IVVN Symposium

# VACCINES FOR AQUACULTURE

6 July 2021
9am to 12.35pm BST

# Programme and abstracts

## Contents

# Programme

Session chairs: **Professor Alexandra Adams** (Aquatic Diagnostics Ltd, United Kingdom) and **Dr Thảo Ngô** (Biotechnology Center of Ho Chi Minh City, Vietnam)

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| 09.00 – 09.05 | **Welcome and introduction**<br>Professor Brian Perry (Universities of Oxford and Edinburgh, UK, and member of IVVN Network Management Board) |
| 09.05 – 09.30 | **Chitosan nanoparticle-based mucosal vaccines delivered against columnaris disease in tilapia**<br>Dr Nopadon Pirarat (Chulalongkorn University, Thailand) |
| 09.30 – 09.55 | **How adjuvants work – progress and challenges for use in fish**<br>Dr Carolina Tafalla (Animal Health Research Centre (CISA-INIA), Spain) |
| 09.55 – 10.20 | **Advances in technologies for fish vaccine development**<br>Dr Nguyen Duc Hoang (Vietnam National University, Vietnam) |
| 10.20 – 10.45 | ***Pichia pastoris* expression platform for the production of novel fish vaccines**<br>Dr Ansgar Stratmann (AQUATRECK Animal Health SL, Spain) |
| 10.45 – 11.15 | **Break and poster session** in breakout groups |
| 11.15 – 11.35 | ***Pseudomonas plecoglossicida* T6SS-1: a key virulence factor required for the pathogenesis in fish and a target for live attenuated vaccine development**<br>Dr Zhen Tao (Ocean University, China) |
| 11.35 – 11.55 | **Prospects for a sealice vaccine**<br>Dr Sean Monaghan (University of Stirling, UK) |
| 11.55 – 12.15 | IVVN pump-priming grant: **Efficacy testing of novel immersion and oral vaccines for *Aeromonas hydrophila* in tilapia and Vietnamese catfish**<br>Dr Thảo Ngô (Biotechnology Center of Ho Chi Minh City, Vietnam) |
| 12.15 – 12.35 | IVVN pump-priming grant: **Development of immunological tools for monitoring the immune response of Nile tilapia**<br>Dr Kim Thompson (Moredun Research Institute, UK) |
| 12.35 | **Close** Professor Brian Perry |

# Sponsors

# Speaker abstracts

## Chitosan nanoparticle-based mucosal vaccines delivered against columnaris disease in tilapia

*Dr Nopadon Pirarat*

*Chulalongkorn University, Thailand*

Columnaris, a bacterial disease caused by *Flavobacterium columnare*, is recognized as one of the most important infectious diseases in farmed tilapia, especially during the fry and fingerling stages of production. The disease is associated with characteristic lesions in the mucosa of affected fish, particularly their skin and gills. Vaccines delivered via the mucosa are therefore of great interest to scientists developing vaccines for this disease. As the surface of living fish is covered by mucus and directly associated with the mucosal immunity. We hypothesized that better adsorption on mucosal surfaces and more efficient vaccine efficacy could be enhanced by biomimetic nanoparticles. Here, we applied a new innovative nanotechnology to potentiate targeted antigen delivery by coating the surface of nanoparticles with mucoadhesive chitosan biopolymer to provide "pathogen-like" properties that ensure nanoparticles binding on fish mucosal membrane. The prepared vaccines were nanosized and spherical as confirmed by scanning electron microscope (SEM). The analysis of hydrodynamic diameter and zeta-potential also suggested the successful modification of nanovaccines by chitosan as indicated by positively charged. The excellent affinity of the chitosan-modified nanovaccines toward fish gills was demonstrated in an *in vivo* mucoadhesive study, confirmed by bioluminescence imaging and fluorescent microscopy. Following vaccination with the prepared nanovaccines by immersion 30 min, the challenge test was performed at 30- and 60-days post vaccination. The relative percent survival (RPS) of vaccinated fish was greater than 70% for mucoadhesive nanovaccine. Histology of the mucosal associated lymphoid tissue (MALT) showed a significantly higher presence of leucocytes and a greater antigen uptake by the mucosal epithelium in vaccinated fish, and there was statistically significant up-regulation of *IgT*, *IgM*, *TNF α*, *IL1-β* and *MHC-1* genes in the gill of the vaccinated fish. Overall, the results of our study confirmed that the chitosan complex nanoparticles achieved better adsorption onto the mucosal surfaces of the fish, elicited great vaccine efficacy and modulated the MALT immune response, demonstrating the feasibility of the mucoadhesive nano-immersion vaccine as an effective delivery system for the induction of a mucosal immune response against columnaris disease in tilapia.

## How adjuvants work – progress and challenges for use in fish

*Dr Carolina Tafalla*

*Animal Health Research Centre (CISA-INIA), Spain*

From all points of view, vaccination is the most adequate method to control infectious diseases that threaten the expansion of the aquaculture industry worldwide. Unfortunately, in fish, most vaccines provide insufficient protection on their own and require the use of adjuvants to increase their efficacy. An added problem in aquaculture is derived from the fact that most commercialized vaccines have to be delivered by injection, usually along with an oleic adjuvant. Despite their relative efficacy, these adjuvants often generate important side effects in vaccinated fish. Furthermore,

vaccinating fish by injection is a labour-intensive activity that provokes a great amount of stress, known to often condition the immune response mounted to the antigen and handling mortalities. In this context, in aquaculture, the search for novel adjuvants that increase the efficacy of the vaccine without generating unwanted side effects, should go along with a search for adjuvants suitable for mass delivery strategies (such as oral or immersion). In this talk, we will review the work undertaken to date in fish concerning both traditional and new generation adjuvants, focusing in the work that our group has been carrying in the past years to identify adjuvants that are suitable for oral vaccination.

## Advances in technologies for fish vaccine development

*Dr Nguyen Duc Hoang*

*Vietnam National University, Vietnam*

*Bacillus subtilis*, a Gram-positive bacterium model, is considered Generally Recognized as Safe (GRAS), endotoxin-free, environmentally friendly, and used as probiotics for animals and aquaculture. This presentation will introduce the expression systems for *B. subtilis* and their potential use as vaccine delivery vectors. We used molecular biology methods to develop expression systems to express recombinant proteins in different locations of *B. subtilis*. As a result, we developed expression vectors for overexpression of recombinant proteins in inducible and inducer-free manners. The proteins could be produced in the cytoplasm and as secretion into the culture medium and on the cell surface vegetative cells and spores. For applications as vaccine delivery vectors, the authors generated *B. subtilis* strains to express a model antigen, heat-labile enterotoxin B subunit (LTB) in the cytoplasm, in the culture medium, on the cell surface of the vegetative cells. The LTB-expressing *B. subtilis* strains were orally administered, and the immune responses, IgG, and secreted IgA levels were measured. The immune response studies showed that the LTB-expressing *B. subtilis* strains delivered orally into mice could induce the sIgA and IgG immune response. The second example was the *B. subtilis* spore displaying toxoid form of alpha-toxin from *Staphylococcus aureus* that could induce the IgG and sIgA inmmuresponse in mice.  As for the third example, we used *B. subtilis* expressing a coat protein of Betanoda virus for immunization and challenge study of sea bass fish. The specific IgM antibodies in the fishes against the Betanoda-coat protein increased when injecting the *B. subtilis* expressing the coat protein. In summary, the results indicated that the genetics tools could be exploited to produce recombinant proteins and generate antigen-expressing *B. subtilis* strains for vaccine development.

## *Pichia pastoris* expression platform for the production of novel fish vaccines

*Dr Ansgar Stratmann*

*AQUATRECK Animal Health SL, Spain*

The fast growing global aquaculture Industry urgently needs highly efficacious affordable vaccines to combat the increased incidence of infections caused by various bacterial, viral and parasitic pathogens. AQUATRECK Animal Health S.L is a new innovative aquaculture pharmaceutical company based in Spain working on a broad spectrum of 2nd generation of vaccines produced in the methylotrophic yeast *Pichia pastoris* (*Komagataella phaffii*) targeting viruses, bacteria or eucaryotic parasites. Our aim is to increase the sustainability and competitiveness of our partners in the global aquaculture industry by developing powerful recombinant vaccines to prevent infections by selected  devastating pathogens.

Examples from our applied research studies portfolio will be presented demonstrating the flexibility and efficiency of our novel technology. The production of VirusLikeParticles (VLP) opens the door for powerful and flexible vaccine development. Through collaborations with many research institutes, we are delighted to present our close to market product originate from VERV and a project under development dealing with SAV and epitope identification by AI. Multivalent vaccine to protect Tilapia against three different viruses was developed by AI, and will be produced as multidomain repetitive artificial protein. For the production of membrane anchored viral glycoproteins we are working on a robust 'surface display' approach, to use *P. pastoris* whole cells for oral vaccination. The aim of this presentation is to give a brief insight in state of the art vaccine development to enable the industrial production of cost effective and powerful vaccines for the global Aquaculture industry.

## *Pseudomonas plecoglossicida* T6SS-1: a key virulence factor required for the pathogenesis in fish and a target for live attenuated vaccine development

*Haoda Ye, Yanyan Li, Amin Yang, Xiaojun Yan, Zhen Tao*

*School of Fisheries, Zhejiang Ocean University, China*

*Pseudomonas plecoglossicida* is one of the most prevalent pathogens affecting the large yellow croaker (LYC; *Larimichthys crocea*), causing a disease characterized by granuloma formation in the spleen, kidney and liver. We identified three type VI secretion systems (T6SSs) in *P. plecoglossicida* XSDHY-P (a fish pathogenic strain), which were named as PP T6SS-1, PP T6SS-2 and PP T6SS-3. To examine if these T6SSs are associated with bacterial virulence to its natural fish hosts, we generated XSDHY-P isogenic multiple-gene mutants ΔT6SS-1, ΔT6SS-2, ΔT6SS-3, and single-gene mutants ΔtssH-1 and ΔtssD-1, and performed fish infection assays to evaluate their virulence in LYC model. The deletion of PP T6SS-1, tssH-1 or tssD-1 nearly 100% attenuated *P. plecoglossicida* virulence, indicating that PP T6SS-1 is a key virulence determinant. Injection vaccination of LYC with the ΔtssD-1 mutant at a dose of 105 CFU per fish resulted in 88% (22/25) survival after wild-type immersion challenge, compared to sham-vaccinated fish (12% survival). In conclusion, analysis of T6SS systems encoded in *P. plecoglossicida* by the gene knockout revealed that T6SS-1 plays an important role in *P. plecoglossicida* pathogenesis, and T6SS-1 deficient strain ΔtssD-1 showed promise as a target for live attenuated vaccine development.

## Prospects for a sea lice vaccine

*Dr Sean Monaghan*

*University of Stirling, UK*

The salmon louse, *Lepeophtheirus salmonis*, represents the greatest disease issue constraining the sustainability and expansion of the Atlantic salmon industry. A number of innovative non-medicinal engineering and biological control approaches are used to control *L. salmonis*, but they face their own welfare challenges. A vaccine would provide a safe and environmentally sound tool.

Research into vaccine development for *L. salmonis* has been on-going for >25 years, since initial reports of protective effects of immunising fish with extract antigens. A bottleneck for ectoparasite vaccinology, however, is the need to discover and produce

targeted protective recombinant antigens to disrupt parasite physiology. Over the last decade there have been major advancements in our understanding of louse biology, host responses and host-parasite interactions. Temporal and spatial impairment of the Atlantic salmon immune system and wound healing ability during infection has been demonstrated through immunological, transcriptomic, genomic and proteomic studies, suggesting a role of pharmacologically active molecules secreted by feeding lice such as proteases and eicosanoids. Additionally, proteins associated with the louse gut have been characterised and have paved the way to more targeted vaccine antigen mining and design to impair feeding. We have evaluated the protective efficacy of >10 vaccine candidates including cysteine and serine native protein extracts as well as recombinant immunomodulatory and digestive louse proteins administered to Atlantic salmon by intraperitoneal injection (IP). IP vaccination has provided limited efficacy on larval lice numbers despite inducing systemic immune responses. Exploiting recent knowledge on fish mucosal immunity and considering the diet of the attached larval chalimus stages being predominantly skin epithelium and mucus, we have recently adopted an oral booster vaccination strategy using nanoparticle technology with the goal of enhancing skin mucosal antibody levels in order to achieve an effective response to sea lice vaccination.

By exploring protective mechanisms of resistant salmonids such as coho salmon, and subsequently the louse proteins associated with modulation of susceptible Atlantic salmon immunity, key targets will be determined for future mucosal vaccine development.

**IVVN PUMP-PRIMING GRANT**

## Efficacy testing of novel immersion and oral vaccines for *Aeromonas hydrophila* in tilapia and Vietnamese catfish

*Thảo Ngô*[1], *Dang Thi Hoang Oanh*[2], *Alaa Eldin Eissa*[3], *Alexandra Adams*[4], *Kerry Bartie*[4], *Andrew Desbois*[4], *Dirk Werling*[5], *Callum Scott*[6]

***Affiliations:*** ***1*** *Biotechnology Center of Ho Chi Minh City, Vietnam;* ***2*** *Can Tho University, Vietnam;* ***3*** *Cairo University, Egypt;* ***4*** *University of Stirling, UK;* ***5*** *Royal Veterinary College, UK;* ***6*** *Benchmark Animal Health Ltd, UK*

In 2014 the contribution of aquaculture to supply food for human consumption overtook that of wild-caught fish for the first time. Aquaculture currently contributes approximately 73.8 million tonnes of aquatic animals with a value of US$130 billion. This has increased by 10-12% from 59 million tonnes in 2011, representing the fastest growing animal production sector. Over 30 species are currently farmed, including tilapia and Pangasius (Vietnamese catfish also known as Tra catfish). These fish species are farmed in low and middle-income countries (LMICs) and provide an important source of revenue for many low income families supplying both the domestic and export market. Tilapia production is rapidly increasing with Egypt the third largest global producer. Tilapia and Vietnamese catfish are both produced in Vietnam. Although Vietnamese catfish production increased dramatically between 2004 and 2008, the export market has since levelled off due to public concern over disease and over use of antibiotics. Currently, disease outbreaks caused by *Aeromonas hydrophila* are having a major economic impact on aquaculture in both countries. No vaccine is currently available in Egypt and despite a vaccine being available for use in Vietnamese catfish, antibiotics remain the treatment of choice due to doubts over vaccine effectiveness (*A. hydrophila* strains are highly diverse) and the high cost of the vaccine (administered by injecting individual sedated fish). The widespread use of antibiotics within farms can encourage antimicrobial resistance, reducing the treatment

options for both fish and human infection. Through a collaboration of scientists from Vietnam, Egypt and the UK we plan to test the efficacy of novel vaccines that can be easily administered (i.e. immersion and oral vaccines), without the need for highly trained personnel and specialist equipment. Such vaccines are urgently needed to help prevent *A. hydrophila* disease outbreaks and reduce antibiotic use in both tilapia and Vietnamese catfish aquaculture.

**IVVN PUMP-PRIMING GRANT**

## Development of immunological tools for monitoring the immune response of Nile tilapia

*Kim Thompson*[1], *Sachdev S Sidhu*[2], *Alexandra Adams*[3], *Alasdair Nisbet*[1], *Hoang Nguyen*[4], *Nguyen Ngoc Phuoc*[5], *Ruth Zadoks*[6]

***Affiliations:*** ***1*** *Moredun Research Institute, UK;* ***2*** *University of Toronto, Canada;* ***3*** *University of Stirling, UK;* ***4*** *Vietnam National University, Vietnam;* ***5*** *Hue University of Agriculture and Forestry, Vietnam;* ***6*** *University of Sydney, Australia*

With the world population projected to increase to over 8.5 billion by 2030, aquaculture has great potential to meet the world's increasing demands for protein. It is the fastest growing animal food production sector globally and now accounts for over 50 percent of all fish consumed. Tilapia is a very attractive species for aquaculture because it can reach harvest size in just 6 months. It is the second most predominant fish species farmed globally after carp. They are farmed in many low and middle-income countries (LMIC) and provide an important source of revenue for low income families. Disease is associated with intensification of aquaculture systems, and both bacterial and viral diseases are severely impacting on the expansion of tilapia farming in LMICs. There is increasing concern about the use of antibiotics to control disease outbreaks and attention is focusing on the use of vaccination for disease control. We need a better understanding of how the cells of the immune response of tilapia respond to infection and vaccination to be able to develop and formulate effective vaccine products for this species. Few reagents are available to allow us do this, however. This project was a collaboration between scientists from Vietnam, Canada and the UK with the aim of developing a suite of synthetic antibodies (sAbs) for key immunological markers to study the response of different immune cell populations in tilapia. Synthetic antibodies are made in the laboratory unlike conventional antibodies, which are produced in animals, thus eliminating the need to use animals to make these reagents. The cell marker targets used for antibody production in this project included CD3Ɛ, CD4, CD8, CD172 (also known as SIRPα), CD45 and CD163. The DNA sequence of each target was successfully identified in the tilapia genome and Fc-fusion proteins produced for each target in HEK293 mammalian cells. As larger quantities of SIRPα, CD45 and CD3ε Fc-fusion proteins were produced compared to the other three targets, Fab-phage binding selection was initially performed for these markers, screening with an antibody phage-display library. Two unique binding phages were identified for tSIRPα, two for CD3 and 19 for CD45. So far five of the 19 phages for CD45 have been cloned into a human IgG vector and transfected into HEK293 mammalian cells to produce anti-CD45 sAbs. The other unique binding phages for SIRPα, CD3ε and CD45 remain to be cloned, and unique binding phages identified for CD4, CD8 and CD163. Three of the five anti-CD45 IgGs work in flow cytometry and indirect immunofluorescence (IIF), but not immunohistochemistry (IHC). These were subsequently used to analyse tilapia leukocyte populations sampled from a vaccination and challenge experiment performed by Vietnamese partners against Streptococcus agalactiae serotype III, sequence type 283, which gave a relative percent survival value of 62.5% in vaccinated

fish. Interestingly, greater amounts of leukocytes were detected in the spleen of non-vaccinated fish compared to vaccinated fish with the CD45 sAbs by flow cytometry. Although further development and optimisation of the sAbs is continuing, it is clear that such tools are important for monitoring the development of adaptive immunity and establishing correlates of protection for the vaccine.

# Poster abstracts

## 1 Enhancing the immunogenicity of *Streptococcus agalactiae* vaccine in tilapia (*Oreochromis niloticus*) with the aid of andrographolide as a novel immunoadjuvant

*Ambily Balakrishnan, Dr Preetham Elumalai*

*School of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Kochi, Kerala, India*

Aquaculture is one of the largest and fastest growing food-producing sectors in the world. Various species of tilapia, like Nile tilapia is one of the major contributors for this as it is cultivated extensively because of their high consumer demand along with the low harvesting time. Tilapia aquaculture in India had faced some challenges recently and high productivity is hampered by outbreaks of some infectious diseases in aquatic environment. Streptococcosis, which is mainly caused by *Streptococcus agalactiae* is one of the most devastating disease among them, which caused mass killing of fishes. Several researches have been conducted world-wide to develop effective *S. agalactiae* vaccines for tilapia, especially with the non-adjuvanted killed vaccines. But it offered protection for a limited period of time.

As the development of effective vaccines should be approached by combining protective antigens together with the application of specific adjuvants, Andrographolide (AG), a diterpene compound extracted from the herb *Andrographis paniculate* is used as adjuvant. It is thought to trigger specific immunological processes, without producing a generalized response with strong side effects. AG which is proved to be quickly absorbed in animals and its high bioavailability with no obvious side effects makes it a promising adjuvant, worthy of further research and development. Studies are planned to unambiguously establish the immunostimulatory potential of these natural adjuvants along with the formalin-killed *S. agalactiae* vaccine potency.

Following immunization with vaccine-adjuvant complex, systemic and mucosal antibody response will be monitored in the fishes. Antibody production and clinical efficacy will be measured in tilapia after immunization with two types of *S. agalactiae* vaccines, consisting of formalin killed cells (FKC) and FKC enriched with adjuvant administered by intraperitoneal injection and along with feed.

1. B.C. Silva, M. L Martin, A.Jatoba A, C.C.B Neto,F.N Vieira,G.V Pereira, G.T Jeronimo, W.Q Seiffert,J.L.P Mourino. Hematological and immunological responses of Nile tilapia after polyvalent vaccine administration by different routes. Pesq Vet Brasil. 2009; 29:874–80
2. Shi-ping Ma, Wei Wang, Jing Wang, Sheng-fu Dong,Chun-hong Liu,Paola Italiani, Shu-hui Sun, Jing Xu, Diana Boraschi and Di Qu . Immunomodulatory activity of andrographolide on macrophage activation and specific antibody response.
3. M.N Abu-Elalaa,A. Samirb, M.Wasfyc and M.Elsayed . Efficacy of injectable and immersion polyvalent vaccine against Streptococcal infections in brood stock and off spring of Nile tilapia (Oreochromis niloticus). Fish and Shell fish Immunology. 2019; 88:293-300

## 2 Mucosal vaccination of fish: A whole body approach

*Cornelius Gunter[1]*, Prof Jeroen Kortekaas[2], Dr Christelle Langevin[3] and Assoc Prof Maria Forlenza[1]*

***Affiliations: 1** Aquaculture & Fisheries Group, Department of Animal Sciences, Wageningen University & Research, Wageningen, The Netherlands; **2** Virology & Molecular Biology, Wageningen Bioveterinary Research, Wageningen University & Research, Lelystad, The Netherlands; **3** Molecular Virology & Immunology, French National Institute for Agricultural Research, Paris-Saclay University, Jouy-en-Josas, France*

This project aims to develop novel mucosal vaccination strategies for warm water fish species. Vaccination is the most effective way to prevent and possibly eradicate diseases which affect aquaculture fish species. Currently, the most effective vaccines are generally delivered by injection, but his method is labour intensive, causes handling stress and is only viable for certain fish species at the right age. Therefore, mucosal vaccination by immersion or oral administration, targeting the gills, skin, intestines or olfactory organs, is preferred.

Mucosal vaccines have been shown to be effective if they mimic the natural route of infection, for example by crossing mucosal surfaces, and if they elicit strong and lasting immune responses. In order to achieve these requirements, this project aims to develop live-recombinant viral vectors and replicon particle vaccines, and to assess their efficacy as mucosal vaccines for fish. For a whole-body approach, we will use a variety of available transgenic zebrafish lines to select promising vaccine candidates and gain insight into the underlying mechanisms of protection. The use of zebrafish which are transparent and/or have labelled immune cells will allow us to investigate *in vivo* and in real-time the kinetics of vaccine uptake and immune cell activation. Promising vaccine candidates will further be used to provide proof-of-principle of their efficacy in commercially relevant species, carp and tilapia.

Altogether the project addresses knowledge gaps on mucosal immune responses and could provide a universal platform for mucosal vaccination of fish.

## 3 Characterisation of genes essential for pathogenesis of fish adapted *S. agalactiae*

*Morena Santi, Adam Blanchard, James Leigh and Sharon A. Egan*

*School of Veterinary Medicine and Science, University of Nottingham*

*Streptococcus agalactiae* or Group B Streptococcus (GBS), is a highly infectious zoonotic pathogen, responsible for streptococcosis in fish, meningitis and septicaemia in humans and mastitis in cattle. In fish, it manifests and septicaemia, resulting in high morbidity and mortality in a number of wild and farmed species, in particular tilapia, sea bass and rainbow trout. A population of GBS mutants were generated using the pGh9:*ISS1* insertional mutagenesis system, to identify conditionally essential genes required for survival in three different conditions related to GBS pathogenesis in fish. The chosen phenotype included growth of a GBS strains in tilapia serum, in media containing hydrogen peroxide and media containing an antimicrobial peptide. Comparative sequence analysis was performed on approximately 100,000 individual mutants for each condition using the PIMMS transposon analysis platform. Among the conditionally essential genes, 3 were shared between the three conditions, *atpB*, *atpC* and *dltX*. *AtpB* and *atpC* are part of ATP synthase or ATPase operon, involved in the regulation of internal pH and the production of ATP, while *dltx* is involved in d-alanylation of teichoic acids

important for charge based regulation of the cell envelope and resistance to the effects of positively charged host immune factors. Other conditionally essential genes were shared only between two of the three conditions, such as the heat shock protein *dnaK* and the protease *clpX*. DnaK is key for the regulation of accurate protein folding whilst clpX removes misfolded or damaged proteins. This study highlights the potential use for transposon mutagenesis to rapidly identify potential vaccine targets for bacterial aquaculture related disease allowing this growing industry to become more sustainable for food production.

## 4 Development of a nanoparticle Tilapia Lake Virus (TiLV) vaccine for tilapia aquaculture in India

*Sreeja Lakshmi*[1], *David Smith*[1], *Kim Thompson*[1], *Sureshkumar Sivan*[2], *Preetham Elumalai*[3]

***Affiliations:* 1** *Aquaculture Research Group, Moredun Research Institute, United Kingdom;* **2** *School of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, Kerala, India;* **3** *Department of Fish Processing Technology, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, Kerala, India*

Aquaculture is the fastest growing food-production sector globally, with over 1 billion people relying on fish as their major protein source. Tilapia (*Oreochromis* sp.) is a major trade commodity for many low to middle-income countries (LMIC), with its production estimated to be around 6.4 million tons per annum (FAO, 2017). The hardiness of tilapia, its adaptability to various production systems and its rapid growth, makes it an excellent fish species for aquaculture. Intensification of tilapia farming has promoted severe disease outbreaks, however, resulting in high mortalities and economic hardship for tilapia farmers. Tilapia Lake Virus (TiLV), a highly virulent and contagious novel orthomyxo-like virus has recently been associated with disease outbreaks in tilapia aquaculture, resulting in massive mortalities in both wild and cultured tilapines. The project addresses to investigate a novel and innovative nanoparticle for delivery of the TiLV vaccine, comparing its efficacy when delivered by immersion, oral or by IP injection and provide an easy and acceptable manner by tilapia fish farmers. Our study focuses on the development of a Tilapia Lake Virus (TiLV) vaccine for tilapia aquaculture in India, using a novel nanoparticle vaccine delivery system. The immune response it elicits, will be evaluated and compared to an inactivated, adjuvanted TiLV vaccine. Fish will be challenged with a virulent strain of TiLV to test the level of protection elicited by the vaccines. Relative percentage survival and antibody titres will be determined for vaccinated fish. The antigenic diversity between Indian TiLV isolates to ensure the vaccine is able to cross-protect between TiLV isolates will be also examined using western blotting with serum from naturally infected fish, followed by contemperory proteomic analysis. The demonstration of the effectiveness of nanoparticle fish vaccine that can be delivered orally or by immersion would represent a significant opportunity for many different aquaculture industries, and this technology could be applied to several diseases in different aquaculture settings, including LMIC countries and even domestic aquaria, where price of vaccination or handling of fish for vaccination is problematic. This is a significant approach helps to pursue the commercialisation of the TiLV vaccine and to advance scientific knowledge on TiLV.

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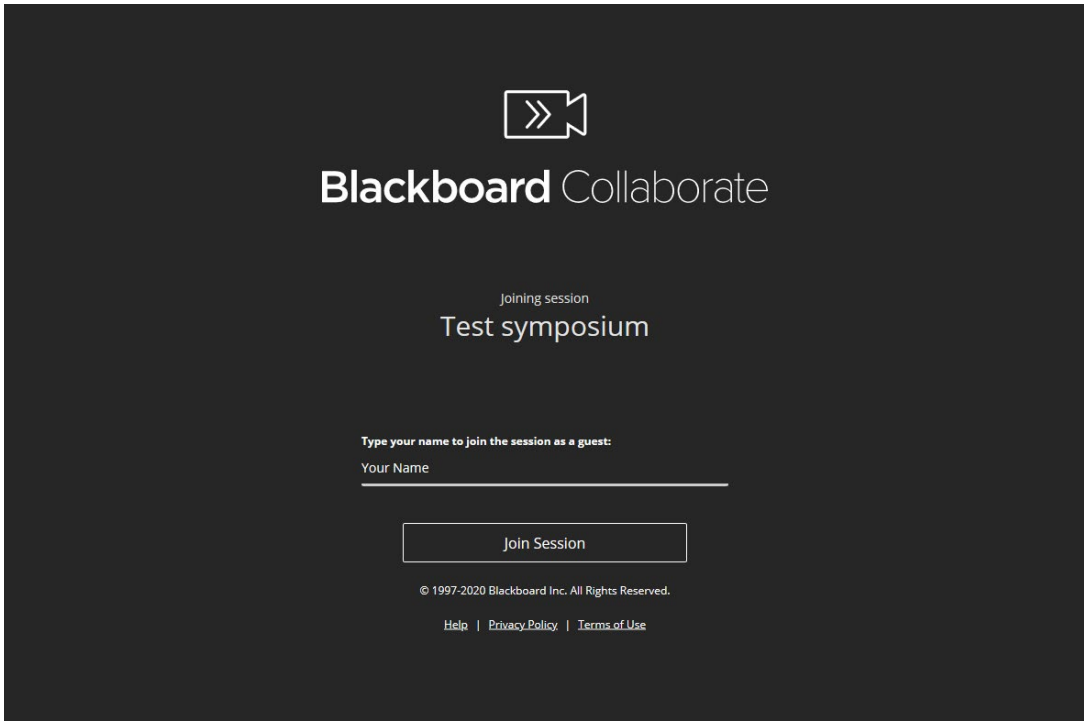


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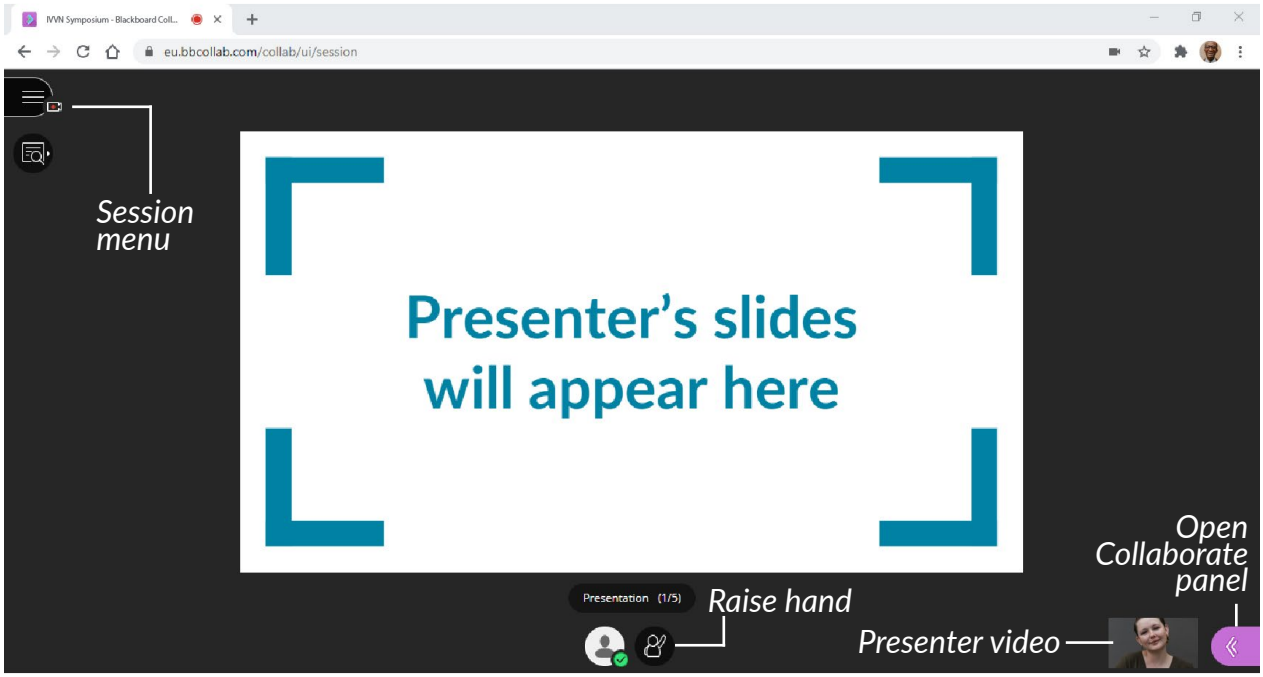


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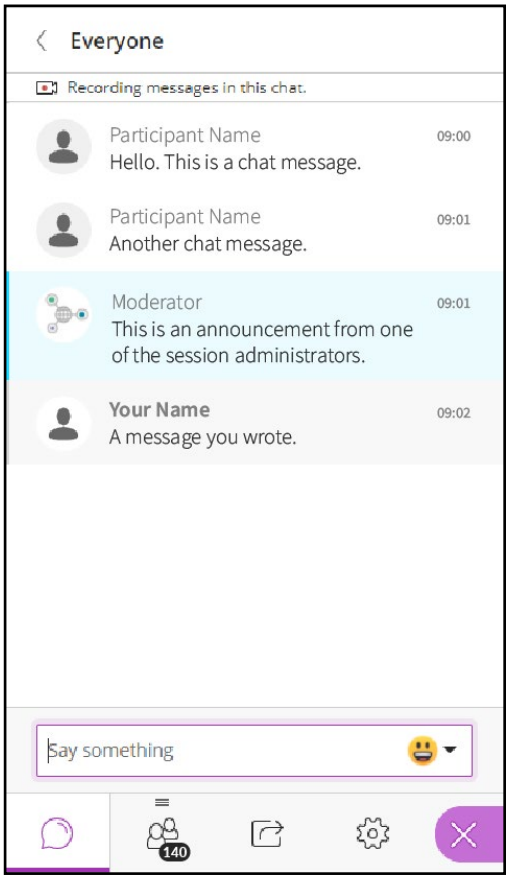


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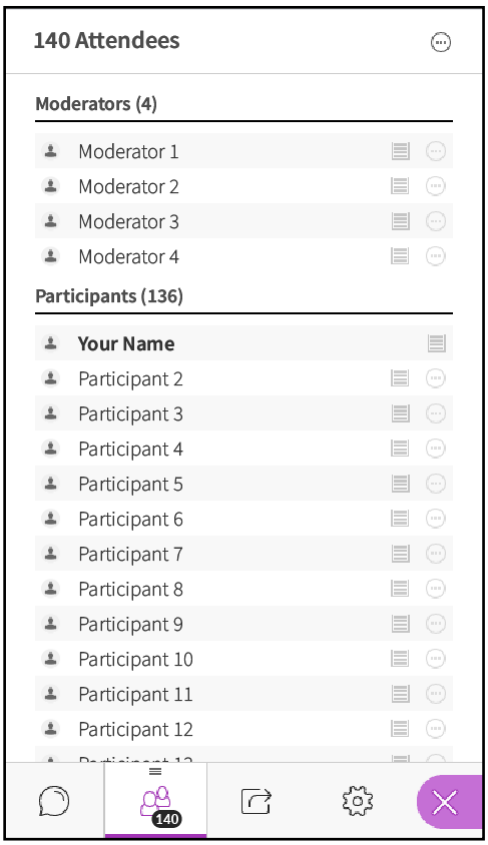


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