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?
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 distinguished by genetic analysis
  - clusters into groups («Subtypes») most likely corresponding to SAV subpopulations that «lived» in geographically separated fish populations pre Aquaculture
• To maximise resolution only a small variable part of the genome is used.
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 distinguishable 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)
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»
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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• Study design
  – Collected antisera from fish populations after experimental infection with 6 different SAV isolates one per 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 $6 \times 6$ tables constructed to analyse reciprocal homologous and heterologous cross-neutralization titres obtained with experimental sera raised against each of the 6 SAV subtypes

<table>
<thead>
<tr>
<th>Serum</th>
<th>SAV-1</th>
<th>SAV-2</th>
<th>SAV-3</th>
<th>SAV-4</th>
<th>SAV-5</th>
<th>SAV-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV1</td>
<td>120</td>
<td>80</td>
<td>60</td>
<td>30</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>SAV2</td>
<td>60</td>
<td>120</td>
<td>30</td>
<td>60</td>
<td>40</td>
<td>$&lt;20$</td>
</tr>
<tr>
<td>SAV3</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>60</td>
<td>120</td>
<td>$&lt;20$</td>
</tr>
<tr>
<td>SAV4</td>
<td>20</td>
<td>$&lt;20$</td>
<td>80</td>
<td>20</td>
<td>60</td>
<td>$&lt;20$</td>
</tr>
<tr>
<td>SAV5</td>
<td>120</td>
<td>80</td>
<td>160</td>
<td>60</td>
<td>240</td>
<td>20</td>
</tr>
<tr>
<td>SAV6</td>
<td>$&lt;20$</td>
<td>30</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>
Conclusions

- The experimental antisera (anti 1-6) showed good cross neutralisation 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.
Conclusions cont

- No virus isolate consistently met the old serology based criteria to be considered separate 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 one «serotype» of SAV
Cross-neutralization studies with salmonid alphavirus subtype 1–6 strains: results with sera from experimental studies and natural infections

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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 neutralisation titres on sera from experimentally infected fish. With the exception of the level of SAV subtype & neutralisation by heterologous sera, good cross-neutralisation 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 neutralisation 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 Pathogenic Alphaviruses) to be considered separate subtypes within an alphavirus species. Only when testing with an SAV subtype-specific monoclonal antibody was a major difference between homologous and heterologous neutralisation 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.

Introduction
Strains of salmon pancreas disease virus (SPDV, Genus Alphavirus, Family Togaviridae), most 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 Rd1 gene sequence data (Finglass et al. 2008). The use of the term ‘subtype’ in this way is consistent with previous studies (Hodneland et al. 2005; Westin 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 neutralisation studies as defined by the Sub-committee on Inter-relationships Among Pathogenic Alphaviruses (STRAAC, Colhoun et al. 1990). Colhoun & Karlsrud (1988). Based on these guidelines, a fourfold or greater difference in cross-neutralisation (virus neutralisation, 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 variants require the use of methods such as monoclonal antibody characterisation to distinguish between these.

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