

Sustainable Performance

AQUAVAC[®]

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Impact of PD-vaccination on viral shedding

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Scientific background – SPDV and shedding

- SPDV has a high potential for water-borne spreading following shedding from infected fish (Lab: Andersen et al, 2010, Virology Journal, 7: 198, Field: Stene et al, 2014, Journal of fish diseases, 37 : 123-134).
- SPDV is detected in mucus and faeces collected from infected fish (Graham et al, 2011, Journal of fish diseases, 34: 273-286; Graham et al, 2012, Journal of fish diseases, 35: 949-951).
- The peak of SPDV shedding correlates with the peak of viremia, which occurs at an early time point in the infection (Andersen et al, 2010, Virology Journal, 7: 198; Graham et al, 2011, Journal of fish diseases, 34: 273-286).
- Leakage of liquid fat from dead infected salmon contains infective SPDV (Stene et al 2015 Journal of fish diseases, *in press*).
- Infection pressure has a strong effect on the probability of recording a PD-outbreak in a cohort (Kristoffersen et al, 2009 Prev. Vet. Med. 90: 127-136).

Can PD-vaccination reduce viral shedding following a SPDV infection?

- Experimental PD-vaccination and SPDV challenge study:
 - Collection of water-, blood- and feces samples at different time points post challenge
 - Detection of virus by means of SPDV specific real time RT-PCR

Experimental set-up (design):

AquaVac
PD7
130 fish

Saline
130 fish

6 wpv (500DD)
SPDV i.m. challenge

AquaVac
PD7
60 + 10
naïve

AquaVac
PD7
60 + 10
naïve

Saline
60 + 10
naïve

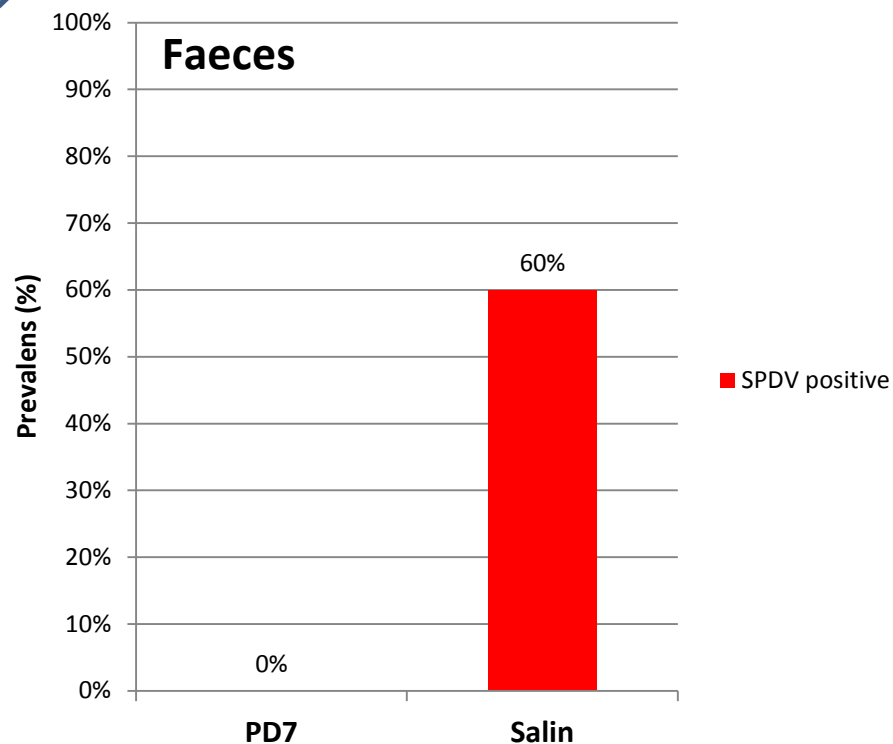
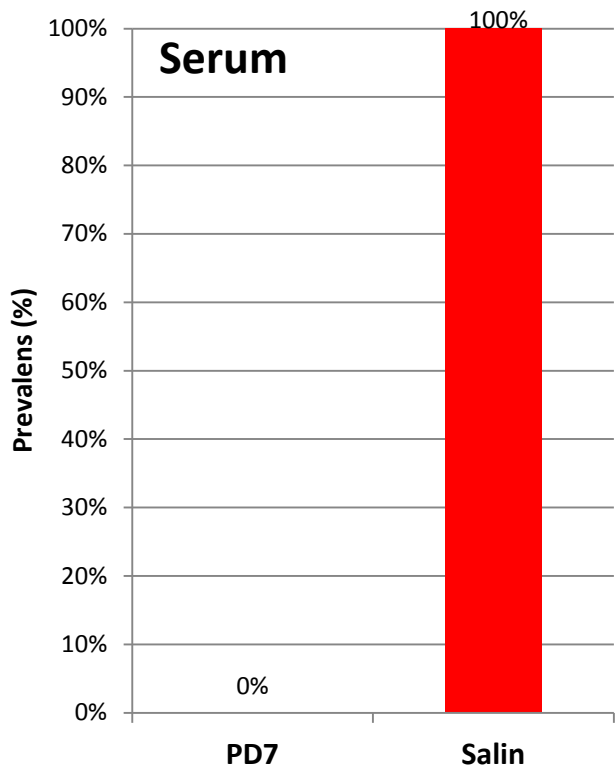
Saline
60 + 10
naïve

Mix:
10 PD7 +
10 saline

T=4 dpc, water sampling (1 L/tank)
T=10 dpc, water sampling (1 L/tank) + faeces og serum (2x10 in mixed tank)
T=14 dpc, water sampling (1 L/tank)
T=18 dpc, water sampling (1 L/tank)
T=24 dpc, water sampling (1 L/tank) + serum from naïve fish (4x10)

Prevalens of SPDV-positive fish (mixed tank) 10 dpc

Miks:
10 PD7 +
10 salin



AQUAVAC PD7 reduces level of infective SPDV in the water – experimental setup:

AquaVac
PD7
130 fish

Saline
130 fish

6 wpv
SPDV i.m. challenge

AquaVac
PD7
60 + 10
naïve

AquaVac
PD7
60 + 10
naïve

Saline
60 + 10
naïve

Saline
60 + 10
naïve

Mix:
10 PD7 +
10 saline

1L water sample added
a fixed ISAv spike and
concentrated by
filtration

T=4 dpc, water sampling (1 L/tank)

T=10 dpc, water sampling (1 L/tank)
+ faeces and serum (2x10 in mixed tank)

T=14 dpc, water sampling (1 L/tank)

T=18 dpc, water sampling (1 L/tank)

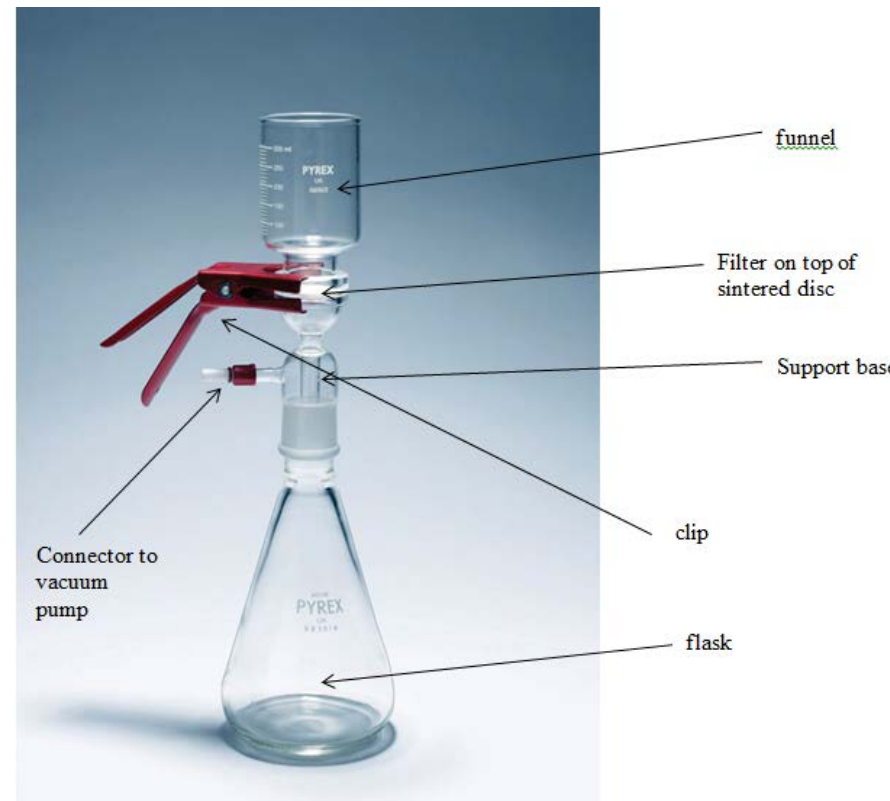
T=24 dpc, water sampling (1 L/tank)
+ serum from naïve fish (4x10)

Detection of SPDV in water samples

Water filtration: modified VIRADEL method*

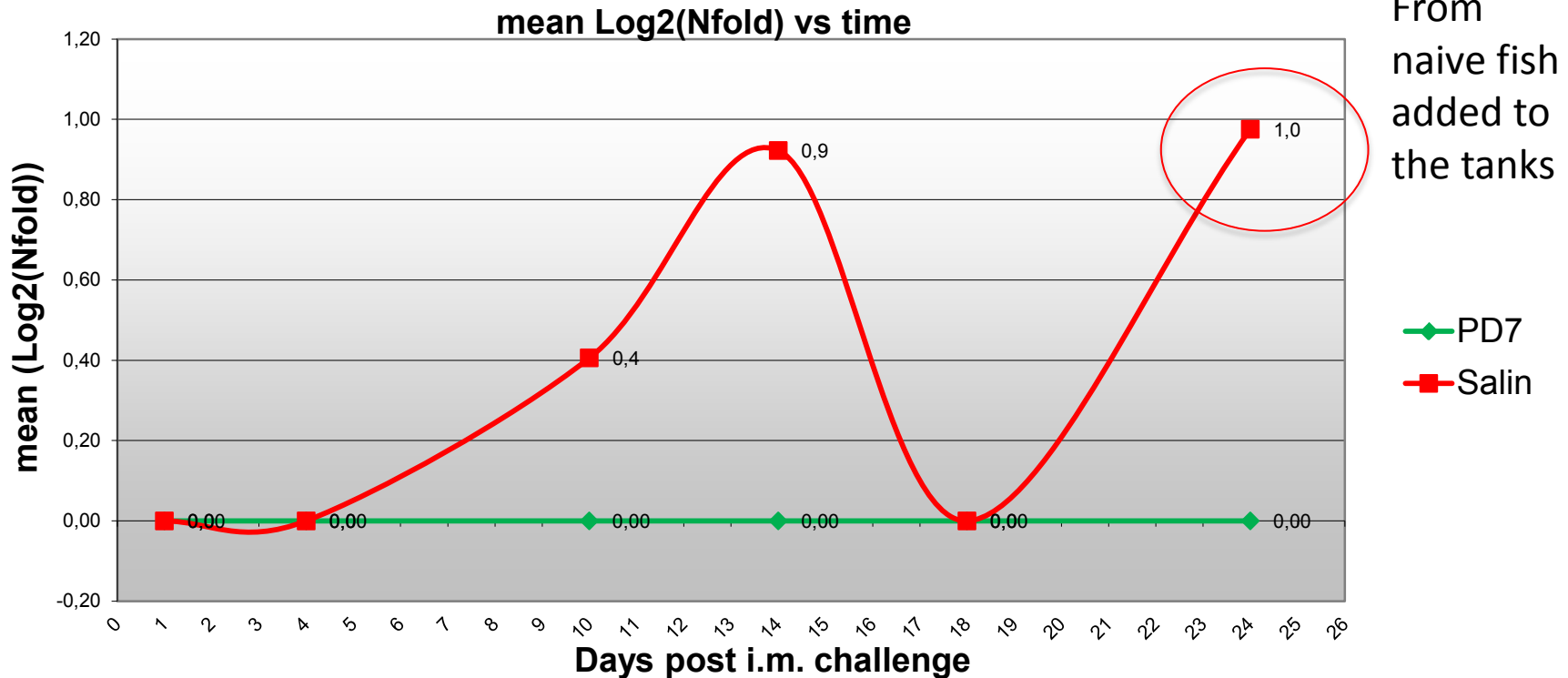
- Adsorption to electropositive filter (1L sample)
- Elution of conc. in lysis buffer (1.4ml)
- RNA extraction and specific real time RT-PCR-analyse for SPDV nsP1 and ISAV seg. 8

*Andersen et al, 2010, Virology Journal, 7: 198



Relative quantification of SPDV-RNA (kinetics).

Log₂ (Nfold) of SPDV-RNA in 1L water collected from 0 – 24 dpc



- PD-vaccination leads to significantly ($p < 0.05$) reduced levels of SPDV in the water after viral challenge

Sampling of naive fish added to the challenge tanks (24 dpc)

AquaVac
PD7
130 fish

Saline
130 fish

6 wpv
SPDV i.m. challenge

AquaVac
PD7
60 + 10
naïve

AquaVac
PD7
60 + 10
naïve

Saline
60 + 10
naïve

Saline
60 + 10
naïve

Mix:
10 PD7 +
10 saline

T=4 dpc, water sampling (1 L/tank)

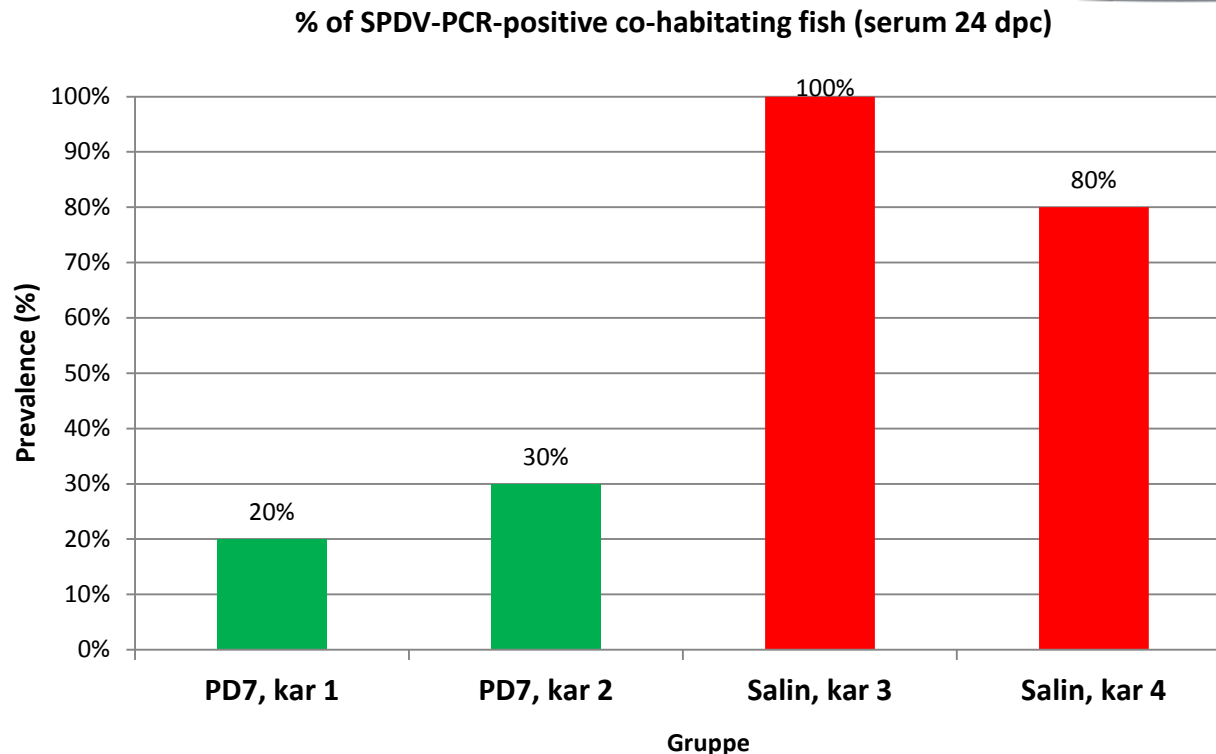
T=10 dpc, water sampling (1 L/tank)
+ faeces og serum (2x10 in mixed tank)

T=14 dpc, water sampling (1 L/tank)

T=18 dpc, water sampling (1 L/tank)

T=24 dpc, water sampling (1 L/tank)
+ serum from naïve fish (4x10)

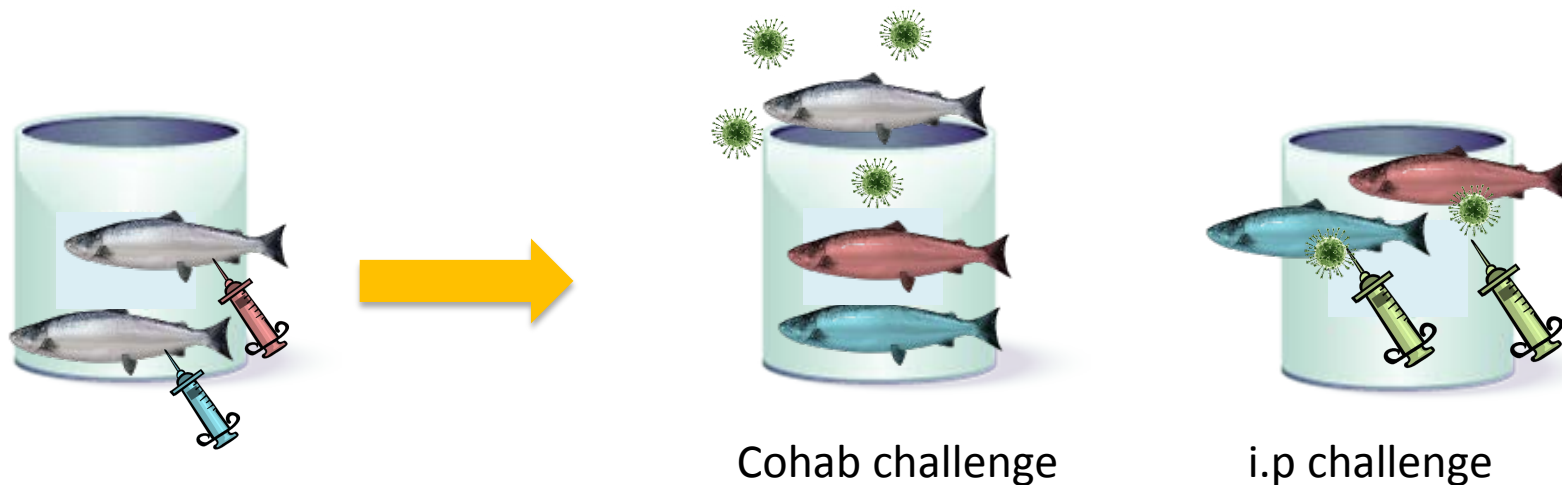
Prevalence of SPDV-positive - naïve fish added to the challenge tanks (serum)



➤ Approximately x5000 more SPDV detected in serum of PCR-positive naïve fish from saline tank than from PD7-tank

- SPDV shedded into the water is infectious
- Significant difference ($p < 0.001$) in number of positive naïve fish added to PD-vaccinated tank compared to un-vaccinated tank

Set-up for long term study for AQUAVAC PD7 (duration of immunity):



T=0

T= 26wpv

T= 33, 50 and 65 wpv

Two equal groups were established at T0:

- One group were vaccinated at T0 with AQUAVAC PD7
- One group were i.p injected with saline

At T= **26wpv**

- Fish were challenged by adding 20% shedders, injected with a Norwegian SPDV isolate

At T= **33, 50 and 65 wpv**

- Fish were challenged by i.p. injecting the fish with a Norwegian SPDV isolate

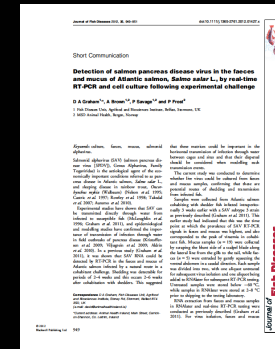
AQUAVAC PD7 reduces shedding of virus through feces up to 65 weeks post vaccination

SPDV positive
SPDV negative

* Time for challenge - weeks post vaccination
** Time for evaluation - weeks post vaccination



Read more about SPDV detection in feces:



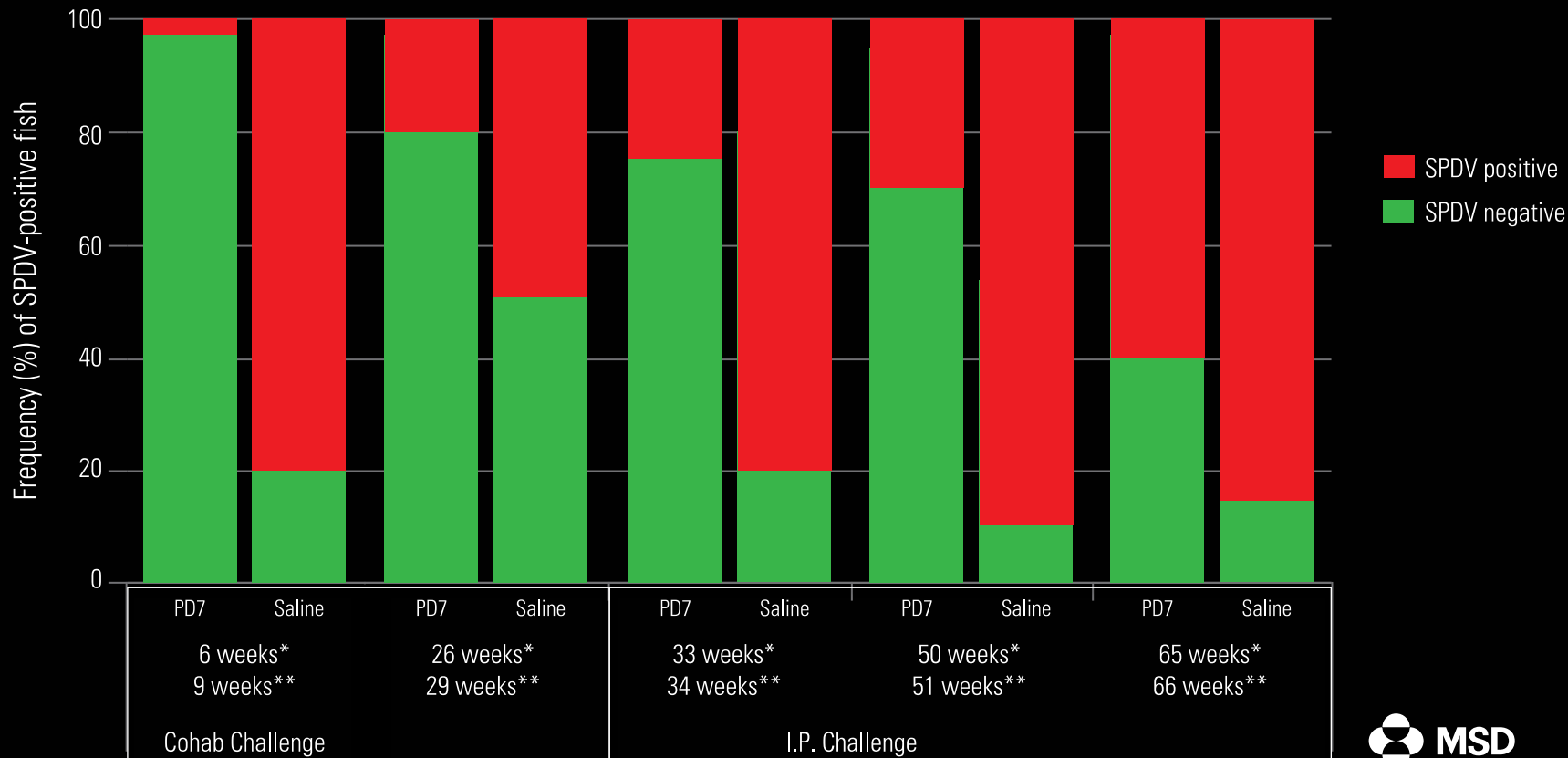
Graham DA, Brown A, Savage P, Frost P (2012a) Detection of salmon pancreas disease virus in the faeces and mucus of Atlantic salmon, *Salmo salar* L., by real-time RT-PCR and cell culture following experimental challenge. J Fish Dis

AQUAVAC PD7 reduced prevalence of SPDV positive fish (viremia) up to 65 weeks post vaccination

- SPDV-positive serum as determined by PCR

* Time for challenge - weeks post vaccination

** Time for evaluation - weeks post vaccination



AQUAVAC PD7 reduces shedding of infective virus to the environment

- **SPDV has a high potential for water-borne spreading following viral shedding from infected fish**
- **Vaccination reduces shedding of infective SPDV to the surrounding environment**
- **For maximum vaccination effect : a high ratio of the population at risk must be vaccinated (R_0 is unknown)**

Thank you for your attention!

Water sampling
requires the most
advanced technology



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