In search for a new vaccine against the salmon louse (Lepeophtheirus salmonis)

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Lepeophtheirus salmonis

The salmon louse is an ectoparasitic copepod, feeding on salmonid fish. Its life cycle is characterized by a high reproduction rate required for the low density of natural occurring hosts. However, the introduction of fish farming in the northern hemisphere with high densities of Atlantic salmon standing in coastal regions all year round has changed the host availability leading to an increased number of reproductive lice making *L. salmonis* a threat for wild salmon and sea trout and a major problem to the fish farming industry.



Live cycle of *L*.salmonis

Atlantic Salmon infected with salmon lice

Adult female L. salmonis



Introduction

Today both chemical and mechanical delousing methods against the salmon louse are used, which resulted in both resistance against common delousing agents as well as shows negative impact on the environment and fish The welfare. demand for the development of new solutions is high. Use of fish vaccines against bacteria and virus diseases has lead to strong

reduction medicine of use in aquaculture. Also a vaccine against the ectoparasitic salmon louse would be the best preventive measure to combat the parasite. However, to develop vaccines against parasites¹, especially ectoparasites is challenging. Until now there is only one commercial available vaccine against an metazoan ectoparasite.

Identification of suitable antigenic targets Problem

Generally demanding to develop vaccines against ectoparasites

- Limited contact area between parasite and the immune system of its host compared to bacteria, virus or enteroparasites
- □ Mechanism for suppression of hosts immune defence (e.g. antigenic variation;





hatching

counteracting the host's bioactive molecules haemostatic, secreted inflammatory and immune mechanisms) have to be bypassed

Requirement

- associated with some **vital function** of the louse
 - □ Antilice effects expressed as lice or egg/offspring mortality, decreased engorgement or egg string length, or inhibition of moulting

Type of antigen:

- **Exposed antigens** that are secreted in saliva during attachment and feeding on a host (proteins or peptides synthesized in the salivary glands, taken up at feeding site by host dendritic cells)
- **Concealed antigens** that are normally hidden from host immune mechanisms (encounters immunoglobulins, typically found on the gut wall and interact with specific immunoglobulins taken up in the blood meal)

Has to trigger the host immune defence

Approaches

Investigating the site of expression

- Gene expression data as source for candidate choice (LiceBase², earlier RNA seq- and microarray experiments, e.g. time series³, microarray tissue⁴, microarray maturation and egg production⁵)
- Confirmation with in situ

Importance

Vaccination and smoltification in common tank (individually marked fish), infection trial in single fish tanks

Results

Lice numbers

Gene expression

²LiceBase: stage

・この4らの~800~234

egg planktonic parasitic

attached

Importance confirmed by RNAi

Fig taken from

6-9) copepodids:

7) 1+2 days

8) 3+4 days

9) 5+6 days

10) chalimus

12) adult female

14) adult male

150

1) Unfert. eggs

4) Nauplia

5) Nauplia II

3) Fert. eggs 2-7d

Oocytes

2) Fert. eggs 0-24h 6) planktonic

≥100 C 50



Density of lice at sampling

Evaluation of reproduction

success

Length measurement,



No significant differences between groups in the amount of lice falling from the fish

Other lice parameters

Reproduction

No reduction could be seen, except a minor effect for test vaccine #2 against control (but not untreated). High variation in lice numbers lead to low statistical power.

D Engorgement



□ RNA interference studies^{6,7}

Gene knock-down of target gene and evaluation of fitness of the louse

Conclusions

- Targets were chosen which fulfilled the ad hoc requirements.
- □ Test vaccines were investigated in vaccine trials.
- However, none of the targets showed the desired effect on lice survival or
 - reproduction of the salmon louse under given circumstances.
- □ High variability in lice numbers lead to low power of the experimental set-up.



#3

#2

Fish parameter

0.5

Untr

Ctr

Effect on fish growth The vaccine did not have an effect on

#1

\succ No significant difference between groups in: Egg string length Hatching success

Engorgement of blood

Antigen production (ELISA)



ACKNOWLEDGEMENTS

This project was funded by the Norwegian Seafood Research Fund (project number 901510) and carried out in collaboration with the SFI-Sea Lice Research Centre (grant number 203513, Research Council Norway).



Sea Lice ADDOD. ADDA. Research Centre

REFERENCES

- 1) Maizels RM. Journal of biology 2009; 8(7):62
- 2) Lice Base: https://licebase.org
- 3) Eichner C, Dondrup M, Nilsen F. Journal of Fish Diseases 2018; 41(6):1005-1029.
- 4) Edvardsen RB, Dalvin S, Furmanek T, Malde K, Maehle S, Kvamme BO, Skern-Mauritzen R. Mar Genomics 2014; 18 Pt A:39-44
- 5) Eichner C, Frost P, Dysvik B, Jonassen I, Kristiansen B, Nilsen F. BMC Genomics 2008(9):126. RNAi
- 6) Dalvin S, Frost P, Biering E, Hamre LA, Eichner C, Krossoy B, Nilsen F. International Journal for Parasitology 2009; 39(13):1407-1415.
- 7) Eichner C, Nilsen F, Grotmol S, Dalvin S. Experimental Parasitology 2014; 140:44-51.
- 8) Heggland, E. I., C. Tröße, C. Eichner, et al., 2019a. Molecular and Biochemical Parasitology, 232, 111197.
- 9) Heggland, E. I., C. Eichner, S. I. Stove, et al., 2019b. Sci Rep, 9(1), 4218.
- 10) Harðardóttir, H. M., R. Male, F. Nilsen, et al., 2019. Comparative Biochemistry and Physiology Part As
- Molecular & Integrative Physiology, 227, 123-133.
- 11) Hamre, L. A., K. A. Glover and F. Nilsen, 2009. Parasitology International, 58(4), 451-460.

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fish growth

