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ERM immersion vaccination and adjuvants

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DAFINET AND PROFISH
WORKSHOP
PATHOGEN – HOST INTERACTIONS AND VACCINE EFFECTS

NOVEMBER 17TH AND 18TH, 2015

Venue:

University of Copenhagen
Frederiksberg Campus
1870 Frederiksberg C
Denmark

Organized by:

DAFINET www.dafinet.dk

The Graduate School for Immunology and Infectious Diseases, KU-SUND

ProFish & TargetFish

Book of abstracts

DAFINET and ProFish Workshop, November 2015
University of Copenhagen

The workshop is supported by:

DAFINET, Danish Fish Immunology Center and Network

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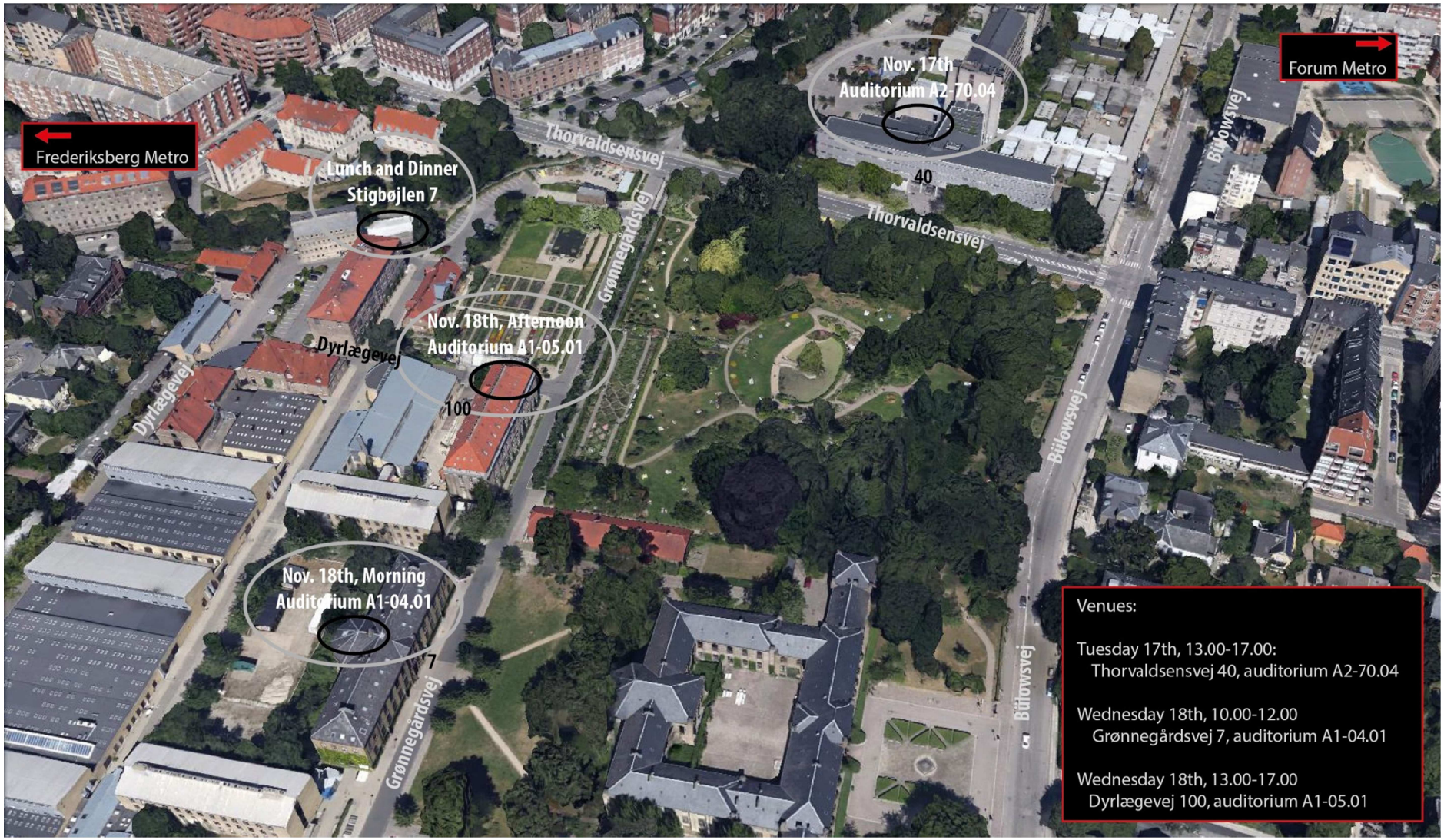
DAFINET AND PROFISH WORKSHOP
PATHOGEN – HOST INTERACTIONS AND VACCINE EFFECTS

NOVEMBER 17TH TO 18TH, 2015

Invited speakers:

Dina Zilberg	**Israel**
Jorge Galindo-Villegas	**Spain**
Louise Jørgensen	**Denmark**
Niels Jørgen Olesen	**Denmark**
Niels Lorenzen	**Denmark**
Scott LaPatra,	**USA**
Sonal Patel	**Norway**
Uwe Fisher	**Germany**


Frederiksberg Metro
Forum Metro
Nov. 17th
Auditorium A2-70.04
Lunch and Dinner
Stigbøjlen 7
Thorvaldsensvej
40
Thorvaldsensvej
Grønnegårdsvej
Nov. 18th, Afternoon
Auditorium A1-05.01
Dyrlægevej
Dyrlægevej
100
Bülowsvej
Bülowsvej
Nov. 18th, Morning
Auditorium A1-04.01
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Grønnegårdsvej
Bülowsvej
Venues:
Tuesday 17th, 13.00-17.00:
Thorvaldsensvej 40, auditorium A2-70.04
Wednesday 18th, 10.00-12.00
Grønnegårdsvej 7, auditorium A1-04.01
Wednesday 18th, 13.00-17.00
Dyrlægevej 100, auditorium A1-05.01

Venues:

All venues are placed at the University of Copenhagen, Frederiksberg Campus. Please see the specific addresses below and the map for physical location on the previous page.

Tuesday 17th, 13.00-17.00:
Auditorium A2-70.04; Thorvaldsensvej 40, 1870 Frederiksberg, Denmark

Wednesday 18th, 10.00-12.00:
Auditorium A1-04.01; Grønnegårdsvej 7, 1870 Frederiksberg, Denmark

Wednesday 18th, 13.00-17.00:
Auditorium A1-05.01; Dyrlægevej 100, 1870 Frederiksberg, Denmar

Lunch and dinner:
Seminar Rooms; Stigbøjlen 7, 1870 Frederiksberg, Denmark

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The Graduate School for Immunology and Infectious Diseases, KU-SUND

PROFISH supported by the Danish Strategic Research Council

In collaboration with TARGETFISH supported by the EU commission (FP7)

Program - Tuesday November 17th, 2015

13:00 Welcome address by DAFINET lea der Kurt Buchmann
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark

13:05 Sonal Patel
Institute of Marine Research, Bergen, Norway
Immunity to Pancreas Disease in salmon

13:50 Scott LaPatra
Clear Spring Foods Inc., Buhl, Idaho, USA
The nasopharynx-associated lymphoid tissue of rainbow trout and the potential as a vaccine delivery site

14:30 **Coffee break**

14:45 Niels Henrik Henriksen
Danish Aquaculture, Denmark
Control and treatment of parasitic diseases in Danish Aquaculture

15:15 Uwe Fischer
Friedrich Loeffler Institute, Insel-Riems, Germany
Cellular immunity against ERM and furunculosis

16:00 **Coffee break**

16:30 Niels Lorenzen & Helle Frank Skall;
Department of Animal Science, University of Aarhus, Denmark
Danish sea reared rainbow trout suffer from furunculosis despite vaccination - How can applied research help to solve the problem?

17:00 Moonika Haahr Marana
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Experimental furunculosis vaccines for Rainbow trout

17:15 Simona Bartkova
National Veterinary Institute, Technical University of Denmark,
Real-time *in vivo* imaging of bioluminescent *Aeromonas salmonicida* in rainbow trout

17:30 Rezgar M. Jaafar
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Effects of adjuvant MontanideTM ISA 763 A VG in rainbow trout injection vaccinated against *Yersinia ruckeri*

17:45 **Wrap up and discussion**

18:30 **Dinner**

Program - Wednesday November 18th, 2015

10:00 Jorge Galindo-Villegas
Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, Spain.
Selective manipulation of the gut microbiota improves immune status in vertebrates

10:45 **Coffee break**

11:15 Niels Jørgen Olsen
National Veterinary Institute, Technical University of Denmark,
VHS virus – present situation

11:45 Kasper Rømer Villumsen
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Th1/Th2/Th17 switches and adjuvants

12:00 **Lunch**

13:00 Louise von Gersdorff Jørgensen
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Antigen uptake in zebrafish (*Danio rerio*)

13:30 Simon Haarder
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Inflammation – zebrafish as a model

13:45 Azmi Al-Jubury
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Characterization of IgD, IgT and IgM postive immune cells associated with *Ichthyophonus* induced granulomas in rainbow trout

14:00 **Coffee break**

14:30 Dina Zilberg
Ben Gurion University, Midreshet Ben Gurion, Israel
Immunization against *Tetrahymena* infection in guppies: a model for systemic parasitosis

15:00 Jacob G. Schmidt,
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
Diluted booster vaccination against enteric red mouth disease in rainbow trout – field trials

15:15 Niccolo Vendramin
National Veterinary Institute, Technical University of Denmark,
Virus Y-PRV2 a new piscine orthoreovirus in rainbow trout: establishment of challenge model and long term pathogenetic study

15:45 Jakob Skov, University of Copenhagen, Denmark
Laboratory of Aquatic Pathobiology, University of Copenhagen, Denmark
ERM immersion vaccination and adjuvants

16:00 **Wrap up, final discussion and conclusions**

Immunity to Pancreas Disease in salmon

Sonal Patel

Institute of Marine Research, Bergen, Norway

Pancreas disease (PD) caused by salmon pancreas disease virus (SPDV), also known as salmonid alphavirus (SAV), is a serious problem in the salmon farming industry in Northern Europe. Clinical cases are mostly seen at the post-smolt stages, and disease intensity depends on several factors such as SAV sub-type, size of fish, immune status, and whether the smolts were set to sea during autumn or spring season. Also, different SAV sub-types have shown varying geographic distribution. Studies covering infection of SAV and vaccination against SAV in salmonids, mostly salmon, but also trout have been carried out in freshwater stages, with very few studies in the sea-water phase. SAV1 infection in freshwater seems to induce early induction of genes associated with immune and defence mechanisms, including genes involved in interferon I and II pathways, and MHC class I and II responses. Histopathological changes in heart and pancreas during early period, and in muscle at later time-points are evident upon SAV infection, and immune cells infiltrating these tissues can be detected. SAV neutralizing antibodies can be detected indicating induction of humoral responses.

The general hypothesis is that the smolt and post-smolt stages are expected to be “leaky” and thus more susceptible to pathogens. Since, salmon often get PD during spring-summer period, which is very soon after sea-water transfer, especially for the post-smolts set out in spring, knowledge about how the biophysical parameters, along with physiological and immunological status of the salmon affects susceptibility to SAV infection during these stages is important. Understanding the mechanisms of SAV infection during a natural route of infection in sea water phase, and immune response to SAV at the cellular and molecular level will be valuable for future planning of preventive measures

Presenting author: Sonal Patel; Sonal.patel@imr.no

The nasopharynx-associated lymphoid tissue of rainbow trout and the potential as a vaccine delivery site

Scott LaPatra[1] and Irene Salinas[2]

[1]Clear Springs Foods, Inc., Research Division, Buhl, Idaho, USA
[2]Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, New Mexico, USA

The nasopharynx-associated lymphoid tissue (NALT) has only been identified in birds and mammals and is thought to be the first line of defense against airborne pathogens. However, the olfactory organ of aquatic vertebrates such as teleost fish is also exposed to waterborne pathogens. We hypothesized that NALT is an ancient and conserved arm of the mucosal immune system of all vertebrates, terrestrial and aquatic. Using rainbow trout as a model, we characterized the main features of teleost NALT at the cellular and molecular level. Trout NALT follows the main canonical features present in other fish mucosa-associated lymphoid tissues including diffuse lymphoid cell populations, a preponderant number of IgT B cells and IgT levels in mucus as well as a diverse associated bacterial community (microbiota). Our results reveal for the first time that NALT is an important arm of the mucosal immune system of fish.

Nasal vaccines are commonly used in domestic and farmed terrestrial animals and humans. Nasal vaccines require small amounts of antigen and stimulate both local and systemic immune responses. Based on our findings we aimed to evaluate the effectiveness of two previously developed vaccines in rainbow trout, a live attenuated infectious hematopoietic necrosis virus vaccine (IHNV) and a killed enteric red mouth (ERM) bacterin. The nasal route was compared to the immersion and injection routes in a series of different experiments. Additionally, delivery of both vaccines into a single nare or each vaccine into separate nares was also tested. Challenge to live pathogens at 7 and 28 days post-vaccination revealed that delivery of IHNV into the left nare and ERM into the right nare is the most effective of all the vaccination regimes tested, including injection of IHNV and immersion with ERM vaccines. Our results demonstrate the nasal vaccines are novel and a potentially new way to effectively control aquatic infectious diseases.

Presenting author: Scott LaPatra; scottl@clearsprings.com

Control and treatment of parasitic diseases in Danish Aquaculture

Niels Henrik Henriksen

The Danish Aquaculture Organisation

Within Europe there is no medicines licensed against fish parasites such as *Ichthyobodo necator*, *Ichthyophthirius multifiliis* and amoebae. Since the ban of malachite green, formaldehyde has been used widely as water disinfectant against these parasites. Due to the carcinogenic properties of formaldehyde Danish researchers have been looking for alternatives. One of the most promising substances has shown to be peracetic acid (PAA). PAA has proven effective in controlling several kinds of parasites on rainbow trout and in fact also *Saprolegnia* on eggs. However, the use of the substance is not as easy as formaldehyde. PAA degrades rapidly, especially in water rich in organic matter. Repeated or continuous dosaging is needed.

Presenting author: Niels Henrik Henriksen; niels@danskakvakultur.dk

Cellular immunity against ERM and furunculosis

T. Yamaguchi[1], G. Kato[2], V. Soto-Lampe[1], H. Miyazawa[2], U. Fischer[1]*

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[2]Tokyo University of Marine Science and Technology, Japan

Enteric red mouth (ERM) disease and furunculosis are important bacterial diseases in rainbow trout aquaculture. While ERM is caused by *Yersinia ruckeri* the causative agent of furunculosis is *Aeromonas salmonicida*. While a number of publications have clearly shown humoral immune responses to bacterial diseases in fish, little is known about the impact of cellular immunity. Cell-mediated cytotoxicity is one of the options the immune system can mount against intracellular infections particularly in viral diseases. However, a facultative intracellular lifestyle has also been suggested for *Y. ruckeri* and *A. salmonicida*.

Here we have used two approaches to investigate the role of cellular immunity in ERM and furunculosis: (1) adoptive transfer of immune cells from vaccinated donors followed by challenge of the recipients and (2) *in vitro* cell-mediated cytotoxicity of immune cells from vaccinated donors against bacterial infected target cells, respectively. Further, bacterin uptake by the gills has been studied for *A. salmonicida*.

As for ERM we have isolated PBL from bath vaccinated clonal trout donors and transferred to naïve genetically identical clonal fish recipients followed by challenge of the latter with live *Y. ruckeri* bacteria. All fish that have received PBL from vaccinated donors survived the challenge infection while control fish injected with leukocytes from non-vaccinated trout exhibited high mortality. Flow cytometry analysis of recipient leukocytes suggest that $CD8^+$ and IgT^+ cells from donors have a preference to home to or to proliferate in the recipient gills. Transfer of memory B cells has been concluded from the fact that IgM titres were higher in *Y. ruckeri* infected recipients injected with immune cells from vaccinated donors when compared to control fish. While this is the first report on adoptive cellular transfer of immunity in a bacterial infection in fish, our data further suggest that immunity in ERM is rather conferred by IgM^+ B cells.

Concerning furunculosis, a new cell line matched in its MHC class I to our clonal trout has been established and intracellularly infected along with an MHC class I mismatched cell line with *A. salmonicida* bacteria. Cell-mediated cytotoxicity of leukocytes isolated from vaccinated donors was rather moderate and NK-like.

In addition, we have performed *A. salmonicida* bacterin uptake studies in the gills. Our flow cytometry and histology data have shown that killed *A. salmonicida* bacteria are taken up by M-like cells based on their lectin staining pattern and that antigen uptake took place in the direct neighbourhood of the interbranchial lymphoid tissue (ILT).

Presenting author: Uwe Fisher, uwe.fischer@fli.bund.de

Danish sea reared rainbow trout suffer from furunculosis despite vaccination - How can applied research help to solve the problem?

H.F. Skall[1], E. Lorenzen[1], T.E. Kjær[1], N.H. Henriksen[2], I. Dalsgaard[3], S.B. Madsen[4], K. Buchmann[5], B. Krossøy[6], N. Lorenzen[1]

[1]Department of Animal Science, Aarhus University, Denmark
[2]Danish Aquaculture, Silkeborg, Denmark
[3]National Veterinary Institute, DTU, Denmark
[4]S. B. Madsen Veterinary Service, Denmark
[5]Department of Veterinary Disease Biology, University of Copenhagen, Denmark
[6]Vaxxinova Norway, Bergen, Norway

Despite vaccination by intraperitoneal injection with oil-adjuvanted vaccines against vibriosis and furunculosis, sea reared rainbow trout in Denmark often develop furunculosis and occasionally vibriosis during warm summer periods. This implies an excessive use of antibiotics and has also decreased some fish farmer's confidence in the commercially available vaccine. This vaccine comprises *Aeromonas salmonicida* subspecies *salmonicida* and *Vibrio anguillarum* serotypes O1 and O2a bacterins emulsified with mineral oil. The bacterin antigens are based on bacteria isolated from Atlantic salmon cultured outside Denmark.

Vaccination and challenge trials performed under experimental conditions suggest that the commercial vaccine provides good protection against challenge with *Aeromonas salmonicida* when the fish are exposed to the bacteria by injection. However, the protection is far less significant when the fish are challenged by cohabitation with infected donor fish followed by elevation of the water temperature. The results demonstrate the importance of optimizing the challenge procedure for evaluation of vaccine efficacy under experimental conditions. In terms of *Vibrio anguillarum* the commercial vaccine failed to protect the fish against an serotype O2 isolate from diseased fish, suggesting that tailoring the antigen composition to Danish/local bacterial variants is needed. Serological examination of the vaccine induced antibody response in the fish is in progress aiming at evaluating the contribution of the humoral immune response to protection as well as at development of an *in vitro* tool for evaluation of vaccine potency.

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Experimental furunculosis vaccines for Rainbow trout

M.H. Marana[1], J. Skov[1], J.K. Chettri[1], B. Krossøy[2],*
I. Dalsgaard[3], N. Lorenzen[4]; P.W. Kania[1], K. Buchmann[1]

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[2]Vaxxinova Norway AS, Bergen, Norway
[2]National Veterinary Institute, Technical University of Denmark, Denmark
[4]Department of Animal Science, Aarhus University, Denmark

Rainbow trout*, Oncorhynchus mykiss* were vaccinated by intraperitoneal (i.p.) injection with vaccines containing bacterin alone or bacterin supplemented with experimental vegetable oil-based adjuvant or a paraffin oil adjuvant. The bacterin consisted of formalin killed bacteria of *Aeromonas salmonicida* subsp. *salmonicida* strain 090710-1/23 in combination with *Vibrio anguillarum* serotypes O1 and O2a. Fish were challenged with the same bacterial strain of *A. salmonicida* either by i.p. or by applying a novel challenge method. Skin lesions were made by puncturing the caudal fin epidermis followed by layering the bacterial culture of *A. salmonicida* on the puncture site for 60 seconds. This challenge method mimics that rainbow trout in fish farms might be infected with *A. salmonicida* through injured epidermis (fin biting) and our new method resembles closely the natural infection route where bacteria gain access to fish through the lesions. The protection, antibody production and side-effects were examined during the trial. The bacterin administered with adjuvant elicited protection and antibody production that was dependent on the antigen concentration in the vaccine. The new infection model proved to be efficient in inducing a more natural disease progression in fish and a stable mortality. The method can differentiate efficacies of different vaccines with regard to adjuvant formulations and content of antigen.

Presenting author: Moonika Harhr Marana; mmarana@sund.ku.dk

Real-time *in vivo* imaging of bioluminescent *Aeromonas salmonicida* in rainbow trout

S. Bartkova, B. Kokotovic, I. Dalsgaard

National Veterinary Institute, Technical University of Denmark, Denmark

Aeromonas salmonicida susbp. *salmonicida,* the causative agent of the disease furunculosis, causes great problems in Danish sea reared rainbow trout (*Oncorhynchus mykiss*) production. Outbreaks occur repeatedly during elevated temperatures and fish harboring *A. salmonicida* can also be covertly infected. A crucial factor for preventing spread of furunculosis is therefore gaining more knowledge of the covert stage, which includes knowing the route of entry and dissemination of the pathogen in the fish. To determine this, one could trace the bacterium using *in vivo* bioluminescence imaging (BLI) that enables visualization and quantification of infections within the same animal over several time points. In this research BLI was used to follow the infection of a virulent Danish *A. salmonicida* isolate transformed with pAK*gfplux*1; a plasmid vector containing the *gfpmut3a* gene from *Aequorea victoria* and the *luxCDABE* operon from *Photorhabdus luminescens*. Since *A. salmonicida* pAK*gfplux*1 displayed similar growth and pathogenicity as the wild type *A. salmonicida*, the transformed bacterium could be used to illustrate a progression of an *A. salmonicida* infection. Fish were infected with *A. salmonicida* pAK*gfplux*1 through an immersion bath and followed over a 24 h period using an IVIS® Spectrum system. Results showed a pattern of favourable pathogen attachment sites, internal passageway and main exit site that will be shown and discussed in the presentation.

Presenting author: Simona Bartkova; sibar@vet.dtu.dk

Effects of adjuvant Montanide™ ISA 763 A VG in rainbow trout injection vaccinated against *Yersinia ruckeri*

R.M. Jaafar[1], J.K. Chettri[1], I. Dalsgaard[2], A. Al-Jubury[1], P.W. Kania[1], J. Skov[1], K. Buchmann[1]

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Enteric redmouth disease (ERM) caused by the fish pathogen *Yersinia ruckeri* is a major threat to freshwater production of rainbow trout (*Oncorhynchus mykiss*) throughout all life stages but vaccination may be a way to control the disease. Injection vaccination of rainbow trout against *Y. ruckeri* infection has been shown to confer better protection compared to the traditionally applied immersion vaccination. It may be hypothesized, based on experience from other vaccines, that adjuvants may increase the protective level of ERM injection vaccines even more.

Controlled comparative vaccination studies have been made to investigate effects of the oil adjuvant Montanide™ ISA 763 A VG (Seppic) when added to an experimental *Y. ruckeri* bacterin (containing both biotype 1 and 2 of serotype O1). Duplicated tanks (2 × 100 fish per group) of fish (mean weight 19 g) were used and tested for protection of 1) non-vaccinated control fish, 2) fish injected with a commercial vaccine (AquaVac® Relera™), 3) fish injected with an experimental vaccine, 4) fish injected with an experimental vaccine + adjuvant and 5) fish injected with adjuvant alone.

It was shown that injection of an experimental vaccine composed of formalin killed *Y. ruckeri* (biotype 1 and 2, serotype O1) (both adjuvanted and non-adjuvanted) conferred a high level of immunity to rainbow trout. Additional experiments using higher challenge dosages showed that the protection was increased by the adjuvant component which in itself induced protection. Injection of an experimental vaccine + adjuvant induced a higher level of antibody in serum, both before challenge and at day 21 following challenge, when compared to non-vaccinated control fish. Expression levels of immune-related genes were more prominent in spleen and liver than in head kidney, but no clear skewing of the immune response towards a Th1, Th2, Th17 or Treg type was seen.

Presenting author: Rzgar M. Jaafar; rezgarhadad@sund.ku.dk

Selective manipulation of the gut microbiota improves immune status in vertebrates

Jorge Galindo-Villegas

Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, Spain.

All animals develop in association with complex microbial communities. It is now well established that commensal microbiota is essential for the correct functionality of each organ in the host. Particularly, the commensal gastro-intestinal microbiota (CGIM) is a key factor for development, immunity and nutrient conversion, rendering them bio-available for various uses. Thus, nutritional inputs generate a positive loop in maintaining host health and are essential in shaping the composition of the CGIM communities. Probiotics, which are live exogenous microorganisms, selectively provided to the host, are a promising concept for manipulating the microbiota and thus for increasing the host health status. Nevertheless, most mechanisms induced by probiotics to fortify the immune system are still a matter of debate. Alternatively, prebiotics, which are non-digestible food ingredients, can favor the growth of specific target groups of CGIM. Several metabolites are produced by the CGIM, one of the most important are the short-chain fatty acids (SCFAs), which emerge from the fermentation of complex carbohydrates. SCFAs have been recognized as key players in triggering beneficial effects elicited by simple diffusion and by specific receptors present thus far only in epithelial cells of higher vertebrates at different GI locations. However, both strategies have shown to provide resistance against pathogens during periods of high stress. In fish, knowledge about the action of pro- and prebiotics and SCFAs is still limited.

Thus, in this talk, I will briefly summarize the mechanisms described on this topic for higher vertebrates and discuss why many of them may operate in the fish gut representing a model for different mucosal tissues.

Keywords: Fish, Host-Microbe, Humans, Immunity, Microbiota, Prebiotics, Probiotics, SCFA, Vertebrates.

Presenting author: *Jorge Galindo-Villegas,* jorge-galindo@usa.net

VHS virus – present situation

Niels Jørgen Olesen[1] and Helle Frank Skall[2]

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[1]Department of Animal Science, Aarhus University, Denmark

Besides being the EU Reference Laboratory for Fish Diseases the fish health section at DTU is also appointed as the OIE reference laboratory for VHS and has as such obligations to keep updated on the emergence and evolution of VHS worldwide.

Geographic distribution:

VHSV can be divided into 4 genotypes and at least 8 subtypes and there is a close linkage between genotypes, geographic range and affected fish species. VHS is still only reported from the Northern hemisphere- and while countries like Denmark, Norway and England have freed themselves for VHS, several countries are still struggling with the disease. An update on the recent VHS outbreaks in rainbow trout in Iran, in olive flounder in Korea, in wrasse in Scotland, in turbot in Turkey, in a number of fish species in the great lakes in USA and Canada, and a general overview of the worldwide distribution of the disease will be given.

Virus evolution:

Recent studies indicate that only a few amino acid changes in the structural proteins of VHSV can change the virulence patterns significantly, thereby coming closer to assessing the risk of none to low virulent viruses becoming high virulent. Virulence factors both depend on the ability of VHSV to enter a cell and on the speed and efficiency of virus replication in the cells. Apparently the viral nucleocapsid protein plays a very important role for the later and seems to be the target for determination of a virulence marker.

Presenting author: Niels Jørgen Olesen; njol@vet.dtu.dk

Can the Choice of Adjuvant Direct T-cell Responses, Minimize Adverse Effects and Still Confer Protection?

K.R. Villumsen[1], D. Christensen[2], E.O. Koppang[3] & A.M. Bojesen[1]

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[2] Vaccine Research & Development, Infectious Disease Immunology, Statens Serum Institut
[3] Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences

For the past two decades, mineral oils have been utilized as a potent adjuvant when formulating injection furunculosis vaccines for intraperitoneal administration in aquaculture of salmonid fishes. Over this period, several field, as well as laboratory studies have demonstrated that these adjuvants improve protection against furunculosis, caused by *Aeromonas salmonicida* subsp. *salmonicida*. However, studies have also demonstrated a range of severe adverse effects associated with the use of mineral oils in fish vaccine formulations, a consequence that warrants further exploration of alternatives to the current *status quo*.

Working with injection vaccines against furunculosis in rainbow trout, this current project aims to shed light on two main questions:

1. Considering a vaccine formulated with mineral oil as the benchmark, would it be possible to obtain a level of protection equal to, or higher than, that benchmark, while at the same time reducing the adverse effects, through the use of adjuvants with a higher expected safety profile?
2. Could the use of specific adjuvants help to direct the T-cell response towards specific Th-profiles, potentially proving one to be superior with regards to protection?

Three formulations of *A. salmonicida* bacterin with either CpG oligodeoxynucleotides, Freund's incomplete adjuvant or cationic adjuvant formulation 01 (CAF01) were prepared to approach these questions. Along with relevant control groups, the vaccine groups are now subjected to an experimental protocol assessing the nature of the respective leukocyte responses, adverse effects, antibody responses, as well as protection from infection of each group. Hopefully, the results will provide valuable insight into the optimal induction of the immune response of rainbow trout, and help to identify safer alternatives to the current adjuvants, paving the way to the formulation of the next generation of furunculosis vaccines for commercially farmed rainbow trout.

Presenting Author: Kasper Rømer Villumsen; krv@sund.ku.dk

Antigen uptake in zebrafish (*Danio rerio*)

L.v.G. Jørgensen, F. Mehrdana, P.W. Kania, M.H. Larsen, D. Frees and K. Buchmann

Department of Veterinary Disease Biology, University of Copenhagen, Frederiksberg, Denmark

In aquaculture, immersion-vaccines are routinely used in rainbow trout against *Yersinia ruckeri* (Yr). The vaccines consist of dead bacteria (bacterin) and following immersion lasting at least 30 seconds, rainbow trout take up the dead bacteria and following 400 degree days they acquire a level of protection. We have used zebrafish as a model organism to study uptake mechanisms and subsequent transport in fish. A gene-modified Yr, developed to constitutively express GFP (green fluorescent protein), was used for bacterin production. Transparent zebrafish (Tra:nac) were immersed in this bacterin for up to 30 minutes. Samples were taken after 1 min, 15 min, 30 min, 2 h, 12 h and 24 h. At each sample point two fish were used for live imaging using a stereomicroscope with UV filters, and three fish sampled for immunohistochemistry (IHC). Results showed that bacteria initially were visible in scale pockets, sometimes on the skin, in the esophagus, in the intestine and in a few instances on the fins. However, within two hours Yr-antigens were visible in the spleen and within 24 hours also in liver and kidney. Bacteria were found to be associated with the gills but were not seen penetrating gill epithelium or inside cells of the gills. These results are in alignment with studies conducted on rainbow trout, and zebrafish may therefore serve as a suitable model for rainbow trout. The next step in this investigation will include the cellular mechanisms responsible for antigen transport.

Presenting author: Louise von Gersdorff Jørgensen; lvgj@sund.ku.dk

Inflammation – zebra fish as a model

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An overview of our in-house developed zebra fish model of inflammatory bowel disease (IBD), a chronic human disease resulting in significantly reduced quality of life, will be given. A number of different techniques have been employed in order to correctly characterize the model, including quantitative-real time PCR (qPCR), Optical Projection Tomography (OPT), goblet cell counting and protein expression by 2-dimensional gel electrophoresis. The findings will be related to other inflammation studies using experimental animals, and the advantages as well as the pitfalls of the model will be discussed. Finally, some thoughts regarding the translatability of the obtained data to the human condition are presented.

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Characterization of IgD, IgT and IgM postive immune cells associated with *Ichthyophonus* induced granulomas in rainbow trout

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Infections with *Ichthyophonus* sp. cause a serious and granulomatous disease in rainbow trout *Oncorhynchus mykiss* affecting several organ systems. The pathology associated with the parasite infection has been described, but no details on specific cellular reactions have been presented. This study was conducted to investigate the cellular immune reaction towards the parasite. By employing immunohistochemical techniques we were able to evaluate to what extent IgD, IgT and IgM positive lymphocytes were involved in the reaction. Samples from heart, liver and head kidney were collected from naturally infected rainbow trout. Positive cells were generally present in all organs. However, it was found that a wide zone around the parasite was devoid of positive cells. This suggests that the parasite induces some immune-depression in the host, a hypothesis which must be further investigated.

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Inducing protection from *Tetrahymena* infection in guppies: a model for systemic parasitosis

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Tetrahymena is a protozoan parasite which infects a wide range of fish species, yet most commonly reported from guppies, in which its effect appears to be the most devastating. Infection appeared to initiate through the skin, creating lesions which extended into the musculature. The parasite then spreads to all internal organs and gills, through tissue destruction and the circulation. Infection affects all internal organs, brain, eye and gills (Pimenta-Leibowitz and Zilberg 2009). Inflammatory response was negligible in guppy (as well as in platy and angelfish; Acanthopterygii superorder), yet it was evident in goldfish and koi (Ostaryophysi super order; Sharon et al., 2014).

Advantages of the infection model include the feasibility to easily culture *Tetrahymena* in vitro, yet regular passage through fish is needed to maintain its pathogenicity (Pimenta Leibowits et al., 2009). Guppies can be available at large numbers (as are regularly sorted out in commercial farms or can be easily produced) and small scale systems are needed for experimentation. Being able to adapt to various salinities, guppy can be used to study both marine and freshwater parasites.

Guppies were successfully immunized against *Tetrahymena* by intra peritoneal injection along with adjuvant (Chettri et al., 2009) and antibody response was demonstrated (Sharon et al., 2015). To evaluate the potential of oral immunization, anal delivery of the antigen was attempted, but it did not lead to protection and resulted in low to no increase in antibody titer (Sharon et al., 2015). Infection with *Tetrahymena* was used to evaluate the effect of dietary supplementation with *Labosphera incisa*, a microalgae which produces high levels of arachidonic acid, and its dihomo-γ-linolenic acid (DGLA) – producing mutant. Their application was demonstrated to reduce infection level and associated mortality from *Tetrahymena* (Khozin-Goldberg et al., 2006; unpublished). *Tetrahymena* infection was also used to evaluate the effect of different commercial diets on fish health (Sharon et al., 2014).

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Diluted booster vaccination against enteric red mouth disease in rainbow trout – field trials

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Enteric red mouth disease (ERM) is caused by the bacterium *Yersinia ruckeri*, and is one of the most common and costly diseases in rainbow trout aquaculture. An effective vaccine is available, and vaccination of fry at around 5 g is common practice on the fish farms. However, the vaccine only offers protection for a few months. This protection can be prolonged by a booster vaccination, which is recommended 4 months after the first vaccination. However, at this time the fish have increased their size approximately tenfold and vaccination is consequently so expensive that fish farmers are reluctant to administer a booster vaccination. It was speculated that a diluted booster vaccine could be sufficiently effective if exposure time was increased. Subsequent laboratory experiments showed that the recommended 1:10 diluted booster for 30 s provided superior protection to a 1:1000 or 1:2000 diluted booster for 1-2 h (Chettri et al 2015). However, the dilute booster still significantly improved survival following challenge with *Y. ruckeri* compared to fish receiving no booster, and could be a realistic alternative for the fish farmer.

To verify these results in a natural situation, field trials were set up at two conventional fish farms, which both experienced regular outbreaks of ERM. A more detailed explanation of the experiment and the results will be presented at the workshop. In short, one of the two fish farms experienced an ERM outbreak two months after the first vaccination, but before the dilute booster was administered. In this case about 5% of unvaccinated fish in a control pond died, while mortality was almost negligible in the ponds with vaccinated trout. Thus, the vaccine worked very well. There were no more ERM outbreaks on this farm. The fish were dilute booster vaccinated 12 weeks after the first vaccination. On the other farm there was an ERM outbreak less than a week after the booster vaccination was administered. While the first vaccination still provided some protection (only approximately 1% of the fish died), there was no mortality in the booster vaccinated fish.

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Chettri et al., 2015. Booster immersion vaccination using diluted *Yersinia ruckeri* bacterin confers protection against ERM in rainbow trout. Aquaculture, vol. 440, pp. 1-5.

Virus Y-PRV2 a new piscine orthoreovirus in rainbow trout: establishment of challenge model and long term pathogenetic study

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During autumn 2013, abnormal mortality was observed in some hatcheries producing rainbow trout in western Norway. The fish, 30-100g, showed signs of a novel disease characterized by circulation failure, pale viscera, anaemia and liquid (ascites) in the abdominal cavity. Haematocrit analysis confirmed severe anaemia. The histopathological examination revealed inflammation of the heart and red muscle and liver necrosis. Moderate to high mortalities have been observed.

Extended microbiological examination performed at the National Veterinary Institute (NVI) in Oslo ruled out known pathogens, while pyrosequecing analysis detected the viral genome of new aetiological agent called virus Y. Further studies have shown the virus belongs to the orthoreovirus family and is distantly related to PRV (piscine orthoreovirus) that most likely is involved in Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon.

A cooperative project between DTU-VET in Copenhagen, NVI and NMBU in OSLO was initiated in order to:

- Assess whether this new virus represent a new high pathogenic pathogen for farmed Rainbow trout in Europe
- Investigate disease pathogenesis.

In cohabitation trials, naïve Rainbow trout were exposed to shedders injected IP with blood pellets from infected fish.

Sampling was carried out at defined time points for 14 weeks.

The virus load in various organs was assessed by RT-qPCR analysis in shedders and cohabitants revealing a fast and active transfer of virus from shedders to cohabitants.

During the second half of the trial it was possible to observe a certain clearance process of the virus which occurred early in the shedders and later in the cohabitants.

Very little mortality and few clinical signs were observed.

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ERM immersion vaccination and adjuvants

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Two candidate adjuvants were tested with a commercial ERM dip vaccine (AquaVac™ Relera, MSD Animal Health) for rainbow trout in an experimental design compatible with common vaccination practices at farm level, *i.e.* immersion of fish in vaccine (±adjuvant) for 30 s. The adjuvants were the commercial product Montanide™ IMS 1312 VG PR (SEPPIC), and a soluble and ≥98% pure β-glucan from yeast (*Saccharomyces cerevisiae*) (Sigma-Aldrich). Hence, five experimental groups in duplicate were established and exposed to vaccine and adjuvants in the following combinations: AquaVac™ Relera (alone); AquaVac™ Relera + Montanide™; AquaVac™ Relera + β-glucan; Montanide™ (alone); and β-glucan (alone). Approximately 450 degree days post-vaccination, the fish were bath-challenged with live *Yersinia ruckeri* to produce survival curves. Blood, skin and gills were sampled at selected time points during the course of the experiment to test for plasma Ab levels and lysozyme activity, and the regulation of immune relevant genes and cells in external, mucosal tissues. Preliminary results show 96% to 100% survival of vaccinated fish with and without any of the two adjuvants, whereas unvaccinated controls and fish exposed to β-glucan alone experienced 58% and 60% survival, respectively (calculated at day 24 post-challenge). Montanide™ alone gave rise to an intermediate level of 72% survival. Lysozyme activity levels in plasma were markedly elevated at day 3 and day 24 post-challenge in fish exposed to Montanide™ alone or β-glucan alone compared to fish from any of the three vaccinated groups.

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