

First Steps of ISAV Recovery from cDNA by Reverse Genetics
Stéphane Biacchesi, INRA, Jouy-en-Josas, France

EADGENE European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

First Steps of ISAV Recovery from cDNA by Reverse Genetics
Stéphane Biacchesi (INRA, Jouy-en-Josas, FRANCE)

This publication represents the views of the Authors, not the EC. The EC is not liable for any use that may be made of the information.

INRA

EADGENE

Infectious Salmon Anaemia Virus : ISAV

- **ISA disease first described in salmon in Norway in 1984.**
→ Listed by the EU as a major threat for salmon.
→ High economic loss with mortality rate up to 90%
→ North Europe, North America and Chile
- **Aetiology recently characterized in 1997 : ISAV.**
→ Falk et al., 1997. JVI.
- **Negative-sense segmented RNA genome = 8 segments.**
→ Family : Orthomyxoviridae
→ Genus : Isavirus

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

INRA

EADGENE

Virus Structure

Structural Proteins

- Envelope Proteins**
 - HE Hemagglutinin Esterase
 - F Fusion
 - M2 Ionic Canal
- Inner Envelope Face**
 - M1 Assembly
 - NS2 or NEP Nuclear Export Protein
- RiboNucleoProtein Complex**
 - NP Nucleoprotein
 - PB1 } Polymerase Complex
 - PB2 }
 - PA }
 - RNA 8 Genome Segments
- Non Structural Protein**
 - NS1 Interferon antagonist

EM by Lepault 50 nm

RNP

HE F M2 KNP

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

INRA

EADGENE

(I) Rainbow Trout Versus ISAV

ISA observed only in Salmon.
→ Adult trout are sensitive but no mortality

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

INRA

EADGENE

Juvenile Rainbow Trout is Susceptible to ISAV Through Different Routes of Infection

ISAV Titers in Waterborne Infected Rainbow Trout

Samples	Days	2, 4 and 7	9	14	18	
Serum	-	-	+ (IFA)	> 10 ⁶ PFU/ml	> 10 ⁶ PFU/ml	
Spleen + Head Kidney	-	-	-	> 5x10 ⁶ PFU/g	ND	
Intestine	-	-	-	> 5x10 ⁶ PFU/g	ND	

IFA, Immunofluorescence assay, ND, Not done.

Biacchesi et al. JFD 2007

INRA

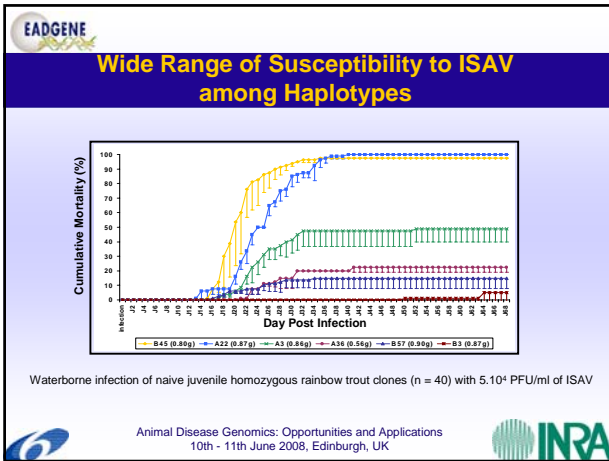
EADGENE

Pathological Signs of ISA in Trout

(A) Pathoclinical signs of ISA in experimentally infected fish (clone A22, 6.2g) by waterborne route.
(B) Exophthalmia in the diseased fish.

INRA

First Steps of ISAV Recovery from cDNA by Reverse Genetics
Stéphane Biacchesi, INRA, Jouy-en-Josas, France



Summary and Perspectives

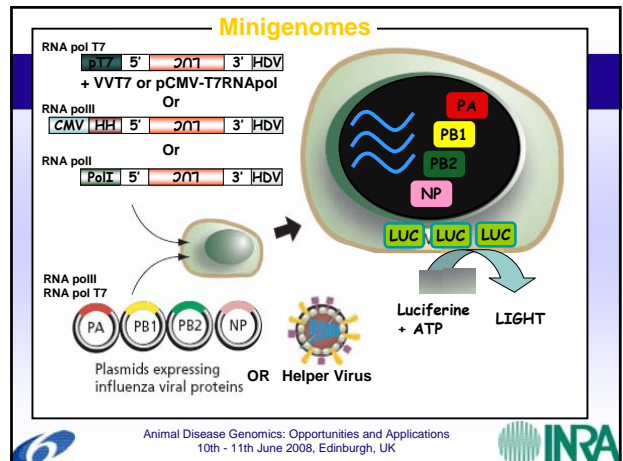
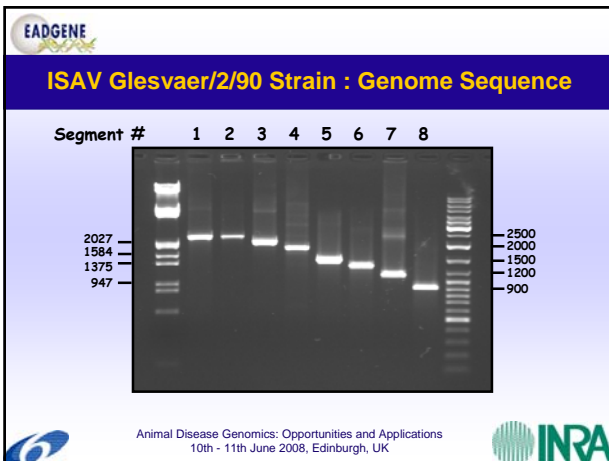
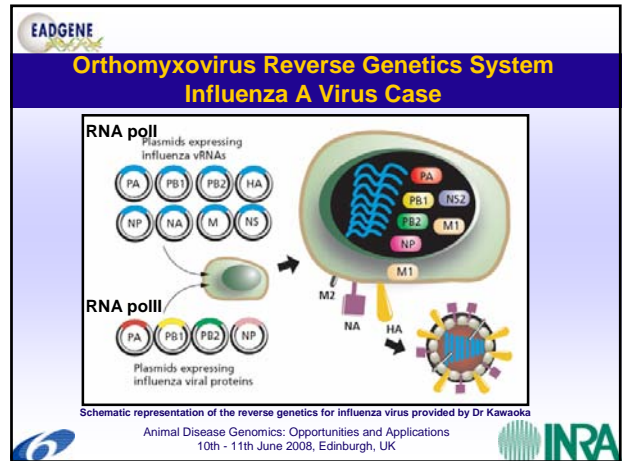
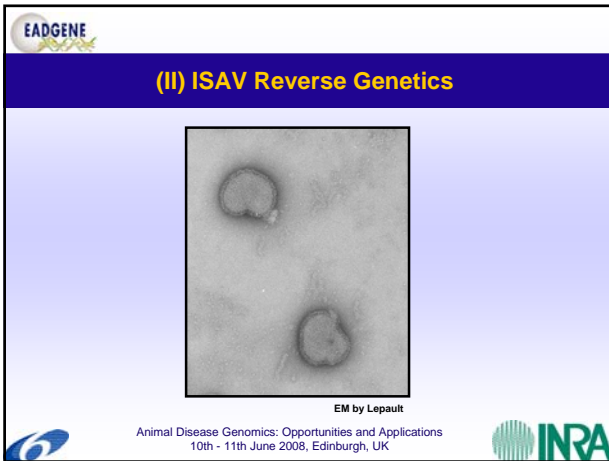
- Juvenile Rainbow trout are sensitive to ISAV waterborne infection.
 - Mortalities: - 80 to 100% by injection
 - up to 30% by waterborne infection
 - ISAV may represent a threat for trout aquaculture
- Wide range of genetic susceptibility to ISAV among haplotypes.
 - Mortalities from 0 to 100% by waterborne infection
 - Trout is a valuable model for research on ISAV

→ RNA samples have been prepared from different organs of infected animals.

→ Hybridization with rainbow trout membranes to identify ISAV-induced genes. (Agenae Mbs first, then possibly with other membranes for comparison)

→ Induction of selected genes/pathways will be investigated in details.

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK



First Steps of ISAV Recovery from cDNA by Reverse Genetics
Stéphane Biacchesi, INRA, Jouy-en-Josas, France

Salmon RNA Poll Promoter mapping

IGS intergenic region
ETS External transcribed spacer
ITS Internal transcribed spacer
18, 5.8 and 28S rRNAs
T1-8 Terminators
P Promoter
UCE Upstream control element

Starting Materials:

- ◆ Lake Trout Genomic Bank from Kent Reed
- ◆ Some sequence of this region and a salmon 18S cDNA fragment

Results:

- ◆ IGS fragment of 6 kb from Lake Trout
- ◆ Fragment of 7.6 kb (IGS (3.5 kb), 18S, 5.8S and 28S) from Salmon
→ Cloning and Transcription assay
→ Determination of the transcription start (+1)

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

Summary

- The genome of ISAV has been entirely sequenced
- All segments and individual genes were cloned and sequenced
- Several minigenomes have been constructed.
- The Pol I promoter is under characterization

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

INRA Participants

Molecular Virology and Immunology:

- Abdenour Benmansour
- Stéphane Biacchesi
- Michel Brémont
- Pierre Boudinot
- Annie Lamoureux
- Monique Le Berre

Fish Facilities:

- Eric Letellier
- Didier Marie
- Dimitri Rigaudeau

Fish genetics:

- Sandrine Le Guillou
- Edwige Quillet

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

Virus Cycle

From Lamb et al. 1994

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK

ISAV Glesvaer/2/90 Strain : vRNA + Proteins

ISAV Glesvaer/2/90					Influenza A/PR/8/34				
Segments	Gene Products				Segments	Gene Products			
#	Size (nt)	Proteins	Length (aa)	Molecular Weight (kDa)	#	Size (nt)	Proteins	Length (aa)	Molecular Weight (kDa)
1	2267	PB2	722	80	1	2341	PB2	759	86
2	2249	PB1	708	81	2	2341	PB1 PB1-F2	757 87	87 11
3	2046	NP	616	68	3	2233	PA	716	84
4	1826	PA	578	65	4	1778	HA	566	61
5	1485	F	444	49	5	1565	NP	498	56
6	1321	HE	391	23 (~43 Glyco)	6	1413	NA	454	50
7	1127	NS1 NEP1 (Unspliced mRNA) NEP2 (Spliced mRNA)	300 173 159	34 19 18	7	1027	M1 M2	252 97	28 11
8	906	M1 M2 (Unspliced mRNA)	241 196	27 22	8	890	NS1 NS2 (or NEP)	230 121	27 14
13227 nt					13588 nt				

Animal Disease Genomics: Opportunities and Applications
10th - 11th June 2008, Edinburgh, UK