



ParaFishControl

A recombinant vaccine targeting the fish parasite *Ichthyophthirius multifiliis*

**ParaFishControl Workshop “North European Fish Parasite
Management Strategies in Aquaculture Farms”**

Berlin, 9th October 2019

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Challenge and Impact

➤ Challenge

Ichthyophthirius multifiliis (*Ich*) is a unicellular parasite that infects almost all freshwater fish species - production fish as well as ornamental fish.

It infects gills, skin and fins and during heavy infections the fish may die.

Within a few days the parasite multiplies intensively depending on temperature.

➤ Impact

Ich causes high mortality in both production and ornamental fish species.

Without treatment, it can kill whole productions within a short time.

Economic losses for the fish farmers both due to fish loss and work labour.

Bottleneck for a production increase of commercial important fish species.

Our approach and our team

- **Brief description of the proposed solution**

AIM: Use artificial intelligence identifying protective antigens to develop a recombinant sub-unit vaccine against the fish parasite *I. multifiliis*

- **Team involved in the development**

- Laboratory of Aquatic Pathobiology, the Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark.
- Department of Cancer and Inflammation Research, University of Southern Denmark, Denmark.
- Evaxion Biotech, Denmark.
- Centre for Medical Parasitology at the Department of Immunology and Microbiology, University of Copenhagen, Denmark
- W42 GmbH, Germany
- Technical University of Denmark, Aqua, Denmark



Our proposed solution (I - Approach) ParaFishControl

A novel technology (EDEN) identifies B-cell antigens using neural networks. The neural network trains on already known B cell antigens – and learns to identify them

The *I. multifiliis* proteome was screened

12 proteins as potential vaccine candidates were considered (out of 7181 proteins)

3 proteins were selected for the experiment based on functionality, subcellular location and putative protective structural domain – and successful recombinant expression



Our solution vs currently employed solutions



➤ Our solution

We need to improve the vaccine

An efficient vaccine presents a safe and efficient way of controlling *Ich*

Cost? Difficult to estimate but mass production in *Pichia* should in theory not be expensive

Accuracy? This vaccine should target all serotypes of *Ich* and no other organisms

➤ Currently employed solutions

Repeated treatments with formalin, hydrogen peroxide and peracetic acid



Our solution has been published
Please check for additional info:



Received: 3 March 2017 | Revised: 6 April 2017 | Accepted: 6 April 2017

DOI: 10.1111/jfd.12653

ORIGINAL ARTICLE

WILEY *Journal of*
Fish Diseases 

Rainbow trout (*Oncorhynchus mykiss*) immune response towards a recombinant vaccine targeting the parasitic ciliate *Ichthyophthirius multifiliis*

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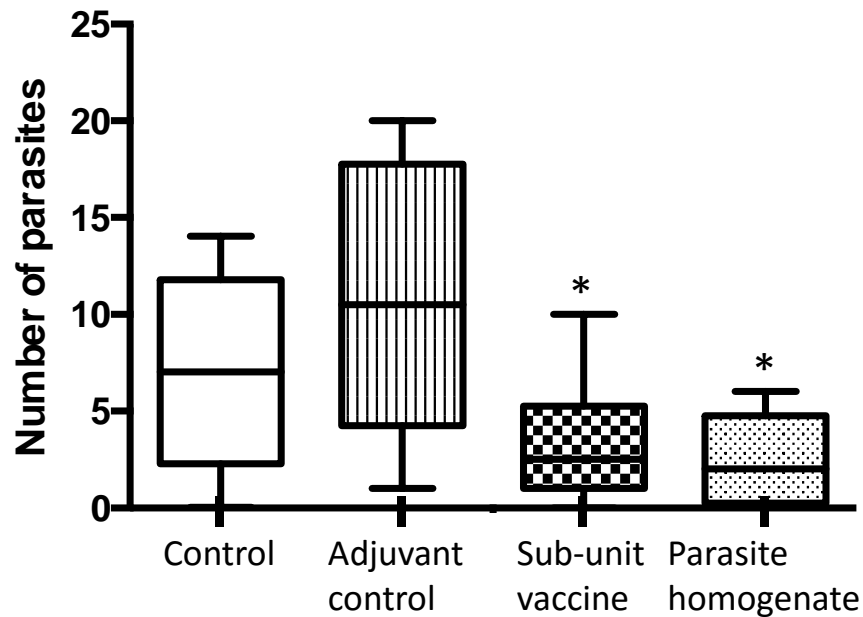
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Abstract

The protective effect in rainbow trout (*Oncorhynchus mykiss*) of an experimental subunit vaccine targeting antigens in the parasite *Ichthyophthirius multifiliis* has been evaluated and compared to effects elicited by a classical parasite homogenate vaccine. Three recombinant parasite proteins (two produced in *E. coli* and one in insect cells) were combined and injected i.p., and subsequently, protection and antibody responses were analysed. Both the experimental and the benchmark vaccine induced partial but significant protection against *I. multifiliis* when compared to con-



Our proposed solution (II - Results)



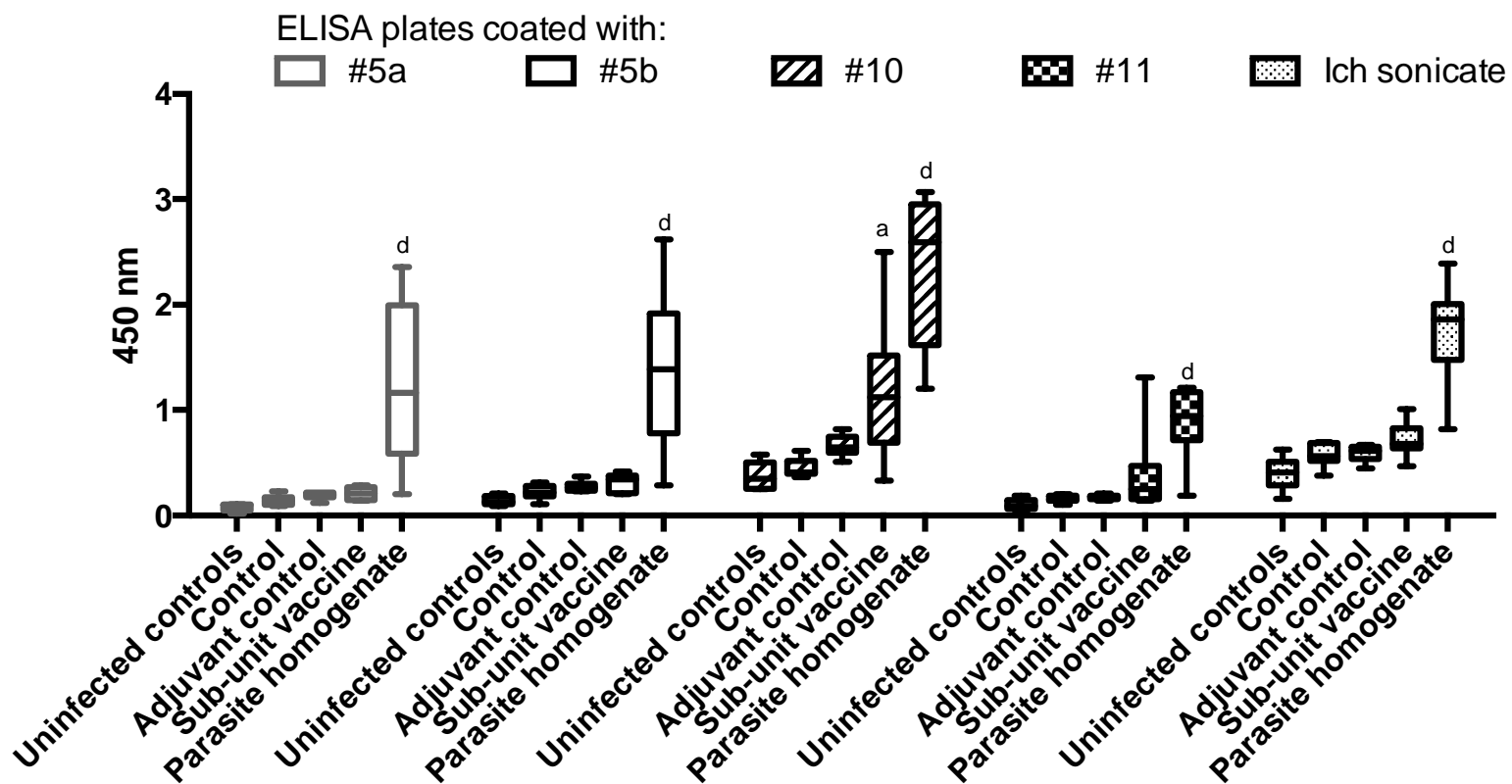
Plasma from the group of fish injected with sub-unit vaccine and parasite homogenate immobilized the infective stage of the parasite moderately. Plasma from control groups did not immobilize the parasites whereas an immune fish (following a natural infection) completely immobilized the parasites.



Moderate protection

Our proposed solution (III)

13 wpi, dilution 1:100



- a = significantly different from uninfected control and control
- b = significantly different from uninfected control and control and adj control
- c = significantly different from uninfected control
- d = significantly different from all other groups

Solution: Use recombinant #10 and further develop the vaccine for a prophylactic approach to protect fish against *Ich*

Expected benefits for the industry

- Prophylactic approach is more feasible than treatments
- Improved health status of European farmed fish
- Improved welfare in fish farms
- Improved sustainability of European aquaculture
- Increased productivity in European fish farms
- Elevated economic result in European aquaculture

Current status and next steps

Patent: EP2017/064899

Improving immunogenicity of the antigens and efficiency of the vaccine

- Discover and include additional novel immunogenic recombinant antigens
- Use a more efficient adjuvant, for example Virus-Like Particles (VLPs)

In collaboration with a company (W42) we have recombinantly expressed #10 in *Pichia pastoris*. The construct is being improved to increase expression.

In collaboration with the Technical University of Denmark we have identified a potential immunogenic epitope from a known protective protein lag52b. lag52b is very problematic to express in its full length recombinantly.

We have fused the epitope to VLP for recombinant expression in *P. pastoris*. The epitope should theoretically be presented in a virus-like conformation and induce an immune response. Expression of this construct has been successful and we are currently running a vaccine trial with #10 and lag52b-VLPs.

Conclusions

We have discovered a novel protective antigen from *Ich* that induce moderate protection in rainbow trout

The vaccine needs improvement and we have recombinantly expressed #10 and an epitope from a known antigen fused to VLPs in *Pichia pastoris*.

Vaccination trials are ongoing

With a cheap vaccine against *Ich* the industry would benefit economically and fish welfare would increase.

Thank You



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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.