

DAFINET WORKSHOP
IN COLLABORATION WITH
BANGFISH AND PARAFISHCONTROL

SECURING FISH HEALTH IN WILD AND CULTURED FISH:
FROM PANGAS AND TILAPIAS VIA CARPS
AND PERCIDS TO SALMONIDS



OCTOBER 25TH, 2017

Venue:

Stigbøjlen 7

University of Copenhagen

1870 Frederiksberg C

Denmark

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BangFish, Danida

ParaFishControl, Horizon 2020

Book of abstracts

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OCTOBER 25TH, 2017

Venue:

**University of Copenhagen
Frederiksberg Campus
Seminar room
Stigbøjlen 7
1870 Frederiksberg C
Denmark**

Program - Wednesday October 25th, 2017

- 09:30 Welcome address by DAFINET leader Kurt Buchmann
Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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- 09:40 Barbara F. Nowak
Institute of Marine and Antarctic Studies, University of Tasmania, Australia
Amoebic gill disease – an emerging disease in European mariculture?
- 10:10 Jiwan K. Chettri
National Veterinary Institute, Technical University of Denmark
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- 10:30 Rzgar M. Jafar
Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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- 10:45 Sussie Dalvin
Institute of Marine Research, Bergen, Norway
Effect of temperature on development of the salmon louse, *Lepeophtheirus salmonis*
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The formula(tion) for success? – balancing protective and adverse effects in three different vaccine formulations
- 11:15 Defang Chen
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- 11:30 Patricia Bwalya
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Institute of Marine and Antarctic Studies, University of Tasmania, Australia
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Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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National Institute of Aquatic Resources, Technical University of Denmark, Denmark
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- 13:30 Foojan Mehrdana
Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
Immunoregulatory effects of *Contracaecum osculatum* ES compounds in a zebrafish model
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Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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Program - Wednesday October 25th, 2017

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Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences
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- 14:45 Moonika M. Marana
Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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- 15:00 Niels O. G. Jørgensen
Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
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Section of Aquatic Medicine and Nutrition, Norwegian University of Life Sciences, Norway
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Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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Program - Wednesday October 25th, 2017

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Section of Aquatic Medicine and Nutrition, Norwegian University of Life Sciences, Norway
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Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
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- 16:30 Seikh R. Islam
Patuakhali Science and Technology University, Bangladesh
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- 16:45 Isaac R. Mulei
Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences
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ABSTRACTS

Securing fish health in aquaculture

Introducing the joint DAFINET workshop 2017

K. Buchmann

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World aquaculture is steadily increasing and has reached an important milestone as aquaculture products now represent the main part of aquatic products used for human consumption. Several hundreds of teleost species from a range of families including among others salmonids, ictalurids, cyprinids and percids are currently being cultured. Health challenges are found in all these major systems. The high population densities in aquaculture production units favour transmission of viral, bacterial and parasitic diseases and in order to secure economic and environmental sustainable farming systems a range of preventive measures should be implemented. High quality environmental conditions should be a prerequisite aided by improved hygiene, management and quarantine procedures. These elements provide a sound basis for the production but where pathogens may encounter the production animals additional measures are needed to reach an optimal fish health management. Immunostimulation is a major prophylactic issue and vaccination is one of the most successful disease preventing approaches met to date.

The present workshop will present a series of researchers disseminating their latest research results related to aquaculture diseases and novel methods for disease prevention will be highlighted.

Amoebic gill disease – an emerging disease in European mariculture?

B.F. Nowak

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Amoebic gill disease (AGD) was first reported from salmonids, Atlantic salmon in Tasmania, Australia and coho salmon in Washington State USA, farmed in marine environment 30 years ago. The causative organism, *Neoparamoeba perurans*, was described more recently by Young et al. in 2007 and Koch's postulates have been fulfilled even later due to the difficulties with culturing the causative agent *Neoparamoeba perurans*. Clinical AGD has been observed in cultured fish in fourteen countries across six continents. Atlantic salmon is the main commercial species affected, but the disease has also been seen in cleaner fish and other fish species farmed in the marine environment. While immune response of salmon affected by AGD has been studied, there is no vaccine available. This presentation reviews our current knowledge of this disease, in particular its emergence in European mariculture.

This presentation summarises our current knowledge (including unpublished information) about the known host range, characteristics of the parasite, case definition and host-parasite relationship. It focuses on the emergence of the disease in European mariculture, in particular Atlantic salmon in Norway, Scotland and Ireland. *Neoparamoeba perurans* appears to be an ubiquitous organism.

While there has been a lot of progress in our understanding of this disease, some fundamental questions remain unanswered. The emergence of AGD appears to coincide with high sea surface temperature and possibly with intensification of mariculture. In contrast to the situation in Tasmania, in Europe AGD is one of many gill conditions affecting Atlantic salmon farmed in sea cages. This means that a new approach is needed for holistic management of gill health in farmed Atlantic salmon.

Passive immunization of rainbow trout fry against *Flavobacterium psychrophilum* and *Yersinia ruckeri*: an overview of passive immunization approach

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Diseases caused by Gram negative bacteria *Flavobacterium psychrophilum* and *Yersinia ruckeri* is a major problem in early stages of rainbow trout, *Oncorhynchus mykiss*. With regard to rainbow trout fry syndrome (RTFS) caused by *F. psychrophilum* there is no commercial vaccine available and even if a vaccine would be available against this disease, protection would be expected to be minimal as fish are not fully immunocompetent at this stage of life when RTFS develops.

The 'IMMFEED' project was conceptualized based on the use of immunoglobulin (IgM), purified from blood waste collected from a fish slaughter house, for incorporation into fish feed. The overall idea was to use immunoglobulin as an immunoprophylactic measure to control diseases in early stages of rainbow trout relying on specific immunoreactivity against *F. psychrophilum* and *Y. ruckeri*. For this purpose, larger rainbow trout (2.5 kg) were vaccinated and serum was collected and tested for IgM titers against specific pathogens. IgM was then purified in large scale, encapsulated in alginate microparticles and used for coating of fish feed. A sandwich ELISA was developed to quantify the total IgM in serum or IgM in re-extracts from alginate microparticles/feed. IgM was successfully incorporated and retained reactivity. Feeding and challenge experiments were then performed to evaluate the effect of medicated feed. The results show minimal or dose dependent effects of incorporated IgM depending on the target bacteria.

Vaccination of rainbow trout (*Oncorhynchus mykiss*) against *Yersinia ruckeri* infections

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One of the main problems in rainbow trout farms is enteric redmouth disease (ERM) caused by *Yersinia ruckeri*, a Gram-negative bacterium. ERM is one of the most contagious infectious diseases causing significant economic losses in the salmonid farming industry. ERM can be treated by use of various antibiotics, but this has some drawbacks. Repeated usage of antibiotic may select for drug resistance in microorganism and leave drug residues in the fish products. Introduction of fish vaccines in aquaculture has shown that this type of immunoprophylaxis can lead to a significant decrease of antibiotic consumption. We evaluated the importance of vaccine administration with special focus on injection (intra-peritoneally), immersion (dip vaccination or bath), or oral vaccine administration. It was found that injection vaccination (0.1 ml vaccine per fish) provides a superior protection of rainbow trout compared to immersion and oral vaccination and the protection was even improved when an adjuvant added to the vaccine. Protection conferred by injection and immersion could be improved by booster vaccination either by re-immersion for 30 second or by bathing fish in a diluted bacterin for at least one hour. Oral immunization may be used as booster after immersion but applied as a single oral application it induced merely a slight response. It is noteworthy that primary oral immunization followed by an oral booster vaccination weakened the response. Adaptive humoral and cellular immune response were judged by following antibody (IgM) levels in trout sera and CD4+ T (T-helper/TH), TH1, TH2, TH17, regulatory T cells, and CD8+ T (cytotoxic T lymphocytes) markers. The kinetics of serum IgM levels differed according to method of vaccination or infection. We observed a positive correlation between IgM antibody titers and protection, which suggests an important role of this Ig class in protection upon challenge. The gene expression analysis, performed by qPCR, showed simultaneous up-regulation of different genes, but no clear trend towards specific subsets of effector T cells. Regulation of genes associated with both humoral and cellular adaptive immunity post-vaccination and upon challenge confirmed the notion of cooperation between these two immunological entities and the presence of memory-B and memory-T cells in rainbow trout.

Effect of temperature on development of the salmon louse, *Lepeophtheirus salmonis*

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The salmon louse (*Lepeophtheirus salmonis*) is a naturally occurring parasite. It lives on the surface of salmonid fish where it eats skin and blood causing direct damage to the fish and expose the fish to secondary infections. Aquaculture of salmonid fish has provided the parasite with access to a much larger number of available hosts and resulted in problems with management of the parasite. Over the years these problems have repeatedly become critical, reflecting the availability of new chemical treatments followed by development of resistance towards the drug. To break this cycle, new knowledge covering the basic biology of the parasite is needed. Here the development of salmon lice was followed on a range of temperatures covering the ecological range of the host. The ability to infect, mortality and reproductive output was also studied. Temperature affected all life stages ranging from egg production over infectivity to ability and speed of development. The number of degree- days required for any given developmental process was constant, but only within a limited range of temperatures. These data are essential to management of lice infestations in aquaculture; assess potential effects on wild fish; and to allow precise experimental design in future studies of salmon lice.

The formula(tion) for success? – balancing protective and adverse effects in three different vaccine formulations

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Funded by the Danish Independent Research Council, the “LOW-TOX” project has focused on the use of adjuvant alternatives to mineral oil in furunculosis injection vaccines for use in rainbow trout. With an experimental vaccine formulation based on mineral oil adjuvant and *Aeromonas salmonicida* subspecies *salmonicida* as the benchmark formulation, alternative formulations with CpG oligodeoxynucleotides or a liposomal adjuvant were investigated. Each vaccine formulation was investigated with regard to conferred protection in an experimental infection setup, presence and severity of adverse reactions and induction of specific antibody titers. Furthermore, the dynamic of the immune responses were addressed by immunohistochemical analysis. While the overall goal is to find an optimal vaccine formulation, offering the best possible level of protection with little or no adverse effects, the picture emerging from this study is slightly more complicated. Nevertheless, the results indicate that the alternative formulations included offer both protection and reductions in observed adverse effects when compared to the benchmark formulation, thus warranting a further investigation of their potential.

Aquacultured fish and associated fish diseases in Sichuan, China

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Sichuan is an inland province in Southwest of China with abundant freshwater resources. During recent years, aquaculture enterprises in this province alone have covered an area of about 210,000 hectares and the annual aquaculture production has reached around 1.33 million tons worth more than \$ 2.9 billion. Pond farming, reservoir farming and paddy field farming are the main aquaculture production types which can be adapted to the local geomorphic characteristics of the mountain-plain.

The aquaculture species comprise mainly grass carp (*Ctenopharyngodon idealla*), silver carp (*Hypophthalmichthys molitrix*), bighead Carp (*Hypophthalmichthys nobilis*), common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), southern catfish (*Silurus soldatovi meridionalis*) and channel catfish (*Ictalurus punctatus*). A range of pathogens may challenge health of these farmed fishes in the local production units. Thus, bacteria including *Aeromonas hydrophila*, *Aeromonas veronii*, *Edwardsiella ictaluri*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae* and viruses such as koi herpesvirus, spring viraemia of carp virus and grass carp reovirus (GCRV) are important pathogens in aquacultured fish in Sichuan. For these diseases treatment involving drug application has been a conventional choice but the successful application of a GCRV vaccine, providing disease control in grass carp aquaculture, has inspired the development of other vaccines.

The research conducted with regard to vaccines for protection against the above-mentioned pathogens will be discussed although most of them are at the laboratory stage. Vaccination is considered to improve food safety and provide environmentally friendly disease control. Considering that Sichuan is located at the upper runs of the Yangtze River, where higher requirements to aquatic habitat protection are being reinforced, the application of vaccines will be a useful way to protect against fish diseases.

A challenge model for *Lactococcus garvieae* in Nile tilapia (*Oreochromis niloticus*)

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From 2014 to date, disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) have been observed on Lake Kariba in Zambia. The disease affects tilapia of 200 grams and larger. Clinical findings include ocular opacity, exophthalmia or endophthalmia and ulcers. *Streptococcus agalactiae* and *iniae* were isolated from diseased fish in 2014 while *Lactococcus garvieae* has been isolated repeatedly since 2015. Bacterial infections negatively affect the production of tilapia and should be managed. As a first step towards the development of a vaccine against *L. garvieae*, the present study was undertaken to develop a challenge model for use in the subsequent vaccine testing.

The antigen used was *L. garvieae* isolated from diseased fish from a farm on Lake Kariba in 2015. The bacteria was characterized using biochemical methods and also by 16S rRNA sequencing. LD₅₀ determination was then done to estimate the challenge dose. The challenge experiment was undertaken at the University of Zambia School of Veterinary Medicine wetlab. 180 Nile tilapia weighing about 20g were allocated into three groups with three replicates each. One group (n=60) was challenged by intraperitoneal injection (IP), the second group by immersion (I) and the last group (control) was injected with PBS. Sampling was done at days 3, 7 and 13.

Clinical signs including ocular opacity, exophthalmia or endophthalmia were observed in the IP group while only ocular opacity was observed in the I group. No clinical signs were observed in the control. Mortalities (n=12) were only observed in the IP group, with none in the I and control group.

Bacterial re-isolation was successfully done in the IP group but not the I or control although by PCR, the immersion and control fish yielded positive results. The implications of these findings will be discussed.

The good, the bad and the ugly - microbiology of fish diseases

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Microorganisms play a wide range of roles in fish health. While usually associated with negative effects, some have been used to promote farmed fish health. Successful health management of farmed fish is essential for sustainable mariculture. The level of potential control of microorganisms is related to the type of aquaculture system. Cage culture offers little control over bacterial or parasitic infections. Additionally, interactions between farmed fish and wild fish are unavoidable in cage culture. Pathogenic microorganisms can cause significant losses and contribute to increased production costs through the costs of treatment and loss in growth of the fish.

Some marine protists are amphizoic – free living but capable of becoming pathogenic. *Miamensis avidus* is a free living scuticociliate which can cause swimmer syndrome in ranched Southern Bluefin Tuna. This condition causes mortality of the infected fish, and there is no known treatment. The triggers causing the free living protists to become pathogenic are unknown.

Epitheliocystis is a condition common in wild and farmed fish caused by intracellular infection of epithelium of gills or skin with bacteria from Chlamydiales. It can cause mortalities, particularly in young fish. While the condition has been known for a long time, it is only recently that we started to identify the causative agents, characterised by host-specificity.

Aquatic animal health research, including microbiology, is crucial for sustainable mariculture.

Parasites in farmed Rainbow trout in Denmark 2016-17

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Parasites in aquacultured fish may have severe economical consequences for the production. Parasite infections of farmed rainbow trout (*Oncorhynchus mykiss*) in Denmark have previously been reported but during the last decade farm design and management practices have changed markedly in Danish farm operations. Therefore continued surveys are needed to establish preventive measures and management of fish health in light of new physical and environmental conditions in different production units.

We here report our preliminary data for rainbow trout cultured in freshwater and marine water in Denmark. Samples of up to 10 fish were taken from each of 13 freshwater farms (113 fish in total) and 9 marine culture farms (90 fish in total) during the period from January 2016 to September 2017. Freshwater rainbow trout sampled sized from 11-27 cm in total length and 69-283 gram in total body weight, while maricultured rainbow trout sized from 25-58 cm in total length and 200-4900 gram in total body weight. Fish were investigated for parasite presence using light microscopical techniques (4-40x magnification in dissection microscope followed by compound microscopy 100-1000x magnification). In addition the musculature was exposed to pepsin/HCl digestion with subsequent sieving for recovery of any zoonotic parasites.

The parasite burden of maricultured rainbow trout was generally very low as merely one cestode (*Eubothrium crassum*) and one sea louse (*Lepeophtheirus salmonis*) was recorded. Freshwater trout were generally infected in skin and fins by *Ichthyobodo necator* and various amoebae. The ciliates *Ichthyophthirius multifiliis* and *Trichodina* spp. were also frequently observed in skin, fins and gills but the occurrence varied considerably. In addition, monogeneans in the genus *Gyrodactylus* (fins and skin) and digeneans in genus *Diplostomum* (eye lenses) were occasionally observed. No zoonotic parasites were detected in any of the fish farms investigated.

The importance for fish farm management for securing fish health will be discussed.

Comparison of prevalence and intensity of infection of the parasitic nematode *Contracaecum osculatum* (liver worm) in Baltic cod from different areas

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The Baltic Sea cod is divided into a western and an eastern stock. For the eastern Baltic stock (EB) weight at age has declined drastically since the mid-2000s resulting in poor condition of individuals while in the meantime number of fish has increased. One of the possible reasons for these changes is the increase of the abundance of grey seal (*Halichoerus grypus*). It has historically been low, but the population size has increased drastically and was estimated to reach 40,000 individuals in the entire Baltic. Grey seal is the final host of the parasitic nematode *Contracaecum osculatum* (liver worm) and cod is one (of several) transport hosts to this parasite. Recent field investigations have documented a marked increase in prevalence (100%) and intensity of infection (up to 370 worms per fish) for this parasite in EB cod in the areas near Bornholm and Christiansø where grey seal haul out. During the 1980s seal abundance was very low and the infection of cod correspondingly low. Information about parasite occurrence in cod stocks from adjacent areas where grey seals are less abundant and harbor seals more prevalent is sparse.

The current study therefore aims to investigate prevalence and intensity of infection with *C. osculatum* (number of parasitic nematodes per infected fish). We showed that the prevalence of infection with third stage larvae of *Contracaecum osculatum* in cod caught near Christiansø in Bornholm Basin and in the Central Baltic was 100% and the intensity of infection 27 ± 4 and 40 ± 4 worms per fish (mean \pm SEM), respectively. Contrary to these high infection rates, prevalence was 51 and 37% in cod from the Sound and the Southern Kattegat respectively, with mean intensity of infection being on average 1 ± 0.1 worm per fish for both these areas. The possible reasons for the lower prevalence and intensity of infection in the Kattegat and the Sound is primarily that grey seals, the primary final hosts of the parasite, are not resident in the Kattegat and the Sound. In addition, the higher salinity and the broader range of prey organisms for cod in these areas may decrease the probability of infection because cod have other food choices than paratenic hosts such as sprat, herring and sand-eels.

Immunoregulatory effects of *Contraecaecum osculatum* ES compounds in a zebrafish model

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Effects of excretory/secretory (ES) compounds from third-stage larvae of the anisakid nematode *Contraecaecum osculatum* on immune gene expression in a zebrafish LPS-induced inflammation model was investigated. ES proteins were injected solely or with LPS.

After 24 hours, injection of ES proteins led to up-regulation of a number of immune-related genes, but generally to a lower degree compared to LPS. However, co-injection of these compounds with LPS exacerbated expression of some genes involving in Th1, Th2, Th17 and innate responses compared to the LPS-injected group. Nevertheless, overexpression of these genes decreased in an inverse dose-dependent manner.

The ability of modulation in the host immune responses by ES products is suggested as an evolutionary survival strategy of larval parasites in the host, which makes them less vulnerable to host immune responses and gives them greater chance to reach the final host.

VHSV infections of Caspian trout: Immune responses following experimental exposure

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Viral Haemorrhagic Septicaemia Virus (VHSV), also termed Egtved virus, is highly pathogenic to rainbow trout. A number of other freshwater and marine fish species are also susceptible to this negative-stranded RNA virus belonging to the rhabdoviridae family. It was isolated for the first time in 1962 and has caused significant losses especially in salmonid farms in European countries, but is occurs worldwide in four different genotypes. The recent introduction of VHSV to rainbow trout farms in Iran has had high impact on the aquaculture industry and in addition, it can be a potential threat for endemic species such as Caspian trout (*Salmo trutta caspius*). This endangered subspecies has been supported by captive breeding and restocking programs since 1983.

Experimental VHSV challenge of Caspian trout (originating from wild and cultivated parentages) elicited more than 50% mortality. The two trout strains showed differences with regard to susceptibility, course of infection, virus load, hematological parameters and immune gene expression. The susceptibility of Caspian trout to VHSV emphasizes the need for protection of wild populations against the virus. Management of VHSV in infected rainbow trout farms should be implemented in a way, which minimizes the risk for infection of *S. trutta caspius* localities.

Detection and molecular epidemiology of tilapia lake virus (TiLV) in wild and farmed Nile tilapia (*Oreochromis niloticus*) from Lake Victoria in East Africa

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Tilapia lake virus (TiLV) is a new viral disease of tilapiines causing morbidity and mortality up to 90 % in wild and farmed tilapia posing great threat to the fishery industry worldwide. Tilapia lake virus disease (TiLVD) or the syncytial hepatitis of tilapia (SHT) was reported (2012) and described (2014) for the first time from the outbreaks in the Lake Kinneret (also known as the Sea of Galilee) and the commercial ponds in Israel. The mortalities in tilapiines have been recorded in the farmed tilapia (*Oreochromis niloticus*), commercial tilapia (*O. niloticus* X *O. aureus* hybrids) and wild tilapia (*Sarotherodon galilaeus*). So far, the virus has been reported in four continents: Asia, Africa and South America.

Our interest in this study was to investigate the presence of the virus from wild and farmed Nile tilapia samples collected from Lake Victoria in East Africa. Based on our diagnosis using PCR, we are reporting for the first time the detection of TiLV infection in the wild and farmed Nile tilapia from Lake Victoria. Twenty-eight out of the 191 fish (14.66%) examined were positive for TiLV by PCR. A total of 442 individual tissues were examined, and the prevalence was highest from the spleen (10.99%, N=191) followed by head kidney (7.69%, N=65) and heart samples (3.45%, N=29). Low prevalence of infection was observed in the liver (0.71%, N=140) and there were no nucleic acids detected from the brain tissues (0.0%, N=17). The search of homology sequences on the National Centre for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLASTN 2.6.1+) and the phylogenetic analysis clustered our sequences together with the recent outbreaks from Israel and Thailand. We are recommending further studies aimed at isolating the virus and experimental studies in Nile tilapia to show its virulence and ability to cause disease in naïve fish.

Subunit vaccine candidates against *Aeromonas salmonicida* in rainbow trout

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Aeromonas salmonicida subsp. *salmonicida* is the etiological agent of furunculosis and a major fish health problem in salmonid aquaculture worldwide. Injection vaccination with commercial mineral oil-adjuvanted bacterin vaccines has been partly successful in preventing the disease, but in Danish rainbow trout (*Oncorhynchus mykiss*, Walbaum) aquaculture furunculosis outbreaks still occur.

In this study we tested the efficacy of experimental subunit vaccines against *A. salmonicida* infection in rainbow trout. We utilized *in silico* screening of the proteome of *A. salmonicida* subsp. *salmonicida* strain A449 and identified potential protective protein antigens, that were tested by *in vivo* challenge trial. A total of 14 proteins were recombinantly expressed in *Escherichia coli*. Three different subunit vaccine combinations were prepared. Rainbow trout were immunized by intraperitoneal (i.p.) injection. The fish were exposed to virulent *A. salmonicida* 7 weeks after immunization. In order to assess the efficacy of the subunit vaccines the immune response were evaluated after immunization and after challenge infection by measuring the antibody levels by ELISA. The survival of fish in different groups was monitored.

The survival of fish at 3 weeks after challenge infection showed that all 3 groups of fish immunized with 3 different protein combinations exhibited significantly lower mortalities (17-30%) compared to the control groups (48% and 56%). The ELISA results revealed significantly elevated antibody levels in fish against several protein antigens, which in some cases were positively correlated to the survival.

Effect of water treatment on growth and flavour quality of pangas and tilapia produced in earthen ponds in southern Bangladesh

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Effects of water treatment by two probiotic (bacterial) products (PondPlus® and AquaPhoto®) and sand filtration were studied on growth performance and flavour quality of tilapia (*Oreochromis niloticus*) and pangas (*Pangasianodon hypophthalmus*) in traditional earthen ponds in Bangladesh.

Compared to untreated ponds, treatments by probiotic products or sand filtration resulted in a higher oxygen content, higher water transparency, less ammonium, and fewer cyanobacteria. Weight gain for individual tilapia was lowest in the AquaPhoto-treated ponds (177 g), while similar gains (188-194 g) occurred in the other ponds. For pangas, the lowest weight gain (627 g) was obtained in the sand filter-treated ponds, as compared to 690-797 g in the other ponds.

Sensory analysis by a Bangladeshi consumer panel indicated a positive effect of the water treatment, especially of the AquaPhoto treatment. In general, depuration of the fish in clean water for 12 to 48 hrs significantly improved the taste of the pond-raised fish.

The study suggests that an improved water quality has beneficial effects on fish production in earthen ponds, but more studies are needed to evaluate whether biological procedures (probiotic treatment) or the simpler sand filtration are the most efficient measures for achieving an improved water treatment.

The 16S rRNA microbiome of wild Nile tilapia (*Oreochromis niloticus*) in Lake Victoria

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Wild Nile tilapia (*Oreochromis niloticus*) is one of the most dominant fish species found in Lake Victoria in East Africa despite being an exotic species introduced in the 1950s. The objective of this study was to determine whether the bacteria composition of the gut of Nile tilapia could be linked to its rapid proliferation to become of the most dominant fish species in Lake Victoria. Data generated from the 16S rRNA Illumina sequencing shows that wild Nile tilapia in Lake Victoria is endowed with a highly rich and diverse microbiome comprising of 17 phyla, 43 classes, 110 families and 110 genera dominated by Firmicutes (51.7%), Bacteroidetes (27.1%), Proteobacteria (10.7%) and Fusobacteria (6.3%). The high Firmicutes:Bacteroidetes ratio points to a high carbohydrate metabolism capacity linked to rapid growth that could be contributing to the rapid growth in Lake Victoria. The phylum Proteobacteria followed by Firmicutes, Bacteroidetes and Actinobacteria all having ≥ 10 families and genera dominated the gut microbiome. The phylum Firmicutes covered a wide range of species inclusive of potential fish pathogens, zoonoses and probiotics while Proteobacteria was dominated by species known to produce antimicrobial compounds and regulators of environmental metabolites. The phylum Fusobacteria were dominated by *Cetobacteria somare* that produces Vitamin B12 while Bacteroidetes were dominated carbohydrate-metabolizing species. In general, the proportion of operational taxonomy units (OTUs) for pathogen bacteria species was lower than the proportion of species linked to probiotic and antimicrobial production, carbohydrate metabolism and environmental regulators. Put together, these findings indicate that the microbiome of the wild Nile tilapia in Lake Victoria could be playing a major role in enhancing its survival and proliferation in Lake Victoria.

Gene expression and transcriptomic analysis focusing on immune-related genes of rainbow trout (*Oncorhynchus mykiss*) exposed to *Ichthyophthirius multifiliis*

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Immune response mechanisms related to parasite infections in rainbow trout are generally poorly understood. The present study was conducted to evaluate the immune response of rainbow trout following exposure to the parasitic ciliate *Ichthyophthirius multifiliis*. We applied controlled experimental infection and elucidated the reactions in the fish using qPCR and transcriptomic analysis. Total RNA from immune tissue samples gill, skin and spleen were collected on day 1 and 8 after parasite exposure. Infection of *I. multifiliis* induced a clear differential expression of immune genes, related both to innate and adaptive immunity. According to our qPCR analysis the host reactions developed with increasing severity of infection. Thus, only a few genes were regulated at 1-day p.i when compared to sampling time at 8-day p.i, at which timepoint most of the immune genes were significantly up-regulated. Gene encoding pro-inflammatory cytokines IL-6 and IL-17 were significantly up-regulated in the skin. Complement factors and chemokines were found to play a role in response to Ich infection since complement C5 and chemokines (IL-8, CK10, CK11, and CK12) were significantly up-regulated in skin and gill. Genes involved in the acute phase response (Serum Amyloid A, Precerebellin, and Hepsidin) and the gene encoding S100A1 were also regulated in response to the parasite infection. The anti-inflammatory or regulating cytokines IL-4/13A, IL-10, and TGF β were significantly elevated during the experiment probably as a response to inflammation caused by infection. The persistent infection activated the expression of IgM and IgT significantly in almost all tissues. However, some of the investigated genes were also significantly down-regulated at later stages of the infection (cytokine IL-1 β , IL-17 and acute phase protein hepcidin). Our transcriptomic analysis of gill samples sampled at day 8 showed differential expression of a total of 3,352 out of 46,585 (7.2%) investigated genes (at least 2-fold change). A total of 1,796 genes were up-regulated and 1,556 genes down-regulated. A total of 230 of the 3,352 (6.86%) regulated genes are functional genes involved in immune processes and 120 of these genes are known to take part in 15 immune-related pathways such as the B cell receptor signaling pathway, Fc-gamma R-mediated phagocytosis, chemokine signaling pathway, antigen processing and antigen presentation (involving 21, 20, 19, and 16 genes, respectively).

Biochemical and genetic characterization of *Aeromonas hydrophila* from different aquatic organism in Asia

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Aeromonas hydrophila, is a gram negative bacteria infecting different aquatic organisms in tropical countries. Vaccine development has been hampered by the existence of variable strains and serotypes *A. hydrophila*. Different molecular markers such 16S rRNA, gyrB and outer membrane protein A (OmpA) have been used to characterize different strains of *A. hydrophila*. In addition, OmpA serves as a promising cross-reactive antigen able to confer protection among different serotypes of *A. hydrophila*. The aim of this study was to compare the biochemical and genetic diversity of *A. hydrophila* isolates obtained from different aquatic organisms and geographic locations in Asia.

A total of 40 isolates from seven fish species from India (*Labeo rohita*, *Catla catla*, *C. batrachus*, *C. carpio*, *C. mrigala*, *C. auratus* and *Oreochromis niloticus*) and three aquatic organisms from Taiwan (*P. sinensis*, *H. ellipticum* and *O. niloticus*) were used in this study. All isolates were cultured in two selective media namely *Aeromonas* Isolation (AI) and Rimler Shotts (RS) agars while hemolytic activity was tested using sheep blood agar. Of the 40 isolates examined, 33 were recovered from AI and RS selective media of which 28 were confirmed as *A. hydrophila* by API-20NE while β -hemolysis was shown in 24 isolates. In terms of biochemical characterization, all isolates were positive for oxidase and tryptone while Arabinose and Maltose showed different results for strains from India and Taiwan. Phylogenetic analyses was done using 16S rRNA, gyrB and OmpA genes while Ribotyping was done by RAPD analysis. Our findings show significant interspecies and inter-geographical variations in the 16S rRNA, gyrB and OmpA sequences of *A. hydrophila* from different aquatic organisms. In general, this study shows that combining biochemical with phylogenetic analyses of different marker genes makes it is easy to characterize *A. hydrophila* strains from different aquatic organisms and geographical regions. The study also shows that phylogenetic comparison of surface proteins, such the OmpA, is useful in identifying immunogenic proteins that are across protective against different strains of *A. hydrophila*.

Challenge studies with Danish *Yersinia ruckeri* O1 biotype 2 in rainbow trout

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The isolate *Yersinia ruckeri* serotype O1 biotype 2 strain 07111224 from a Danish outbreak of ERM (enteric redmouth disease) in rainbow trout (*Oncorhynchus mykiss*) was tested for virulence in by different fish sizes (5, 19, 25 and 52 g) and challenge methods (bath, intraperitoneal (IP) injection, anal intubation and cohabitation). The results from the study confirm that rainbow trout susceptibility to *Y. ruckeri* strain 07111224 is dependent on fish size. Bath challenge by *Y. ruckeri* 07111224 (10^8 CFU/ml) caused 79% mortality in 5 g fish, while the mortality in 19 – 52 g fish was around 26%. In IP injection challenges, 10^5 – 10^6 CFU of *Y. ruckeri* 07111224 caused high mortality to fish of all sizes. In anal intubation challenges, 10^9 CFU of *Y. ruckeri* 07111224 induced 82% mortality in 5 g fish and decreased, as the fish got larger. Cohabitation challenges induced 45% mortality in the 5 g test fish when test fish were cohabitated with equal number of shedders, while mortalities in the test fish of five other challenge groups were lower (5 – 25%).

These results showed that *Y. ruckeri* 07111224 is virulent enough to cause the mortality in 5 – 52 g rainbow trout by three of infection routes (bath, IP injection and anal intubation). The bath and cohabitation challenge experiments showed that 5 g fish are very susceptible to horizontally transmitted *Y. ruckeri* 07111224 therefore waterborne methods are applicable as the model of natural infection route.

The relationship between water quality parameters and phytoplankton abundance in pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*) ponds in Bangladesh

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Phytoplankton and zooplankton retort rapidly to any alterations in nutrient changes in water bodies indicating the growing nutrient pollution. On one hand where phytoplankton serves as food source, on the other hand some forms causes fish poisoning due to release of toxins during blooms.

The present study was carried out to analyze the water quality and plankton diversity of 4 selected pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*) ponds from two different regions of Bangladesh; Bogra and Khulna. Monthly sampling was done for a period of 12 months, July 2016 to June 2017. Samples were analyzed for physico-chemical parameters. Quantitative and qualitative analysis of plankton. Plankton populations in the water of the experimental ponds were found to be consisted of 91 genera belonging 94 species under 13 planktonic groups composed of 9 groups of phytoplankton and 4 groups of zooplankton. Temperature, nitrate, phosphate, ammonia were positively correlated and pH, DO, transparency were negatively correlated with the plankton abundance. Results shows that the water is fairly good for fish culture. Nutrients of water enhanced the plankton growth which can lead to bloom. Therefore, it needs some corrective measures to maintain the water chemistry of the pond which will help to keep the plankton community in control.

First detection of infectious hematopoietic necrosis virus in farmed rainbow trout in Kenya

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Rainbow trout (*Oncorhynchus mykiss*) was introduced to Kenyan rivers in 1920s primarily for sport fishing. However, it later gained economic importance with farmers establishing commercial farms for rearing the fish. The Kenyan government set up a hatchery to supply fry and fingerlings to farmers in 1948. Over the years, increased demand for stock from farmers has led to importation of fertilized eggs from Europe and America. Importation of breeding materials has potential to facilitate pathogen transfer. Infectious hematopoietic necrosis (IHN) is a notifiable fish disease that has not previously been reported in Kenya.

The objective of the present study was to screen some farms in Nyeri county following reports of mortalities. Eight farms were visited and fish observed in the ponds. Those manifesting ill-health and healthy looking fish were collected for tissue sampling (n=20 per farm). Samples of headkidney, stored in RNAlater[®] were used for extraction of RNA which was then used to synthesize cDNA. Polymerase chain reaction (PCR) of the samples yielded a product of about 680 bp. Sequencing of this product confirmed for the first time, the presence of IHNV in rainbow trout in Kenya.

Morphological, biochemical and genetic comparison of group B *Streptococcus agalactiae* biotypes I and II

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Tilapia production is expanding rapidly to be the leading fish industry on the globe. However, the expansion of this industry in aquaculture has brought with it the increase in fish diseases. Among the diseases, the most important emerging disease in tilapia is *Streptococcus agalactiae*. This bacteria species has several strains that have the ability to infect a wide range of hosts including fish. In fish, three strains namely biotype I, II and III, have been characterized and isolated from different countries. Biotypes I and II are pathogenic to tilapia, whereas biotype III is non-pathogenic. Hence, only biotype I and II were used to study the morphological, biochemical and genetic variation of *S. agalactiae* strains in the Nile tilapia (*Oreochromis niloticus*) in this study.

As a first step, we used the morphological and biochemical tests to demonstrate that both isolates were members of the group B *Streptococcus* (GBS). Both strains were gram-positive cocci shaped bacteria with characteristic long chains seen in GBS bacteria. Only biotype I was able to show the hemolytic activity on 5% sheep blood agar whereas biotype II was non-hemolytic. The CAMP-test showed potentiation of the hemolytic activity of *Staphylococcus aureus* on sheep blood agar. The API-20 Strep system gave a profiling index of 3663010 and 3663410 indicating that the two isolates were separate strains classified as biotypes I and II, respectively, belonging to GBS under the species *S. agalactiae*. Genetic characterization using the 16S ribosomal RNA confirmed that both isolates were members of the *S. agalactiae* species. Phylogenetic analysis of virulence and immunogenic proteins such as the CAMP factor (cfb), fibrinogen-binding protein A (fbsA), c5a peptidase gene (scpB), pilli island gene (PI-2b) and surface immunogenic protein (SIP) also showed similarity of both strains used in this study with other GBS members deposited in the Public databases from different host species.

In summary, this study shows that both biotypes I and II strains from Nile tilapia used in this study have similar morphological, biochemical and genetic properties with other GBS.

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