence might have happened around 1900.

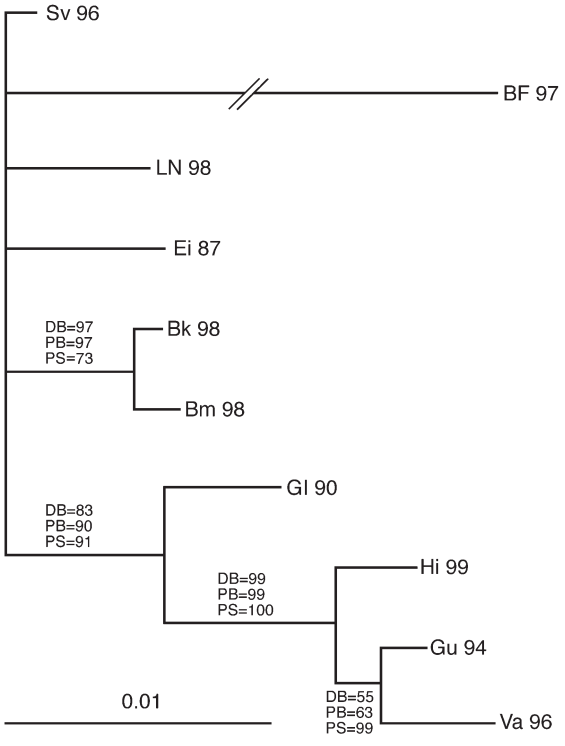

Fig. 1. A 50% majority rule consensus tree of 10 000 puzzling steps or bootstrap replicates performed with PAUP* 4.0 (Swofford 1998). Branch lengths were calculated using maximum likelihood and the Hasegawa-Kishino-Yano (HKY) model of substitution. The true branch length of the BF 97 isolate should be 0.28 according to the scale bar in the lower left corner. Numbers on the tree indicate percentage support for that topology with the different optimality criterions used; DB: distance bootstrapping; PB: parsimony bootstrapping; PS: puzzling steps

DISCUSSION

The pairwise analysis of gene segment 2 and 8 from 8 different Norwegian isolates sampled in different geographical area during a 12 yr period supports previous reports suggesting that the North American isolates are significantly different from the European isolates (Blake et al. 1999, Cunningham & Snow 2000, Kibenge et al. 2000). Among the European isolates, segment 8 appears to be very conserved, showing few nucleotide changes during the 12 yr period studied here. The findings that segment 8 has 88% identity at the nucleotide level, and 71 and 96% identity for the 2 putative proteins encoded by this segment, is in accordance with Blake et al. (1999). The possible functions of these predicted proteins have not been studied. The 2 proteins encoded by segment 8 (NS1 and NS2) in the influenza A virus show the same pattern as observed for the 2 deduced products from segment 8 of ISAV, i.e. the NS2 protein is more conserved than the NS1 protein (Kawaoka et al. 1998, Suarez & Perdue 1998). However, it remains to be shown that the predicted proteins of segment 8 of the ISAV correspond to the NS1 and NS2 proteins of influenza viruses.

The phylogenetic analysis based on the polymerase sequences resulted in trees that clustered together 4 of the Norwegian isolates with relative high bootstrap support. Two of these isolates, Gl 90 and Va 96, were sampled from farms located relatively close to each other, while the Hi 99 and Gu 94 isolates were sampled at more distant locations. The phylogenetic analysis also clustered together the Bk 98 and Bm 98 isolates, which were isolated the same year on locations relatively close together. Whether this can be explained by transportation of fish or equipment is uncertain, as movement of fish at this time was restricted as part of the measures taken to control the disease in Norway (Håstein et al. 1999) and no link between the 2 outbreaks in 1998 has been found. It is not known whether this virus might be spread with migrating wild fish populations or not, but that could be possible.

The phylogenetic relationship between the Scottish LN 98 isolate and the different Norwegian isolates could not be elucidated from the study of these 2 genes. To obtain a better evaluation of the phylogenetic relationship between the different European isolates more variable genes than the 2 included in this study are needed. Genes encoding surface proteins would probably be good candidates to examine in such a study.

Of the total of 23 amino acid substitutions found when comparing all the other sequences to the oldest Norwegian isolate (Ei 87), 15 were found in the Canadian BF 97 isolate. Nine of these 15 substitutions were found clustered between the 2 highly conserved polymerase motifs A and B found in most viral RNA-dependent RNA polymerases (Poch et al. 1989). A similar clustering of amino acid substitutions has also been observed in a comparison of PB1 protein sequences from influenza A viruses isolated from different hosts (Kawaoka et al. 1989), demonstrating the functional constraints in the polymerase protein.

The mutation rate for the polymerase gene was calculated to be 0.96×10^{-3} nucleotides site^{-1} yr^{-1}, which is close to the mutation rate of 0.87×10^{-3} nucleotides site^{-1} yr^{-1} reported for the PB1 gene from human influenza A virus (Kawaoka et al. 1989, Webster et al. 1992). The finding that most substitutions are synonymous is in accordance with the findings of Saitou & Nei (1986). They reported that the rate of nucleotide substitution in influenza A virus genes is much lower at the first and second positions than at the third position of codons, except for 1 part of the H3 hemagglutinin in which all 3 nucleotide positions had essentially the same substitution rate. The latter observation was explained by little purifying selection on this gene and by the fact that the substitution rate therefore occurs at the same rate as does the mutation rate. Based on the calculated substitution rate, the divergence date between the Norwegian Gl 90 and North American BF97 isolates was calculated to be around 1900. One should, however, be cautious about this dating since evolutionary rates are not always linear with time (Scholtissek et al. 1993). In addition, the time-scale of virus isolation and number of isolates included will probably affect estimated evolutionary rates, thus influencing the estimated divergence date. With this in mind, it is nevertheless an interesting point that an extensive transportation of eggs from salmonid fish was carried out between Europe and North America in the late nineteenth century (Welcomme 1988). One of the species introduced to Europe from North America was rainbow trout *Oncorhynchus mykiss*, probably originating from the McCloud River in California (Benkhe 1992, Gall & Crandell 1992). This species was first introduced to Europe in 1879, when eggs were imported to France (Welcomme 1988). In 1882 rainbow trout was also imported to Denmark (Rasmussen 1967), from which location eggs were transported to Norway in 1902 (MacCrimmon 1971). It is known that ISAV can replicate in rainbow trout without causing disease (Nylund et al. 1997). The virus has also been found in sex products of Atlantic salmon (Melville & Griffiths 1999) and can probably be found in sex products of other salmonids which are host to this virus. It is therefore possible that the virus could have been transferred with eggs that were not disinfected. However, it is also possible that the ISAV may have been transported from Europe to North America with sea trout *Salmo trutta* that were introduced to North America from Europe in 1883–1884 (Corteney et al. 1984, Welcomme 1988). At present it is therefore not possible to suggest a geographical origin of the ISA virus.

Acknowledgements. We thank Ingvild Samdal and Jegathes Thevarajan for technical assistance and Dr Rebekka Cox Brokstad for improving the English of the manuscript. Financial support for this work was provided by grant 107131/122 from the Norwegian Research Council and from Hydro Seafood.

LITERATURE CITED

Benkhe RJ (1992) Native trout of western North America. American Fisheries Society Monograph No. 6, Bethesda, MD

Blake S, Bouchard D, Keleher W, Opitz M, Nicholson BL (1999) Genomic relationships of the North American isolate of infectious salmon anemia virus (ISAV) to the Norwegian strain of ISAV. Dis Aquat Org 35:139–144

Courteney WR Jr, Hensley DA, Taylor JN, McCann JA (1984) Distribution of exotic fishes in the continental United States. In: Courteney WR Jr, Stauffer JR Jr (eds) Distribution, biology and management of exotic fishes. Johns Hopkins University Press, Baltimore, p 41–77

Cunningham CO, Snow M (2000) Genetic analysis of infectious salmon anaemia virus (ISAV) from Scotland. Dis Aquat Org 41:1–8

Dannevig BH, Falk K, Namork E (1995) Isolation of the causal virus of infectious salmon anemia (ISA) in a long-term cell line from Atlantic salmon head kidney. J Gen Virol 76: 1353–1359

Devold M, Krossøy B, Aspehaug V, Nylund A (2000) Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout Salmo trutta after experimental infection. Dis Aquat Org 40:9–18

Evensen O, Thorud KE, Olsen YA (1991) A morphological study of the gross and light microscopic lesions of infectious anaemia in Atlantic salmon (*Salmo salar*). Res Vet Sci 51:215–222

Gall GAE, Crandell PA (1992) The rainbow trout. Aquaculture 100:1–10

Gojobori T, Moriyama EN, Kimura M (1990) Statistical methods for estimating sequence divergence. Methods Enzymol 183:531–550

Håstein T, Hill BJ, Winton JR (1999) Successful aquatic animal disease emergency programmes. Rev Sci Tech OIE (Off Int Epizoot) 18:214–227

Kawaoka Y, Krauss S, Webster RG (1989) Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J Virol 63:4603–4608

Kawaoka Y, Gorman OT, Ito T, Wells K, Donis RO, Castrucci MR, Donatelli I, Webster RG (1998) Influence of host species on the evolution of the nonstructural (NS) gene of influenza A viruses. Virus Res 55:143–156

Kibenge FSB, Lyaku JR, Rainnie D, Hammell KL (2000) Growth of infectious salmon anaemia virus in CHSE-214 cells and evidence for phenotypic differences between virus strains. J Gen Virol 81:143–150

Krossøy B, Hordvik I, Nilsen F, Nylund A, Endresen C (1999) The putative polymerase sequence of infectious salmon anemia virus suggests a new genus within the Orthomyxoviridae. J Virol 73:2136–2142

MacCrimmon HR (1971) World distribution of rainbow trout. J Fish Res Board Can 28:663–704

Melville KJ, Griffiths SG (1999) Absence of vertical transmission of infectious salmon anemia virus (ISAV) from individually infected Atlantic salmon *Salmo salar*. Dis Aquat Org 38:231–234

Mjaaland S, Rimstad E, Falk K, Dannevig BH (1997) Genomic characterization of the virus causing infectious salmon anemia in Atlantic salmon (*Salmo salar* L.): an orthomyxo-like virus in a teleost. J Virol 71:7681–7686

Mullins JE, Groman D, Wadowska D (1998) Infectious salmon anaemia in salt water Atlantic Salmon (*Salmo salar* L.) in New Brunswick, Canada. Bull Eur Assoc Fish Pathol 18: 110–114

Nylund A, Kvenseth AM, Krossøy B, Hodneland K (1997) Replication of the infectious salmon anaemia virus (ISAV) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 20:275–279

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Poch O, Sauvaget I, Delarue M, Tordo N (1989) Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. EMBO J 8:3867–3874

Rasmussen CJ (1967) Håndbog i ørretopdræt. Rhodos International Science Publishers Copenhagen (in Danish)

Rodger HD, Turnbull T, Muir F, Millar S, Richards RH (1998) Infectious salmon anaemia (ISA) in the United Kingdom. Bull Eur Assoc Fish Pathol 18:115–116

Saitou N, Nei M (1986) Polymorphism and evolution of influenza A virus genes. Mol Biol Evol 3:57–74

Scholtissek C, Ludwig S, Fitch WM (1993) Analysis of influenza A virus nucleoproteins for the assessment of molecular genetic mechanisms leading to new phylogenetic virus lineages. Arch Virol 131:237–250

Suarez DL, Perdue ML (1998) Multiple alignment comparison of the non-structural genes of influenza A viruses. Virus Res 54:59–69

Swofford DL (1998) Phylogenetic analysis using parsimony and other methods, version 4.0, Sinauer Associates, Sunderland, Massachusetts

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Thorud K (1991) Infectious salmon anaemia. Transmission trials. Haematological, clinical chemical and morphological investigations. Thesis, Norwegian College of Veterinary Medicine, Oslo

Thorud K, Djupvik HO (1988) Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.). Bull Eur Assoc Fish Pathol 8:109–111

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (1992) Evolution and ecology of influenza A viruses. Microbiol Rev 56:152–179

Welcomme RL (1988) International introductions of inland aquatic species. FAO Fish Tech Pap No. 294

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

Submitted: August 23, 2000; Accepted: November 3, 2000
Proofs received from author(s): December 29, 2000