Cross neutralization studies with SAV 1-6 strains: results with sera from experimental studies and natural infections

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= To what extent do the immune-response against one strain of SAV recognize other strains of SAV?

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SAV strains and subtypes

- SAV (=SPDV) is one single virus species that gives one qualitatively well described disease (PD=SD) in salmonids
- SAV isolates are currently distinguist by genetic analysis
  - clusters into groups («Subtypes») most likely corresponding to SAV subpopulations that «lived» in geographically separated fish populations pre Aquaculture

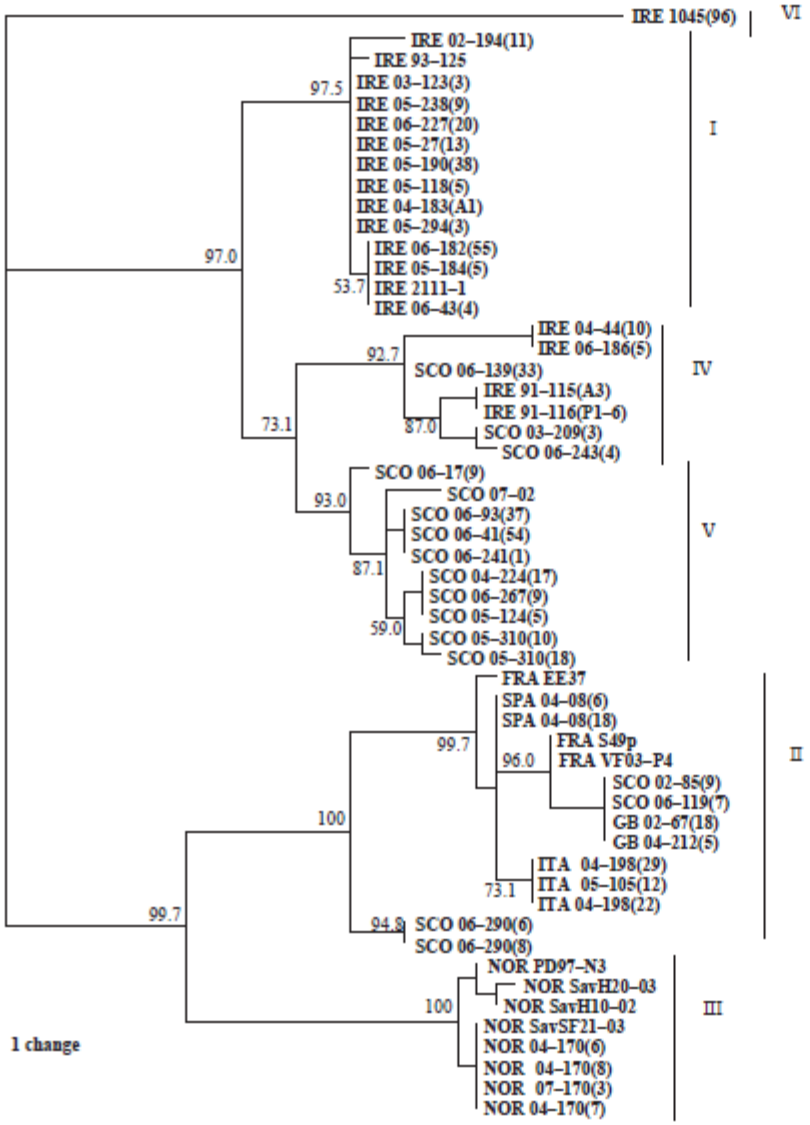

Figure 1 Phylogenetic tree showing relationships between salmonid alphavirus strains based on nucleotide sequence comparisons of the E2 gene fragment.

- To maximise resolution only a small variable part og the genome is used.

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SAV strains and phylogenetic subtypes

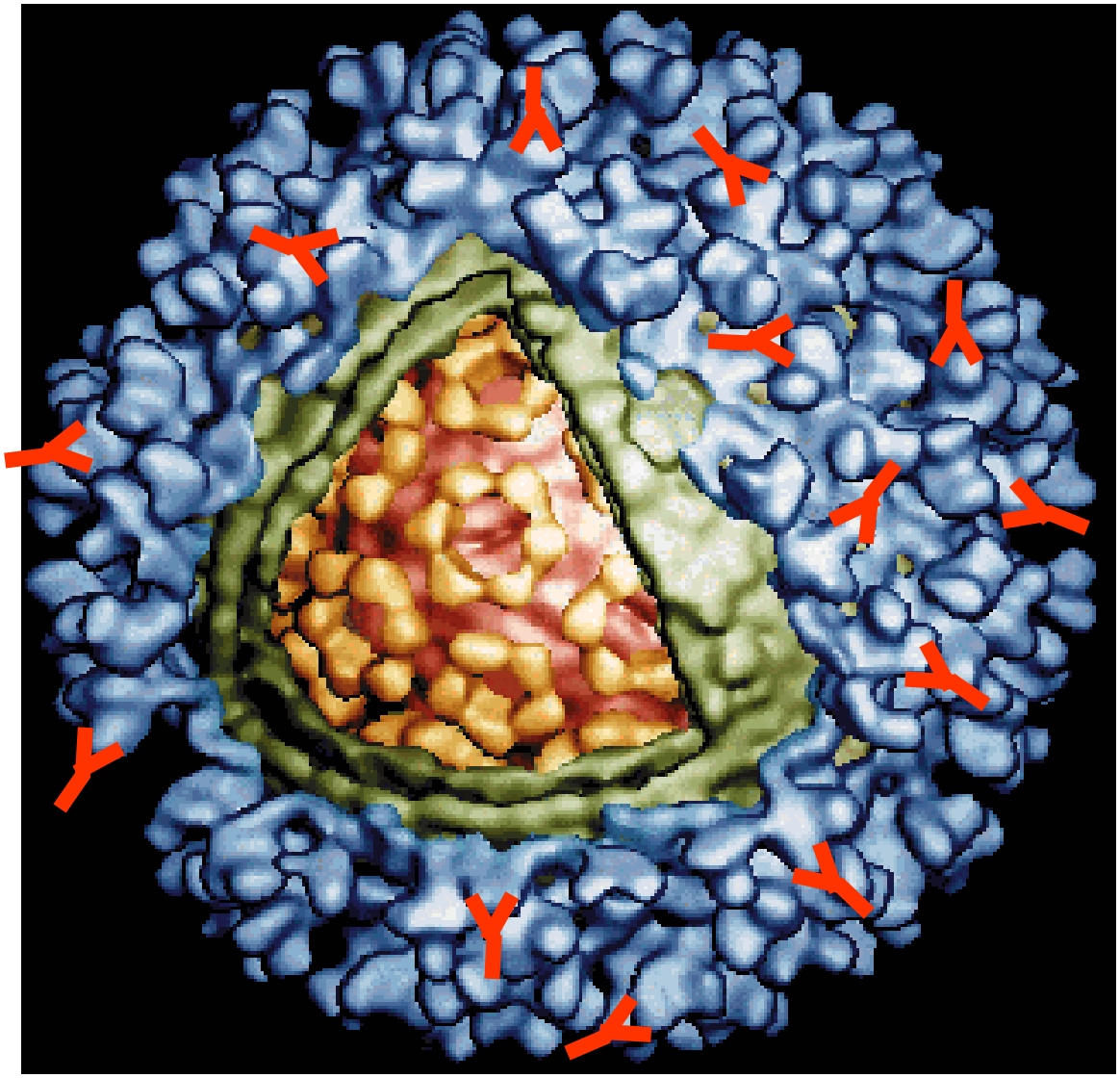
- Phylogenetically SAV isolates clusters into groups («Subtypes»)
  - Genetic characterisation can distinguish everything, also virus isolates that are biologically indistinguishable
    - Genetically distiguishable and biologically different is not the same
  - Phylogenetic characterisation is primarily* valuable for
    - virus evolution studies
    - Isolate archives and tracking down the origin of an infection
      - Cermaqs SAV3 cases in Northern Norway (ref K Otterem Feb 4th)
      - Where did the old SAV2 isolate introduced to mid Norway come from?

* Until we identify genetic markers that correlates with specific biological properties

Are there variation in biological features between SAV isolates?

- Anything else would be a scientific surprise
- But to find what and to what extent we need to do other things than «sexy» phylogenetic analyses
  - Pathogenicity differences? (ref presentation Torunn Taksdal)
  - To what extent do the immune-response against one strain of SAV recognize other strains of SAV? (this presentation)

SAV particle



Salmon antibodies recognise and binds to small structures on virus surface

Many different structures is recognised by many different antibodies like «a key in a lock»

Binding of some of the antibodies to some key structures makes the virus uninfectious.
The virus is «neutralized» by «virus neutralizing antibodies»

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To what extent do the immune-response against one strain of SAV recognize other strains of SAV?

- Virus neutralisation test with antibodies is a very sensitive method to detect variation on virus isolate surface structures that are relevant for immunity. = «Serotyping»

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Cross-neutralization studies with salmonid alphavirus subtype 1–6 strains: results with sera from experimental studies and natural infections

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- Study design
  - Collected antisera from fish populations after experimental infection with 6 different SAV isolates one pr subtype. 66 sera in total.
  - Collected sera from field after infection with isolate of 3 different subtypes (1, 4 and 5), 18 sera in total.
  - Analyzed all sera for their capacity to neutralize isolates of SAV isolates representing all the 6 subtypes.

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Table 4 Example of the 6 × 6 tables constructed to analyse reciprocal homologous and heterologous cross-neutralization titres obtained with experimental sera raised against each of the 6 SAV subtypes

	SAV subtype used in VN assay					
Serum	SAV-1	SAV-2	SAV-3	SAV-4	SAV-5	SAV-6
SAV1	**120**	80	60	30	80	*30*
SAV2	60	**120**	*30*	60	40	*<20*
SAV3	30	30	**40**	60	120	<20
SAV4	20	<20	80	**20**	60	<20
SAV5	120	*80*	160	*60*	**240**	*20*
SAV6	<20	30	60	30	30	**20**

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- Conclusions
  - The experimental antisera (anti 1-6) showed good cross neutralistion capability of isolates of all the other subtype groups (with exception of the level of SAV6 neutralisation).
  - A similar pattern was evident from field sera (anti SAV1, 4 and 5) except that cross neutralisation of SAV6 was more evident

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- Conclusions cont
  - No virus isolate consitantly met the old serology based criteria to be considered seperate subtypes within an alphavirus species.
    - What matters regarding protective immunity across SAV isolates is not a high resolution genetic grouping system (the subtypes) but to what extent SAV isolates are serologically different. This study shows that SAV isolates from each subtype are serologically very closely related.

      There is only only one «serotype» of SAV

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Abstract

The serological reactivity between strains of each of the six currently genetically defined subtypes of salmonid alphavirus (SAV) was examined by comparison of homologous and heterologous virus neutralization titres on sera from experimentally infected fish. With the exception of the level of SAV subtype 6 neutralization by heterologous sera, good cross-neutralization was detected between all subtypes, albeit with variation in geometric mean titres when each subtype-specific serum set was tested against the panel of virus subtypes. A similar pattern was evident with field sera, except that heterologous neutralization of the SAV6 strain was more evident. In only 23% of available pairwise comparisons was the homologous titre recorded with an experimentally derived serum fourfold or greater than the heterologous titre, and in only two instances was this difference demonstrated in both directions. No virus strains consistently met the old serology-based criteria (Sub-committee on Inter-relationships Among Catalogued Alphaviruses) to be considered separate subtypes within an alphavirus species. Only when testing with an SAV subtype-2-specific monoclonal antibody was a major difference between homologous and heterologous neutralization capacity evident. These results provide new direct or indirect information in terms of SAV classification, vaccine efficacy and the selection and validation of reagents for serological and immunological diagnostic purposes.

Keywords: heterologous, homologous, salmonid alphavirus, serology, subtype, virus neutralization.

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Introduction

Strains of salmon pancreas disease virus (SPDV, Genus *Alphavirus*, Family *Togaviridae*), more frequently referred to as salmonid alphavirus (SAV), identified to date have been assigned to six subtypes by phylogenetic studies (designated SAV subtypes 1–6) based on the analysis of partial E2 gene sequence data (Fringuelli *et al.* 2008). The use of the term 'subtype' in this way is consistent with previous studies (Hodneland *et al.* 2005; Weston *et al.* 2005) and is accepted by those working with SAV.

Prior to the development of molecular typing techniques, the degree of relatedness between alphaviruses was determined by serological analyses based on serological cross-reactivity in tests such as haemagglutination, complement fixation and neutralization studies as defined by the Sub-committee on Inter-relationships Among Catalogued Alphaviruses (SIRACA; Calisher *et al.* 1980; Calisher & Karabatsos 1988). Based on these guidelines, a fourfold or greater difference in cross-neutralization (virus neutralization, VN) titres in both directions when homologous virus/serum pairs are tested is consistent with their belonging to different *Alphavirus* species, while a fourfold difference in titre in one direction indicates that the viruses are different subtypes of the same virus species. Lesser differences associated with antigenic varieties require the use of methods such as monoclonal antibody characterization to distinguish between these.

This approach was used by Brault *et al.* (1999) to distinguish four lineages within eastern equine encephalitis viruses, the only species of the EEE genetic complex. More recently, the serologically

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