

# Isolation and characterisation of salmonid alphavirus from wild caught ballan wrasse off the coast of Ireland

Neil Ruane, Fish Health Unit





The use of cleaner fish as a biological control of sea lice has increased in recent years

Ireland – wild caught wrasse (ballan, goldsinny, corkwing, rock cook) and hatchery reared lumpfish





## Health Screening

In Ireland the first 60 wrasse from each Bay are screened for pathogens prior to use on marine sites:

- Cell culture on BF2 & EPC (pools of 5)
- Histopathology
- Bacteriology – SWA, CBA+, TCBS
- Molecular – heart & kidney

Species identity is confirmed by sequencing of the mitochondrial control region (Almada *et al.*, 2017; Royal Society Open Science 4, 160773).



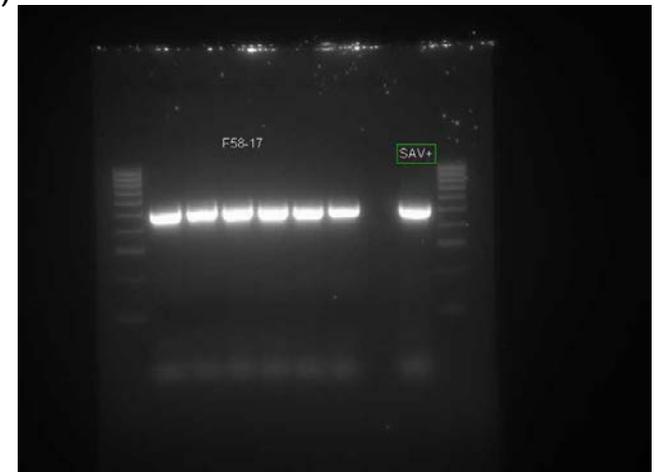


Sample F/58/17: 60 wrasse

- CPE observed on d14 in 1/12 pools (BF2 cells)
- VHS & IPN ELISA negative
- IHN IFAT negative

#### PCR

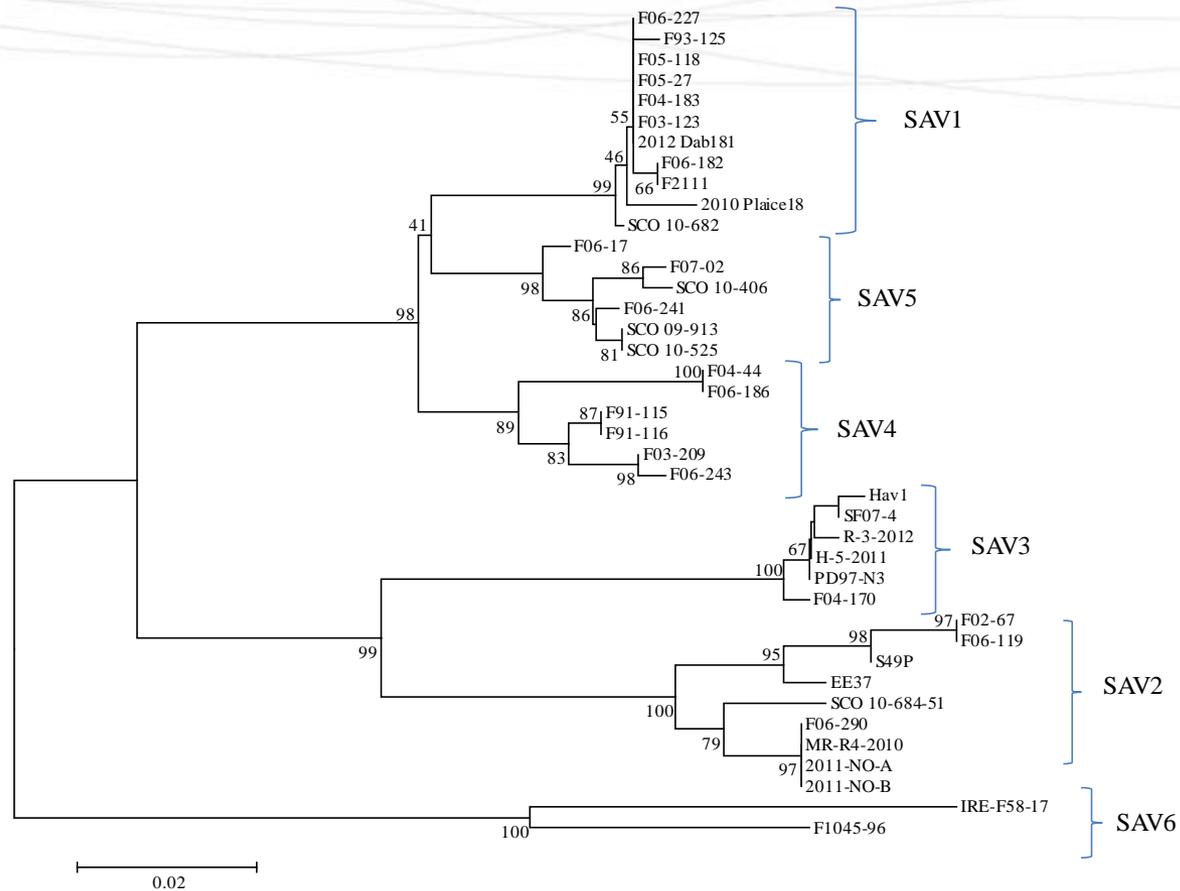
- Ranavirus negative
- Aquatic birnavirus negative
- Rhabdovirus (inc. vesiculovirus) negative
- VHS/IHN negative
- Salmonid alphavirus positive (+qRT-PCR)



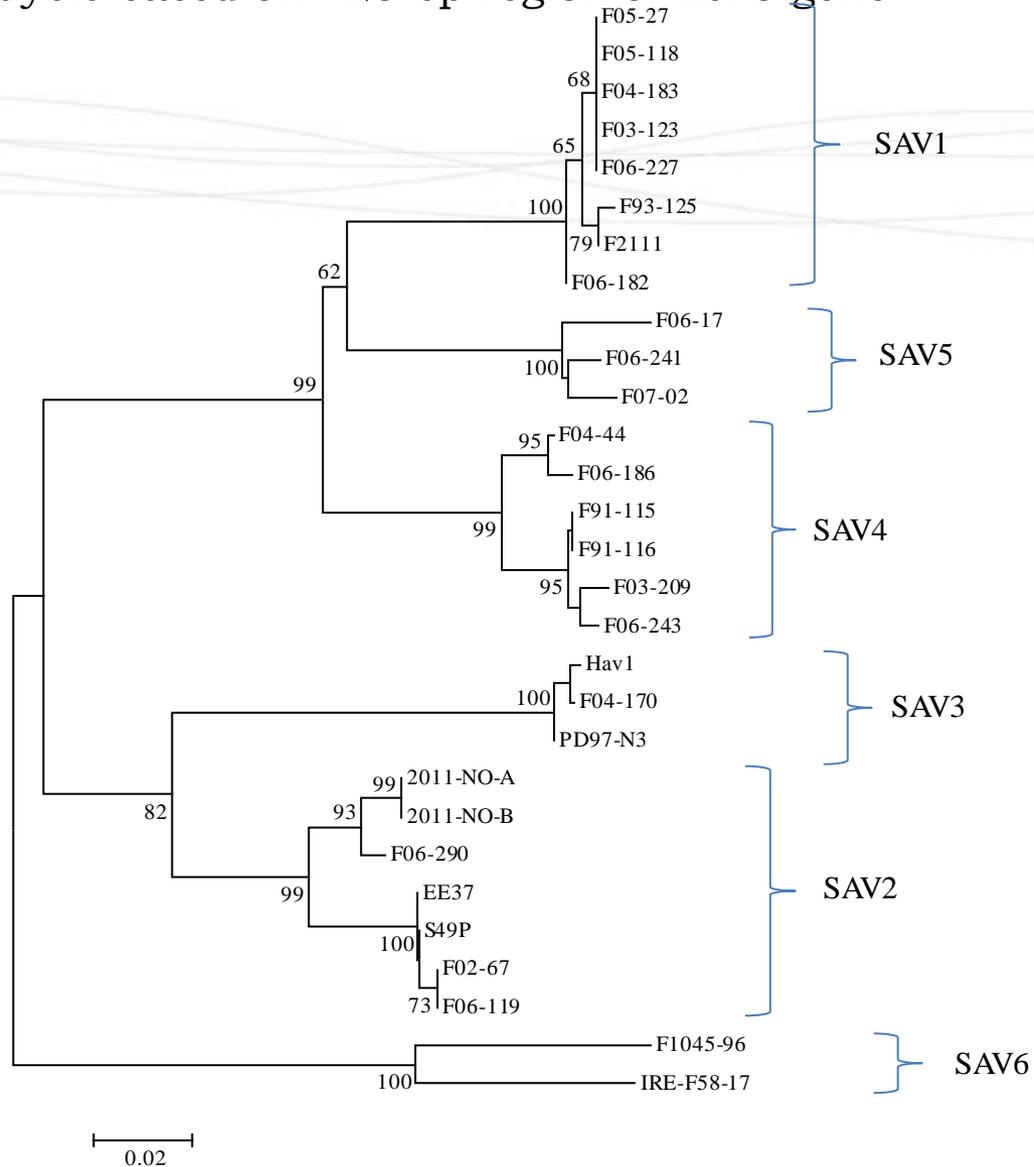
#### Species

- All five fish in the pool confirmed as Ballan wrasse

# Phylogenetic analysis based on 359 bp region of E2 gene



# Phylogenetic analysis based on 423 bp region of nsP3 gene





## nsP3 AA comparison

	30	40	50	60	70	80	90	100	110	120	130
F1045-96 (SAV6)	PAILRAASMGARSVRSVQSGPAGHRVVPAPPSVAGAASVAGPARQPSRPPSSVNMPAVIRGLTRDQFSAVRVRARRDLELERSEHGSQASFRSGSLVVGAAAAGSIASGY										
IRE-F58-17 (SAV6)	PAILRAASMGARSVRSVQSGPAGHRVAPAPLSVAGAASVAGPARQPSRPPSGVNMPAVIRGLTRDQFSAVRVRARRDLELERSEHGSQASFRSGSLVVGANAAGSI-SGY										
F93-125 (SAV1)	PA--RAASAGARSVRSVQSGSTGHR-----GAFSVAGSVRQPSGPPSSVSTPAAIRGLTRDQFDAVRVRARRNLELEGSEHGSQSSFHSGSLA-----GSSASSY										
S49P (SAV2)	PAILRAASTGARSVRSVQSGLTGHR-----DAVSVAGSVRQPSGPPSSVSTPAAPRGLTREQFGAVRARARRDLELEGSEHGSQASFRSGSLV-----GSTASSY										
HAV1 (SAV3)	PA--RAASTGARSVRSVQSGSAGHR-----DVASVAGSAG-----PRGLTRDQFGAVRARARRDLELEGSEHGSQTSFRSGSLM-----ESTASGY										
F91-115 (SAV4)	PAILRAASAGARSVRSVQSGSAGHR-----SAFSVASSARQPSGPPSSVSTPAATRGLTRDQFDAVRVRARRNLGLEGSEHGSQASFRSGSLA-----GSSASSY										
F06-17 (SAV5)	PAILRAAGAGARSVRSVQSGPAGHR-----GAFSVAGSVRQPSGPPSSVSTPAAPRGLTRDQFDVVRARARRNLEPEGSEHGSQASFRSGSLT-----GSSASSY										

Comparison of the amino acid sequences of a partial nsP3 region from the salmonid alphavirus isolate IRE-F58-17 with an isolate from each of the six main SAV subtypes. Sequence alignments were performed by ClustalW analysis and amino acid deletions are indicated with a dash.



## Original SAV6 detection: F1045-96

Date	Name	Sample type	Date of Incubation	Date of Pass	Number	Result
20/6/96 (6)	[REDACTED]	salmon: 26x post smolts 1-3 23x guppies (6-6)	20.6.96	26.6.96	1032	Negative mixed - white sp. 10x ✓
20/6/96 (6)	[REDACTED]	30x post smolt salmon 5x smolts (1-3) 15x smolts (4-6)	20.6.96	26.6.96	1033	Negative ✓
26/6/96	[REDACTED]	8 guppies, 2x salmon 10x salmon	28.6.96	5.7.96	1034	Negative
27/6/96 (6)	[REDACTED]	30x salmon (1-4) post smolts 1, 2, 3 included	28.6.96	5.7.96	1036	Negative ✓
27.6.96 (6)	[REDACTED]	Salmon trout x? (1-6) no 2 of 6 collected, 2 in no brown on head, 1 salmon - eq. 2x	28.6.96	5.7.96	1037	Negative ✓
27.6.96 (6)	[REDACTED]	post smolt Salmon x? (1-5) small smolts - 1st sp. 1st, 2nd sp. 2nd - 2x	28.6.96	5.7.96	1038	Negative ✓
27.6.96	[REDACTED]	5x trout fingerlings	28.6.96	5.7.96	1039	Negative ✓
28.6.96 (6)	[REDACTED]	30x post smolts 1-3	28.6.96	5.7.96	1042	Negative ✓
28.6.96 (6)	[REDACTED]	30x post smolts (1-3)	28.6.96	5.7.96	1043	Negative ✓
28.6.96 (6)	[REDACTED]	30x post smolts 1-3	28.6.96	5.7.96	1044	IMMUNO - NEG
28.6.96 (6)	[REDACTED]	10x salmon guppies (1-2)	28.6.96	5.7.96	1045	IMMUNO - NEG 1045 1045 LIKE VIEWS
28.6.96	[REDACTED]	10x post smolts (1-2)	28.6.96	5.7.96	1046	Negative ✓

Ankous June '99  
IMMUNO - NEG  
reanalysis from 1st head original  
with CPAGE 1216-2 16.6.99 +  
1.4.99 CDC reanalysis TRD.



Original SAV6 detection: F1045-96

28.6.96 [REDACTED] 10 x salmon growers (1-2)  
 Co. Galway

<p>Neut. 20.8.</p> <p>Neut 27.8 Bfz/cuse.</p> <p>28.6.96</p>	<p>Neut. 16.7.96</p> <p>2<sup>nd</sup> Pass 12.7.96</p> <p>5.7.96</p> <p>Neut. 12.7.96 / 16.7.96</p>	<p>in FLASKS 2.8.96</p> <p>9.8.96</p> <p>16.8.96</p> <p>10G5</p> <p><b>TOGA LIKE VIRUS</b></p>	<p>Aarhus June '99 *</p> <p>IMMUNO - NEG.</p> <p>inoculation from filtered originals onto CUSE + RTG-2 16.4.97 + 17.4.97 (PE PRODUCED) ⇒ T.E.D.</p>
--	--	--	---



## Conclusion

Second isolation of a SAV6 subtype.

SAV(1,2,5) has been detected in flatfish and SAV5 was isolated from dab.

Increasing evidence that SAV is not restricted to salmonids.

No epidemiological link with aquaculture for the current isolate.



## Conclusion

Evolution of alphaviruses – host switching and geographic introductions<sup>1</sup> with a marine origin<sup>2</sup>.

A wild reservoir (North Sea?) could account for multiple introductions of SAV leading to independent outbreaks or self-sustaining epizootics<sup>3</sup>.

Is SAV6 an ancestral or wild-type SAV?

Has aquaculture production led to competitive exclusion of SAV6 in favour of endemic subtypes SAV1, 2 & 3?

- Health screening of all fish prior to deployment in fish cages is important.

1. Powers et al. 2001. J. Virol. 75, 10118-10131.
2. Forrester et al. 2012. J. Virol. 86, 2719-2738.
3. Karlsen et al. 2014. J. Gen. Virol. 95, 52-59.

## Acknowledging the work of the FHU:

- Dave Swords
- Teresa Morrissey
- Michelle Geary
- Cathy Hickey
- Eve Collins
- Fiona Geoghegan
- Fiona Swords

