

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

Genomics for Animal Health: Outlook for the Future
13- 14th October 2009, Muséum National d'Histoire Naturelle, Paris, France

Fish pathogen research

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Fish pathogen research

- This project focus on salmonids (Atlantic salmon and rainbow trout) and on the viruses:
- Infectious Salmon Anaemia virus (ISAV), Infectious Pancreatic Necrosis Virus (IPNV) and Salmonid Alphavirus (SAV).
- The partners involved have been: INRA, RIBFA, FLI, DJF, ROSLIN, NSVS.

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Viral diversity

- In the ocean it is ~ 3×10^9 viruses/litre
- The volume of oceans of the world is ~ $1,3 \times 10^{21}$ litres
- ~ 4×10^{30} viruses in the ocean
- Assuming an average virus to be approx 100 nm big –and if the viruses in the ocean were stretched end to end they would span ~ 10 mill light years or 100 times the distance across our own galaxy

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EADGENE Virologie Moléculaire des poissons U892 VIM INRA

Infectious salmon anemia virus

NP, F, HE, MI

ISAV

Microscopy

Panel of mAbs

Minigenomes

RNA pol T7 + VVT7 or pCMV-T7RNAPol
RNA polII Or
RNA polI Or
RNA pol T7

Plasmids expressing Influenza viral proteins OR Helper Virus

Luciferase + ATP LIGHT

Full genome sequencing for Ant
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Infectious salmon anemia virus

Short communication

Fish genotype significantly influences susceptibility of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), to waterborne infection with infectious salmon anaemia virus

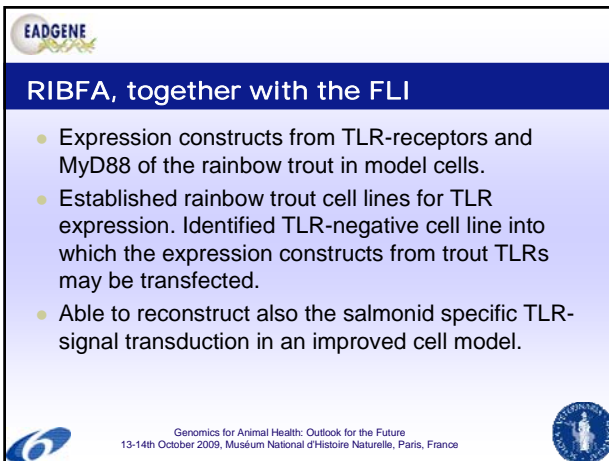
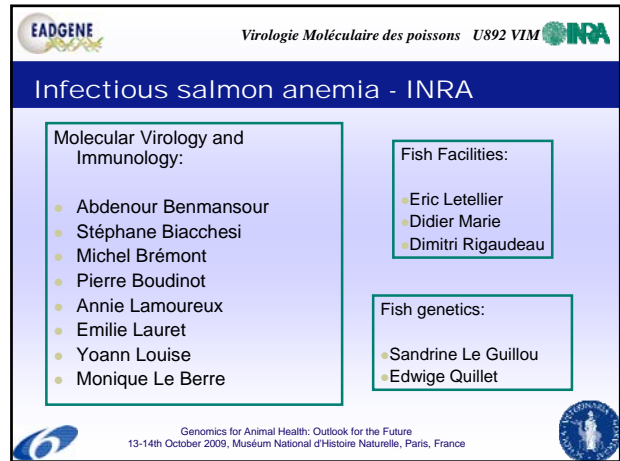
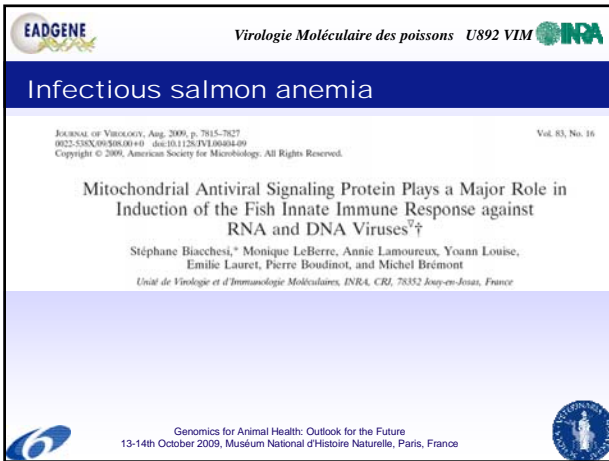
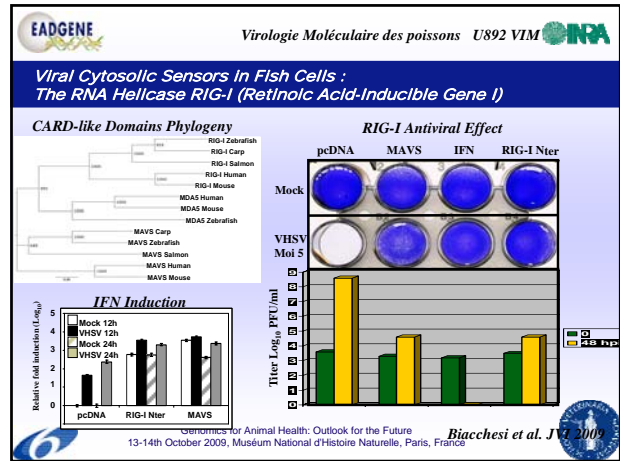
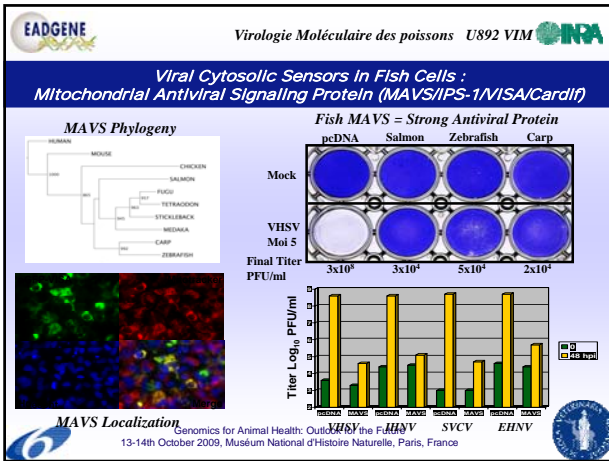
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Control

Infected

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People involved at RIBFA and FLI

Research Institute for the Biology of Farm Animals RIBFA :

- Alexander Rebl
- Tom Goldammera
- Hans-Martin Seyfert

Friedrich-Loeffler-Institute (FLI):

- Uwe Fischer
- Bernd Köllner

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Analysis of the transcriptome profile of IPNV vaccinated and challenged fish using microarray

- 3 different vaccine groups (1 reference pool; normal fish)
- 12 individuals in each vaccine group for each time point
- Made pools of 3 individuals in each pool (P1-P4) for each time point
- 3 time-points analyzed
 - weeks 10, 14 and 20
 - 2 time points, Week 10 and Week 14 (from the non-vaccinated group)

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Microarray data analysis, IPNV vaccines

- CA768207 BAF (9)
 - BAF complexes are mammalian chromatin-remodelling complexes
- CB506151 CLECT (3)
 - C-type lectins – involved in inflammation
- CB511048 CLECT DC-SIGN (10)
 - DC-SIGN possibly play a role for hepatitis C for entry into DC
 - Dendritic-cell specific intercellular adhesion molecule-3 grabbing non-integrin – involved in inflammation
 - Receptors for dengue virus (in DCs)
 - DC-SIGN also binds to ICAM3 of T cells
- CA064578 CATHEPSIN D (7)
- CD152334 CATHEPSIN K (7)
- CA051900 CATHEPSIN L1 (7)
- CA053623 CATHEPSIN S (10)
 - Cathepsins are proteolytic enzymes involved in lysosome protein breakdown and protein processing
 - Cathepsin S in macrophages only
- EG860270 CD81 ANTIGEN (9)
 - Entry of hepatitis C into cells
- CA054828 Ig mu chain C region membrane-bound form (10)
 - Immunoglobulin – adaptive responses
- CA048716 Integrin beta-1 (10)
 - Involved in cell attachment and also signaling between cells
- CA058281 Macrophage mannose receptor 1 (3)
 - Scavenger
- CA037526 Protein chibby homolog 1(6)
 - Transcription factor

These genes were differentially regulated in the kidney in the group indicated over a time period of 4 weeks (i.e. week 14 was compared to week 10) and would thus be an indication of a response in a group of fish with 40% survival rate (72% control mortality)

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People involved at NSVS in IPNV work

- Inderjit Mercy
- Øystein Evensen

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Transcriptional analysis of Immune-related genes Atlantic salmon experimentally challenged with salmonid alphavirus (SAV3)

- Salmon 35 g size. T 10-13 °C
- Two breeds of salmon were used. One with high resistance towards ISA, and one with low resistance against ISA
- ISAV, like SAV is a ssRNA virus. Indicating that some parts of the early innate immune responses could be related.
- Cohabitant challenged fish.
- Simulates a natural infection route but exact time of infection can only be estimated.

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SAV challenge

Fish breed	Serum		Heart tissue	
	3 wpc	4 wpc	4 wpc	8 wpc
Control/breed	75	87	93	
ISA-High	65	67	87	
ISA-Low	75	100	93	

Prevalences of SAV-positives

Histopathology score: No mortality

Photo: T. Poppe

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SAV challenge


Real-time qPCR (relative quantification, EF1)

Innate immunity:

- TLR8
- TLR9
- STAT1
- Mx
- IFN γ type 1
- IFN γ
- CXCL10
- Viperin
- MHC I
- MHC II
- CD8

Adaptive immunity:

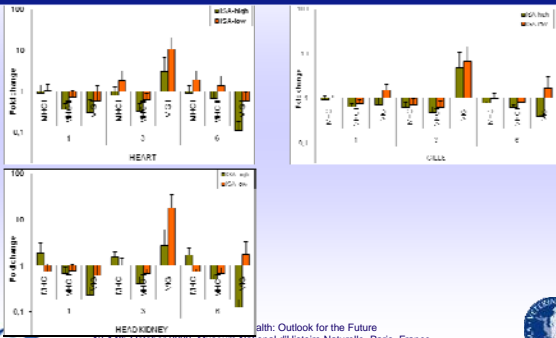
- IgM



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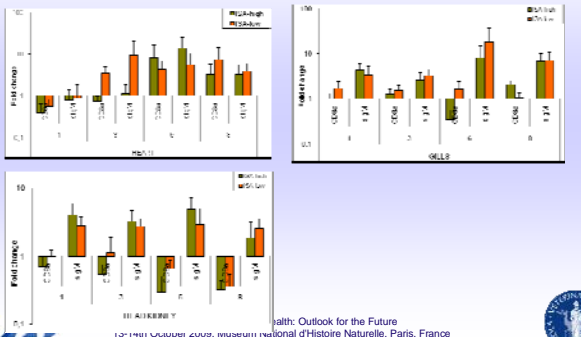
Immune gene expression in SAV infected ISA-high/low resistant fish.



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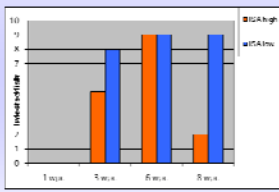


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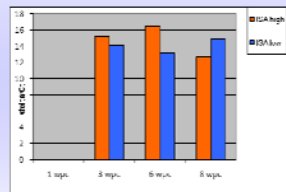
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SAV in hearts

Number of SAV positive fish



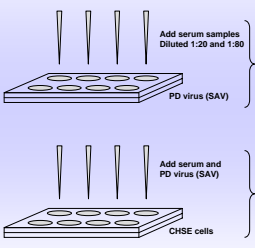
Relative quantification of SAV versus EF1



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SAV Neutralization assay



	ISA high (H)	ISA low (L)
0 wpc	<28	<28
1 wpc	<28	<28
3 wpc	>28	>28
6 wpc	>28	>28
8 wpc	>28	>28

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	1 wpc	3 wpc	6 wpc	8 wpc
	ISA-H ISA-L	ISA-H ISA-L	ISA-H ISA-L	ISA-H ISA-L
TLR8	Head kidney	Heart		
TLR9	Head kidney	Heart		
STAT2	Head kidney	Heart		
Mx	Head kidney	Heart		
IFN 1	Head kidney	Heart		
IFN γ	Head kidney	Heart		
CXCL10	Head kidney	Heart		
Viperin	Head kidney	Heart		
MHC1	Head kidney	Heart		
MHC2	Head kidney	Heart		
CD8	Head kidney	Heart		
IgM	Head kidney	Heart		
Virus in heart				
Pathological changes in heart				
Neutralizing antibodies				

UP 2-10 fold
UP >10 fold
Down 2-10 fold
Down >10 fold
italic - results from pooled samples

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SAV challenge - responses

- The ISA_{low} group
 - Moderately more histopathological changes,
 - Higher prevalence virus positive fish (serum 3 wpc, heart 4 and 8 wpc),
 - Lower level of neutralising antibodies 8 wpc.
 - More virus in heart samples 8 wpc
 - Mx was upregulated in organs tested at 3 wpc
- The ISA_{high} group
 - IgM, head kidney, higher than ISA low at 1,3 6 weeks

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SAV challenge - responses

- For both ISA_{low} and ISA_{high} groups:
- Few of the response genes tested showed consistent change at translational level for both groups.
- Viperin (Vig1) and IFN γ was upregulated in all organs tested, at 3 wpc
- IgM was upregulated for both groups, all organs, from 1, 3, 6 and 8 wpc in gills and head kidney.

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People involved in the SAV and ISAV work, Norway

<u>NSVS</u> Stine Braaen, Lars Austbø, Erling Olaf Koppang, Mohasina Syed, Espen Rimstad.	<u>UiT</u> Hanna Leena Thim, Jorunn Jørgensen
<u>NVI</u> Marie Løvoll, Søren Grove	<u>Intervet-Norbio</u> Petter Frost, Dag Knappskog
	<u>SalmoBreed</u> Rune Stigum Olsen

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