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Development of a model system for studying cell-mediated immune responses to salmonid alphavirus

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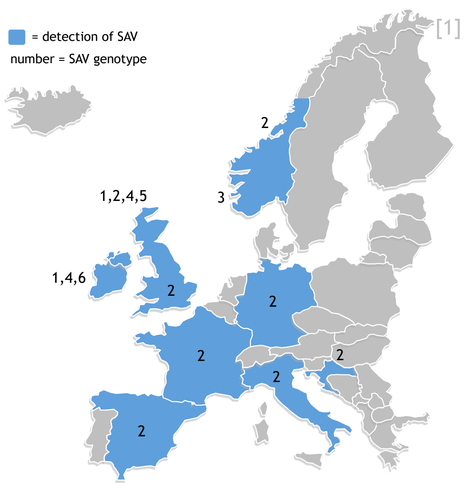
Introduction

Salmonid alphavirus (SAV) is an economically relevant pathogen causing pancreas disease (PD) in Atlantic salmon (*Salmo salar*) and sleeping disease (SD) in rainbow trout (*Oncorhynchus mykiss*).

It is transmitted horizontally via shedding into the water column and outbreaks last on average 3 months.

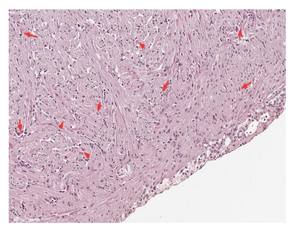
Outbreaks may occur at any time during on-growing, and at any time of year.



Symptoms include inappetence, abnormal swimming behaviour, and mortality.

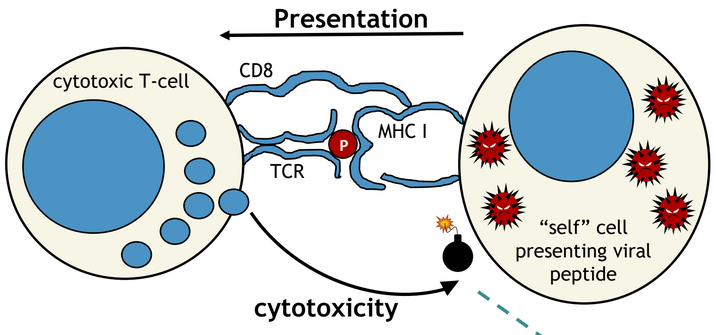
Pathological changes typically include a loss of exocrine pancreatic tissue, myocarditis and inflammation and/or degredation of skeletal muscle.

Surviving fish have been found to have significantly lower growth than uninfected fish [2].



PRESENTLY IN-DEPTH KNOWLEDGE OF VIRAL PROPERTIES, PATHOGENICITY AND THE INTERPLAY BETWEEN VIRUS AND HOST IMMUNITY REMAIN ELUSIVE

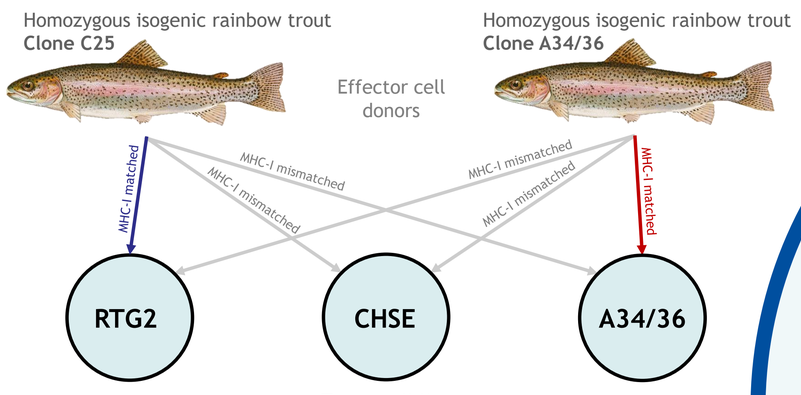
Model system



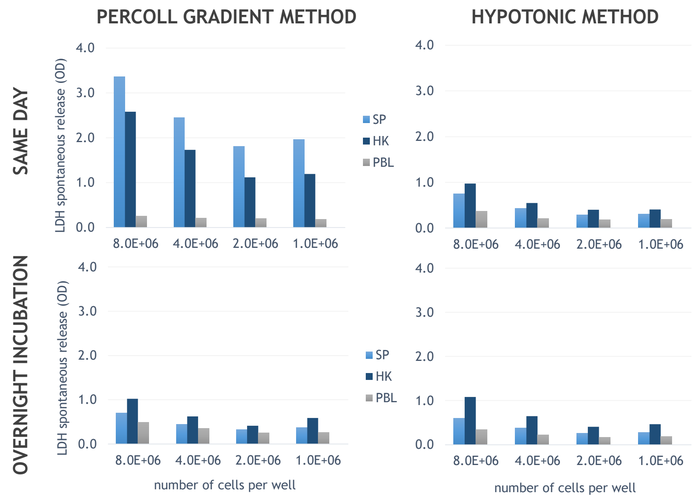
Fish leucocytes are able to execute cell-mediated cytotoxicity (CMC) against altered/infected self-cells and foreign cells via cytotoxic T-cells.

Virally infected cells will present peptides bound to MHC-I to TCR+ CD8+ cytotoxic T-cells. Specific CMC is consequently dependent on the successful binding of histocompatible TCR with MHC-I (MHC-I restriction).

OBJECTIVE: DEVELOP AN EFFECTOR/TARGET CELL SYSTEM FROM CLONAL RAINBOW TROUT AND SAV INFECTIBLE MHC-I MATCHED AND MISMATCHED TARGET CELLS



Effector cell optimisation

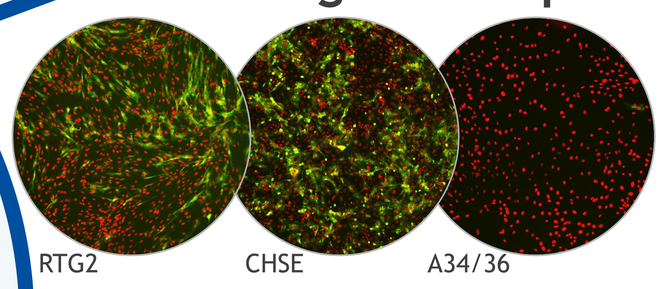


Effector cells were isolated using the Percoll gradient method and the hypotonic lysis method [3].

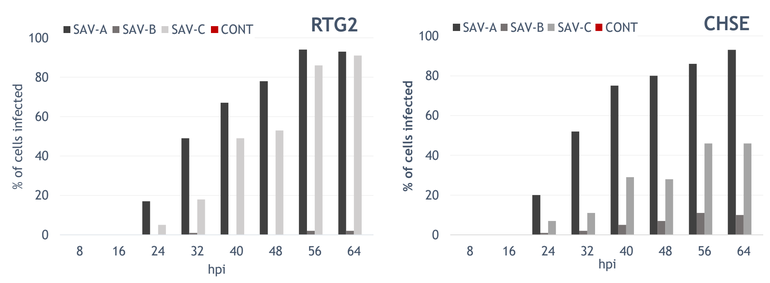
Spontaneous LDH activity was measured immediately after cell isolation, or after an overnight incubation.

RESULT: SPONTANEOUS LDH RELEASE FROM EFFECTOR CELLS CAN BE REDUCED BY USING THE HYPOTONIC METHOD

Target cell optimisation



RESULT: A34/36 CELL LINE IS NOT INFECTIBLE WITH SAV.

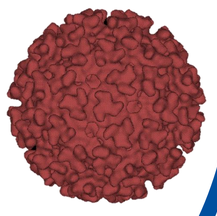


RESULT: SAV ISOLATE A IS OPTIMAL FOR TARGET CELL INFECTION

Salmonid Alphavirus

Family *Togaviridae*, Genus *Alphavirus*

Nucleotide and amino acid sequence identities are relatively low with previously described mammalian alphaviruses.



It is an enveloped, positive-sense, single stranded RNA virus ~12 kb in size which encodes 8 proteins.

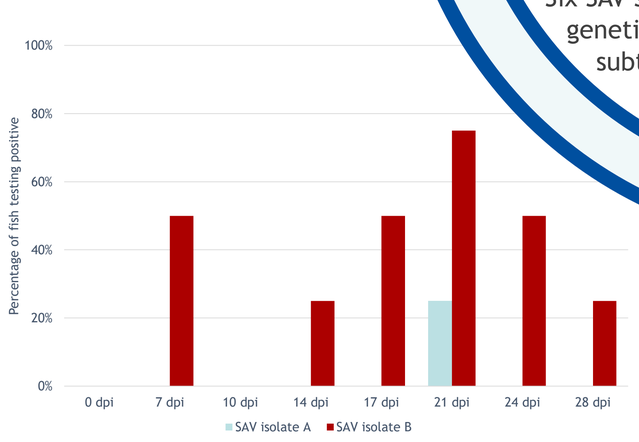
Six SAV subtypes have been described based on genetic differences. Studies show these subtypes are homologous and serologically closely related [4, 5].

Infectibility of rainbow trout

Rainbow trout were i.m. injected with SAV isolate A (**high passage /cell culture adapted strain**) or B (**low cell culture passage**).

Fish were sampled over 4 weeks.

Virus presence in sera was detected by RTqPCR and by histopathology (score 1-4).

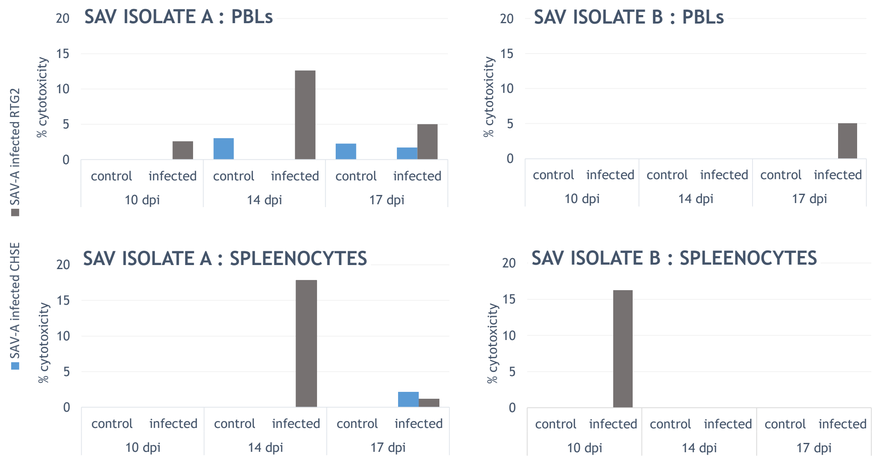


	SAV isolate A infection													
	0 dpi		7 dpi		10 dpi		14 dpi		17 dpi		21 dpi		24 dpi		28 dpi	
	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas
Mean score:	0	0	0	0	0	0	0	0	0	0	0	0,3	0	0	0	0,3

	SAV isolate B infection													
	0 dpi		7 dpi		10 dpi		14 dpi		17 dpi		21 dpi		24 dpi		28 dpi	
	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas	Heart	Pancreas
Mean score:	0	0	0	0	0	0	0	0	0	1	0	2	1	2	1,8	2

RESULT: RAINBOW TROUT ARE MORE SUSEPTIBLE TO INFECTION BY SAV ISOLATE B

In vitro measurement of virus-induced cytotoxicity



Rainbow trout (**clone C25**) were infected with SAV isolate A or B at doses known to induce pathogenesis but not mortality.

Effector cells were added at a ratio of 12:1 to MHC-I mismatched CHSE and MHC-I matched RTG2 cells which had been infected with SAV isolate A.

CONCLUSION: THE MODEL SYSTEM IS EFFECTIVE AT MEASURING SAV-INDUCED CMC

[1] map template: yourfreetemplates.com . [2] Jansen et al. (2017). *J Fish Dis* **40**: 141-155. [3] Hu et al. (2018). *Dev Comp Immunol* **88**: 104-113. [4] Taksdal et al. (2007). *J Fish Dis* **30**: 545-558. [5] Graham et al. (2015). *J Fish Dis* **37**: 683-691.

MSD Be well