Sequence variation in the full genome of PMCV

sampled from field outbreaks of CMS

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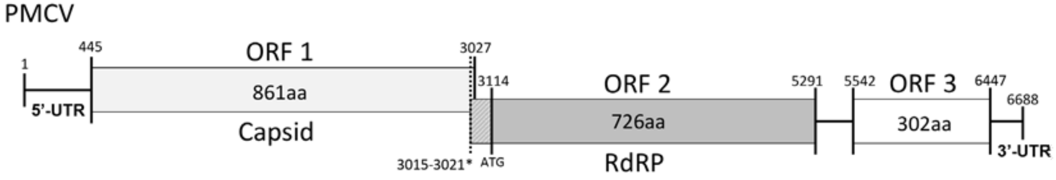
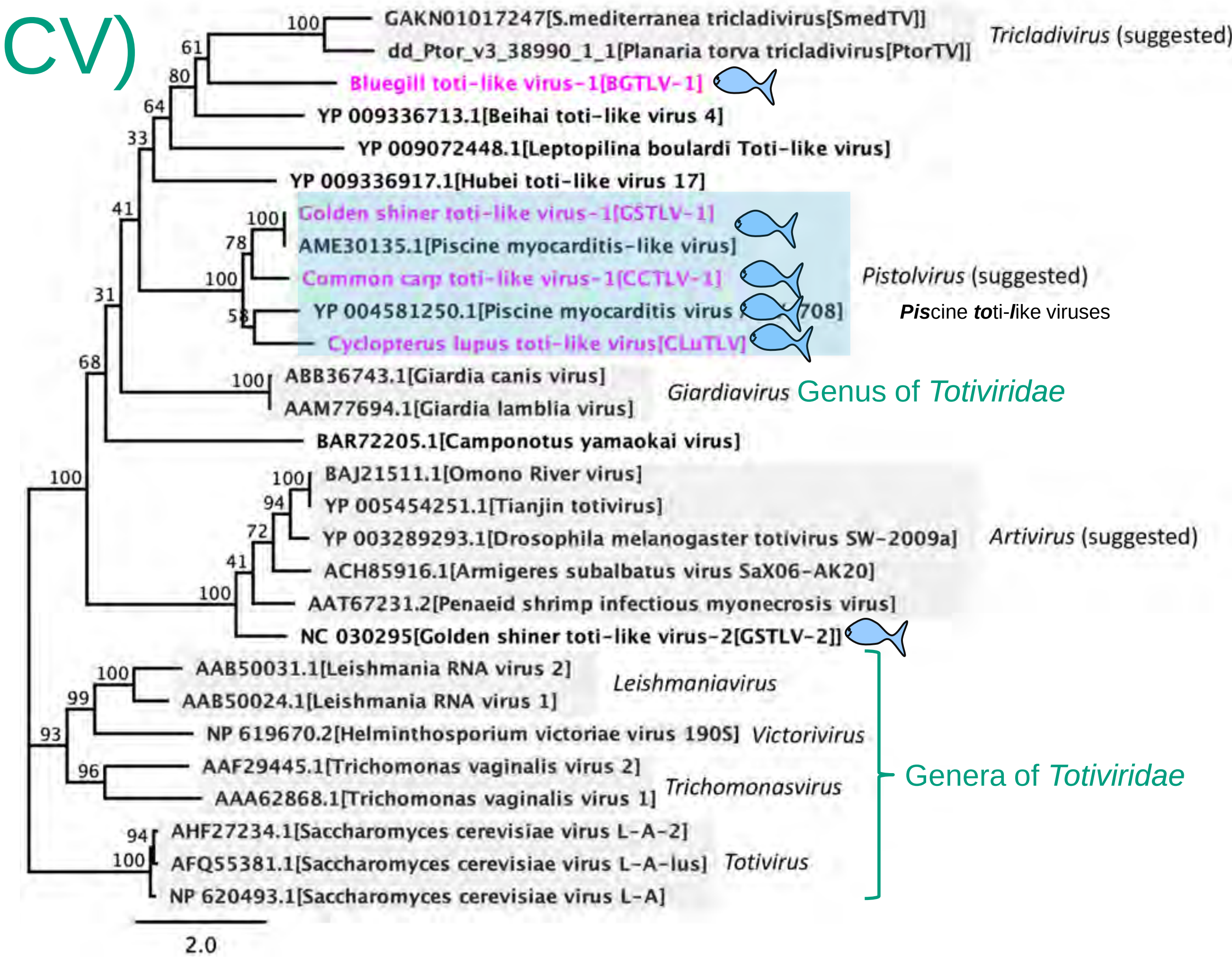
Piscine myocarditis virus (PMCV)



- dsRNA genome of 6688nt
- Simple virus encoding only 3 proteins
- Similarities to *Totiviridae* – i.e. «toti-like virus»
  - Similar capsid and RdRp arrangement
  - Longer genome due to additional ORF3
    - PMCV from salmon
    - GSTLV-1 from golden shiner
    - CCTLV-1 from common carp
    - CLuTLV from lumpsucker



***Pis**cine **to**ti-**l**ike viruses*

Genus of *Totiviridae*

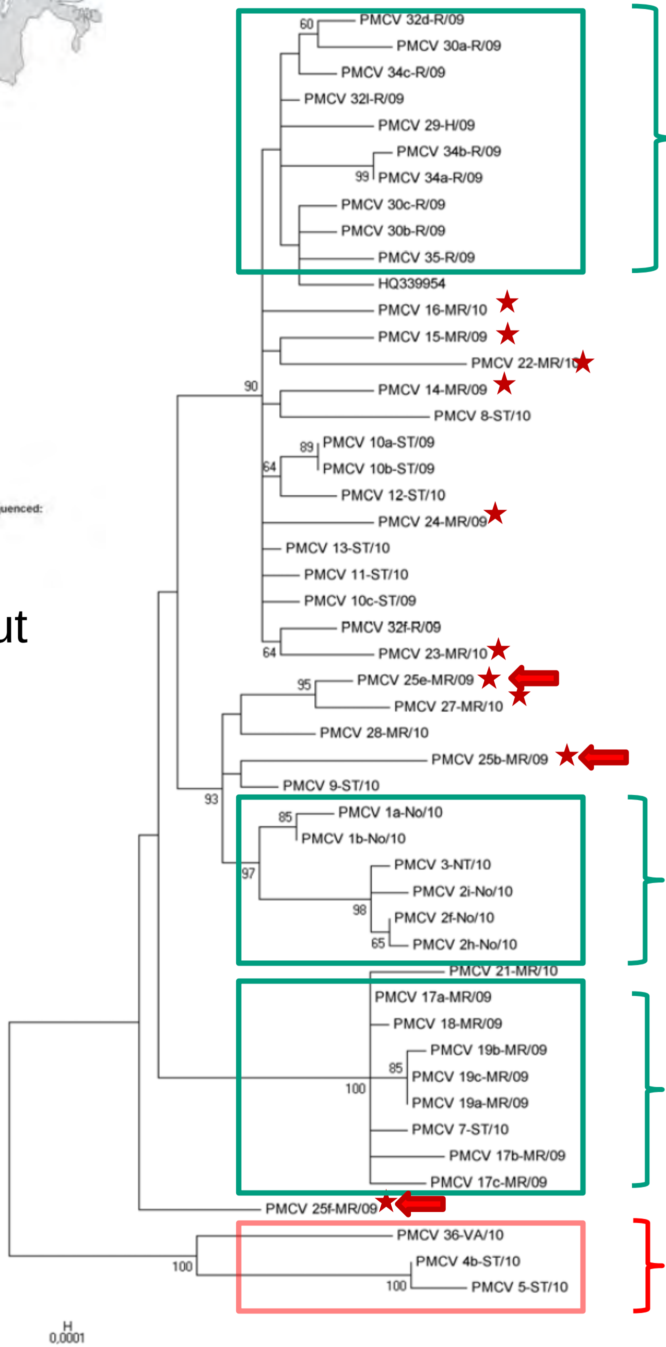
Genera of *Totiviridae*

Figures from Sandlund et al "Comparative molecular characterization of novel and known piscine toti-like viruses" – Submitted, March 2021

Background

Genetic variation - PMCV

- Norwegian strains are highly similar[1]
  - Most divergent shares 97,7% nt identity
  - Capsid 99,3% identical amino acids
  - ORF3-protein 97,7% identical amino acids
- «Some relation» between phylogeny and geographical origin, but also contradictions
- Similar findings for Irish strains[2]
  - Most divergent among Norwegian and Irish strains shares 97,7% nt identity



Rogaland and Hordaland

★ Strains from Møre og Romsdal spread over 3 of 4 groups

Same site/outbreak/ time point

The three northern most strains

Close sites in Møre and Romsdal

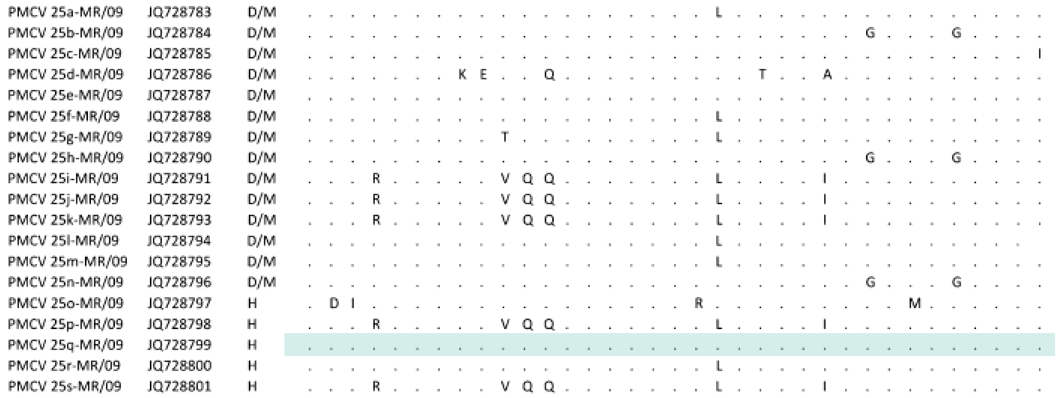
Two close strains from Trøndelag together with the most southern strain (from Vest-Agder)

1) Wiik-Nielsen 2012 and results from FHF-project 901179 (manuscript in preparation)
2) Scholz 2017 and Tighe 2019

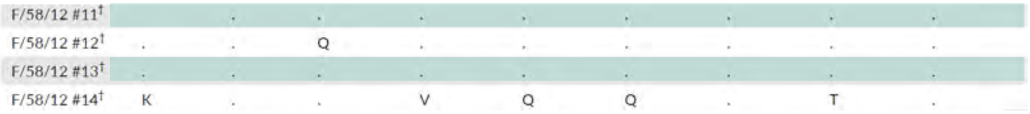
Background

Genetic variation – PMCV ORF3-encoded protein

- One outbreak consists of/results in several virus variants and ORF3 equal to ALV-708 strain[1] is usually represented[2]
  - Within one disease case
    - Up to seven different variants present between individuals
  - Variants different from ALV-708 is never found in more than one outbreak
  - The position with change is usually exclusive to individual cases
    - Only 4 positions with change in more than one outbreak



PMCV 25a-MR/09	JQ728783	D/M	. . . . L . . . .
PMCV 25b-MR/09	JQ728784	D/M	. . . . G . . . G . . . .
PMCV 25c-MR/09	JQ728785	D/M	 I
PMCV 25d-MR/09	JQ728786	D/M	. . . . K E . . Q . . . . T . . A . . . .
PMCV 25e-MR/09	JQ728787	D/M	. . . .
PMCV 25f-MR/09	JQ728788	D/M	. . . . L . . . .
PMCV 25g-MR/09	JQ728789	D/M	. . . . T . . . . L . . . .
PMCV 25h-MR/09	JQ728790	D/M	. . . . G . . . G . . . .
PMCV 25i-MR/09	JQ728791	D/M	. . . R . . . . . V Q Q . . . . L . . . . I . . . .
PMCV 25j-MR/09	JQ728792	D/M	. . . R . . . . . V Q Q . . . . L . . . . I . . . .
PMCV 25k-MR/09	JQ728793	D/M	. . . R . . . . . V Q Q . . . . L . . . . I . . . .
PMCV 25l-MR/09	JQ728794	D/M	. . . . L . . . .
PMCV 25m-MR/09	JQ728795	D/M	. . . . L . . . .
PMCV 25n-MR/09	JQ728796	D/M	. . . . G . . . G . . . .
PMCV 25o-MR/09	JQ728797	H	. D I . . . . R . . . . M . . . . . .
PMCV 25p-MR/09	JQ728798	H	. . . R . . . . . V Q Q . . . . L . . . . I . . . .
PMCV 25q-MR/09	JQ728799	H	. . . .
PMCV 25r-MR/09	JQ728800	H	. . . . L . . . .
PMCV 25s-MR/09	JQ728801	H	. . . R . . . . . V Q Q . . . . L . . . . I . . . .



F/58/12 #11†		.	.	.	.	.	.	.	.
F/58/12 #12†	.	.	Q	.	.	.	.	.	.
F/58/12 #13†	.	.	.	.	.	.	.	.	.
F/58/12 #14†	K	.	.	V	Q	Q	.	T	.

1) Haugland 2011

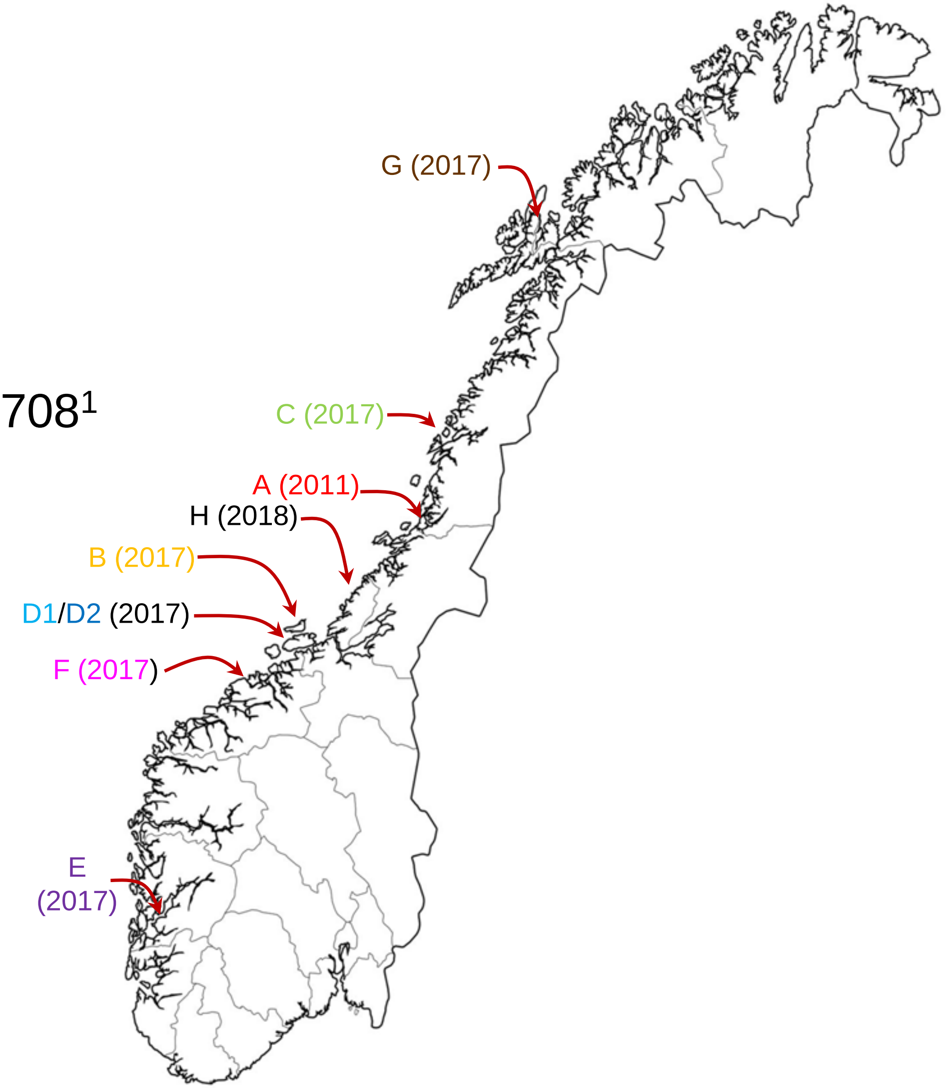
2) Wiik-Nielsen 2012 and results from FHF-project 901179 (manuscript in preparation)

Mat & Met

Sequence studies of PMCV full genome

- Full genome sequence only available for one isolate: PMCV ALV708[1]
  - ORF1/RdRp: 16 full ORF + 21 partial
  - ORF2/Coat: 1 full ORF from ALV708
  - ORF3: 28 full ORF + 35 partial
  - UTRs: Only available from PMCV ALV708

Case	Sampling	Geographic location	# individuals
A	05/04/11	Nordland	5
B	13/06/17	Frøya, Trøndelag	6
C	27/06/17	Dønna, Nordland	6
D1	28/08/17	Hitra, Trøndelag	5
D2	05/12/17	Hitra, Trøndelag	8
E	05/08/17	Jondal, Hordaland/Vestland	4
F	19/09/17	Kristiansund, Møre og Romsdal	6
G	04/01/18	Harstad, Troms og Finnmark	5
H	17/12/18	Frøya, Trøndelag	12



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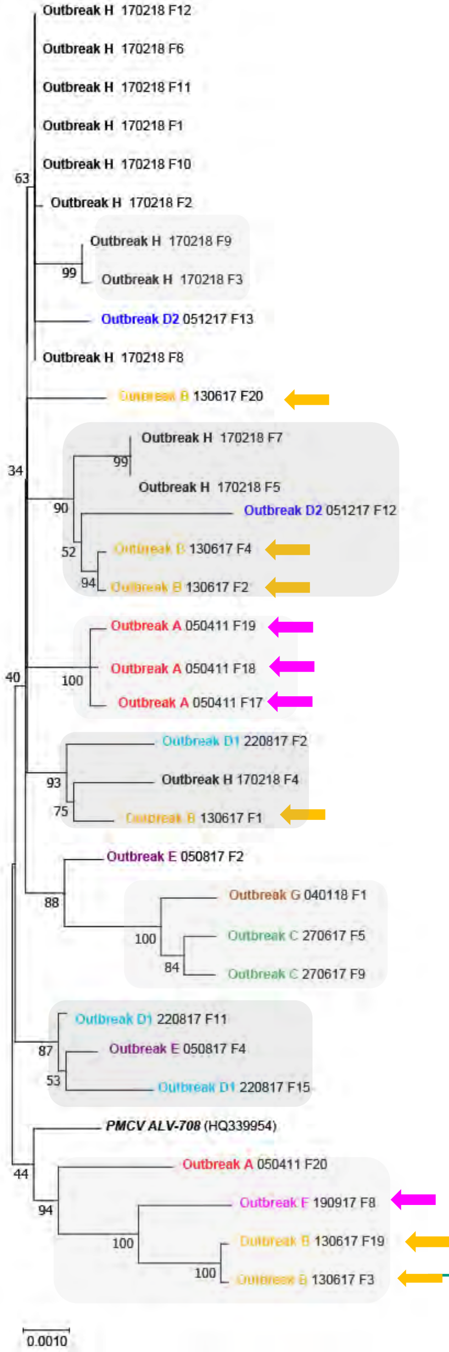
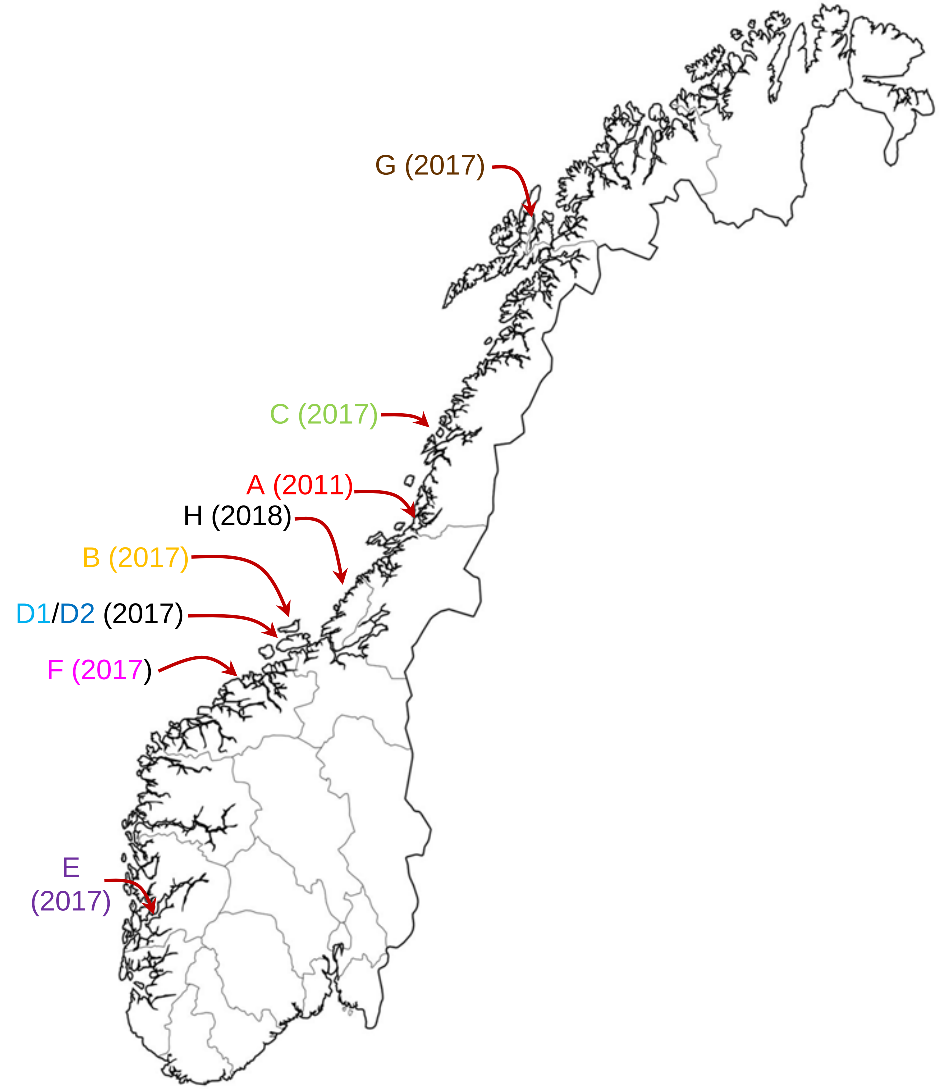
1) Haugland 2011

Results

Sequence studies of PMCV full genome

- Near full genome sequence was achieved for samples from 33 individuals
    - PMCV strains are highly similar over the full-length 6688nt genome
    - Most divergent isolates share 99,2% nts
    - One outbreak usually includes several strains

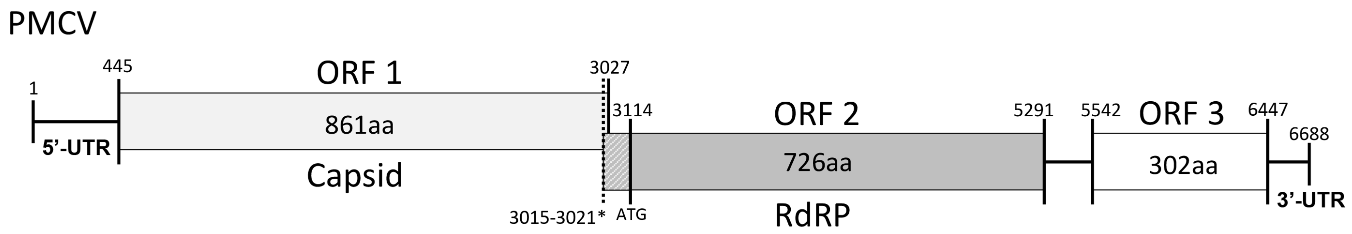




Results

Sequence studies of PMCV full genome – most divergent strains

- Most divergent strains by nucleotides and amino acid sequence on each ORF, UTR and encoded proteins:



	5’UTR	ORF1/Capsid	UTR ORF1-2	ORF2/RdRp	UTR ORF2-3	ORF3/ encoded protein	3’UTR
Identical **nucleotides**	98,7%	99,1%	**96,4%**	99,1%	98,4%	98,5%	99,0%
Identical **amino acids**		99,1%		99,3%		**96,4%**	

- 100% identical strains were found in individuals from same outbreak, but never from different outbreaks
- The strains with highest identity between two outbreaks shared 99,8% nts

Summary

Sequence studies of PMCV full genome

- PMCV strains are highly similar over the full-length 6688nt genome
    - Highest variability in ORF3-encoded protein
- One outbreak usually includes several strains – why?
    - The variability among individuals probably reflects variability in each individual
    - One strain infects the fish and mutates rapidly throughout the infection and results in a cloud of variants?
    - A cloud of variants infects the fish and the most fit strain(s) result in disease?
    - Multiple infection by several strains?

- Sequencing of PMCV is not possible to use for tracking virus to study origin of infection or other epidemiological aspects (geographical relation, historical etc.)

Questions?

Acknowledgements:

- Håkon Torsvik and Sandra Radunovic, Aquamedicine unit, for technical help with field sampling and sampleprep and PCR
- Turhan Markussen, Aquamedicine unit, NMBU, for phylogenetic analyses
- Liv Sandlund and Stian Nylund, Pharmaq analytiq, and Nick Phelps and Soumesh Padhi, University of Minnesota, for collaboration on piscine toti-like viruses
- **Funding**
  - Norwegian centennial chair (NOCC) – project «Comparative studies of genetic variability and in vitro virulence comparison between the piscine toti-like viruses found in Norwegian Atlantic salmon and golden shiner from Minnesota", 2018-2020
  - FHF – project 901179 "CMS – markører for opptreden av klinisk sykdom»

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