

**Monday September 3<sup>rd</sup> – Archibald / Campbell  
Tilapia Health 1**

**Moderators – Win Surachetpong ( Kasetsart University ) Paola Barato ( Copavet – Colombia )**

1:15 PM	<b>Tilapia Health 1</b>	<u>Ramirez-Paredes</u> - The Potential Of Autogenous Vaccines For Controlling Infectious Diseases In Tilapia Aquaculture
1:30 PM		<b><u>Delphino</u> - Seasonal Dynamics Of Bacterial Pathogens Of Nile Tilapia Farmed In A Brazilian Reservoir</b>
1:45 PM		<u>Barato</u> - Outbreaks Of <i>Edwardsiella anguillarum</i> -Associated Edwardsiellosis In Farmed Tilapia ( <i>Oreochromis Sp.</i> ) In Colombia
2:00 PM		<u>Lafrentz</u> - Resistance Of Nile Tilapia <i>Oreochromis niloticus</i> To <i>Streptococcus Iniae</i> And <i>S. agalactiae</i> Is Heritable But Not Correlated
2:15 PM		<b><u>Pulpipat</u> - Phenotyping, Genotyping, And Pathogenicity Of <i>Francisella noatunensis</i> Subsp. <i>orientalis</i> Isolated From Cultured Tilapia <i>Oreochromis Sp.</i> In Taiwan</b>
2:30 PM		<u>Surachetpong</u> - Current Situation Of Tilapia Lake Virus: What Arte The Impact To Tilapia Aquaculture?
2:45 PM		<u>Gómez-Sánchez</u> - Tilapia Lake Virus ( Tilv ) Emergency Disease In Wild Tilapia In Peru
3:00 PM		<b>Refreshments</b>



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## **The Potential of Autogenous Vaccines for Controlling Infectious Diseases in Tilapia Aquaculture.**

J. Gustavo Ramirez-Paredes\* and Timothy S. Wallis

Ridgeway Biologicals, Compton, Berkshire, England U.K.

[gus.ramirez-paredes@ridgewaybiologicals.co.uk](mailto:gus.ramirez-paredes@ridgewaybiologicals.co.uk) [tim.wallis@ridgewaybiologicals.co.uk](mailto:tim.wallis@ridgewaybiologicals.co.uk)

The farming of tilapia species (*Oreochromis niloticus*, *O. aureus*, and *O. mossambicus*) is one of the most important sectors in aquaculture worldwide. This activity has spread throughout dozens of developing countries in Africa, Asia and Latin America where it boosts local economy and constitutes an affordable source of animal protein for human consumption. Losses due to infectious disease are one of the major challenges that this industry currently faces. Outbreaks of disease are predominantly caused by co-infections where two or more pathogens trigger mortalities after husbandry handling procedures. The most common tilapia pathogens are: *Streptococcus* spp., *Flavobacterium columnare*, *Edwardsiella tarda*, *Aeromonas* sp., *Plesiomonas shigelloides*, *Francisella noatunensis orientalis* and Tilapia Lake Virus. Despite the relevance that these agents possess there are no licensed vaccines commercially available to prevent or treat these coinfections. Autogenous vaccines (AV) are farm-specific inactivated formulations that have the potential to be rapidly developed and deployed when novel pathogens emerge and off-the-shelf fully licensed vaccines do not exist. In this context the use of AV arises as the most efficacious solution to control mortalities caused by these pathogens in the field. Ridgeway Biologicals Ltd (RBL) is the UK leading supplier of AV's for veterinary use and holds a range of mono and multivalent aquaculture vaccines. These include bacterins for tilapia successfully used in West Africa and the first viral auto vaccine for farmed fish i.e. a Nodavirus AV for European sea bass widely used in Greece. In an attempt to gain a better understanding on the predominant pathogens affecting tilapia farmed in Latin America, RBL has conducted a series of bacteriological and viral surveys in farms suffering mortalities in Colombia, Honduras, Peru and Mexico. In the present study a summary of these outcomes will be presented and compared to our results from Africa where regular disease surveillance has been established since 2016 by RBL. Moreover the significance of maintaining these surveillance programmes and their impact on the success that auto-vaccines have will be discussed and exemplified with a study case from Ghana.

**Conference Session Designation:** ( Immunology-Vaccines or Cleaner Fish )

**Presentation Format:** ( Oral )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Seasonal Dynamics of Bacterial Pathogens of Nile Tilapia Farmed in a Brazilian Reservoir

Marina K V C Delphino<sup>1,3\*</sup>, Carlos A G Leal<sup>2</sup>, Ian A Gardner<sup>3</sup>, Gabriella B N Assis<sup>2</sup>, Georgia D Roriz<sup>1</sup>, Fernando Ferreira<sup>4</sup>, Henrique C P Figueiredo<sup>2</sup>, and Vítor S P Gonçalves<sup>1</sup>

- <sup>1</sup> Veterinary Epidemiology Laboratory (EpiPlan), Faculty of Agronomy and Veterinary Medicine, University of Brasilia, Campus Darcy Ribeiro, Brasília, DF, Brazil  
[vitorspg@unb.br](mailto:vitorspg@unb.br) [mdelphino@upe.br](mailto:mdelphino@upe.br)
- <sup>2</sup> National Reference Laboratory of Aquatic Animal Disease (AQUACEN), Ministry of Fisheries and Aquaculture, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil [leal.cag@gmail.com](mailto:leal.cag@gmail.com) [figueiredoh@yahoo.com](mailto:figueiredoh@yahoo.com)
- <sup>3</sup> Health Management Department, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada [iagardner@upe.ca](mailto:iagardner@upe.ca)
- <sup>4</sup> Department of Preventive Veterinary Medicine and Animal Health, Faculty of Veterinary Medicine and Agronomy, University of São Paulo, Cidade Universitária, São Paulo, SP, Brazil [fernando@vps.fmvz.usp.br](mailto:fernando@vps.fmvz.usp.br)

Fish aquaculture is rapidly growing in Brazil. Nile tilapia is the most cultivated species, mainly through an intensive production system carried out in floating cages installed in large reservoirs. However, fish pathogens pose a major challenge to production chain sustainability, and tilapia farmers often have limited knowledge of prevailing health problems and rarely implement biosecurity practices to prevent introduction of economically important infectious agents. This study aimed to identify the key disease risks of tilapia farming in a tropical reservoir and characterize the dynamics of the prevalent pathogens, as a basis for development of effective control measures for tilapia health and surveillance programs. From August 2015 to October 2016, a longitudinal study was carried out at the Três Marias reservoir, in the municipality of Morada Nova de Minas in the southeast of Brazil. Daily and monthly data were collected from six out of 32 existing fish farms, including fish samples, mortality counts, and measurements of temperature and water quality parameters. The main bacteria detected were *Streptococcus agalactiae*, infecting mostly adult tilapia throughout the period, with higher frequency as the average temperature increased, and *Francisella noatunensis* subsp. *orientalis* (Fno), infecting mainly younger tilapia, only during the cooler months. Coinfections with multiple pathogens were detected in 33 fish. The detection of Fno in one farm in two consecutive winters, after months of unfavorable water temperature conditions and without evidence of sustained introduction of infected stock, strengthens the case for investigating if this pathogen can survive and remain infective causing new outbreaks. Furthermore, variation in mortality was likely associated with the dynamics of the studied pathogens.

**Conference Session Designation:** ( Tilapia Disease )  
**Presentation Format:** ( Oral )  
**Student Presentation:** ( Yes )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Outbreaks of *Edwardsiella anguillarum*-Associated Edwardsiellosis in Farmed Tilapia (*Oreochromis* sp.) in Colombia

Paola Barato<sup>1\*</sup>, Matt Griffin<sup>2</sup>, Laura Pachón<sup>1</sup>, Miguel Montufar<sup>1</sup>, Susan Yun<sup>3</sup>, Esteban Soto<sup>3</sup>

<sup>1</sup> Corporación Patología Veterinaria, CORPAVET, Calle 25A # 38A – 16, 111321, Bogotá, Colombia  
[paola.barato@corpavet.com](mailto:paola.barato@corpavet.com)

<sup>2</sup> Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, PO Box 197, 127 Experiment Station Road, Stoneville, MS 38776, USA  
[matt.griffin@msstate.edu](mailto:matt.griffin@msstate.edu)

<sup>3</sup> Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA, [sotomartinez@ucdavis.edu](mailto:sotomartinez@ucdavis.edu)

Edwardsiellosis in fish can be caused by members of the genus *Edwardsiella*, including *Edwardsiella ictaluri*, *E. tarda*, *E. piscicida* and *E. anguillarum*. While *E. ictaluri* and *E. tarda*-associated edwardsiellosis have been reported in tilapia (*Oreochromis* sp.), these reports occurred prior to the recent reorganization of *E. tarda* and recognition of *E. piscicida* and *E. anguillarum* as valid taxa. Herein, we describe two outbreaks of *E. anguillarum* associated edwardsiellosis in farmed tilapia in Colombia. The first outbreak was reported by the Departmente of Meta in January of 2017, a mortality event in tilapia (*Oreochromis* sp.) raised in a biofloc system approached 40%. Similarly, in November 2017 in the Department of Huila, mortality in pond-reared red (*O. mossambicus* X *O. niloticus*) and Nile tilapia (*O. niloticus*) alevins approached 30%. For each outbreak, 15 live tilapia alevins (~10g) were submitted for diagnostic workup. Fish were euthanized upon submission and immediately subjected to post-mortem examination. Brain, eyes, gills, heart, liver, spleen, stomach, intestine, kidney and skin were processed for histopathological analysis. Also, spleen, liver, brain and eyes from five fish were pooled aseptically, homogenized and aerobically cultured on blood agar for microbiological analysis. Recovered isolates consistent with *Edwardsiella* spp. were archived for later molecular analysis. In both cases, histopathology demonstrated systemic granulomatous infection (granulomatous splenitis, hepatitis, nephritis, encephalitis and choroiditis) compatible with previous reports of edwardsiellosis. Three isolates were recovered from the Biofloc case: two were identified molecularly as *E. anguillarum*, which was confirmed molecularly by PCR and *gyrB* sequence analysis; while one isolate was confirmed as *E. tarda*. One *E. anguillarum* isolate was recovered from the Huila case with the same molecular analysis. One of the confirmed *E. anguillarum* isolates was used to fulfill Koch's postulates by intragastric (IG; 10<sup>7</sup> CFU/fish), intracoelomic (IC; 10<sup>7</sup> CFU/fish) and immersion (IMM; 10<sup>8</sup> cfu/ml) challenges in Nile tilapia fingerlings. Each exposure group consisted of 10 tilapia alevins (approximately 8g). All tilapia exposed by IC died during the first 24 h post-challenge. Similarly, all fish exposed by IG died within 72 h. Comparably, four tilapia exposed by IMM infection died within 72 h. The six remaining survivors were euthanized 7 days post challenge. Only one control fish (IC challenge) died. Tissues from fresh dead IG and IMM infected fish, as well as the survivors from the IMM challenge and controls were evaluated by histopathology. Systemic granulomatous infection was observed in all exposed fish, with no relevant lesions present in any controls fulfilling Koch's postulates. This is the first description of *E. anguillarum* associated edwardsiellosis in tilapia.

**Conference Session Designation:**

( Tilapia Diseases )

**Presentation Format:**

( Oral )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Resistance of Nile Tilapia *Oreochromis Niloticus* to *Streptococcus Iniae* and *S. Agalactiae* is Heritable But Not Correlated

Benjamin R. LaFrentz<sup>1\*</sup>, Craig A. Shoemaker<sup>1</sup>, Carlos A. Lozano<sup>2</sup>, Julio C. García<sup>1</sup>, Esteban Soto<sup>3</sup>, De-Hai Xu<sup>1</sup>, Benjamin H. Beck<sup>1</sup>, Morton Rye<sup>2</sup>

<sup>1</sup> USDA-ARS, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832 USA  
[benjamin.lafrentz@ars.usda.gov](mailto:benjamin.lafrentz@ars.usda.gov)

<sup>2</sup> Akvaforsk Genetics Center AS, Sjølsengvegen 22, N-6600 Sunndalsøra, Norway

<sup>3</sup> University of California, Davis, School of Veterinary Medicine, Tupper Hall, Davis, CA 95616 USA

Tilapia (*Oreochromis* sp.) are an important source of protein with an economic value approaching US \$8 billion yearly. Streptococcal disease, caused by *Streptococcus iniae* and *S. agalactiae* are emerging or re-emerging diseases that negatively affecting tilapia aquaculture worldwide. Because of the difficulty controlling these pathogens in tilapia production, selective breeding for resistance *S. iniae* and *S. agalactiae* is a potential tool to limit the impact of streptococcal disease. The objectives were: 1) to verify additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *S. iniae*; 2) to determine if realized genetic gain in resistance and/or susceptibility to *S. iniae* is possible following positive assortative mating based on estimated breeding values (EBV); and 3) to determine if resistance to *S. iniae* and *S. agalactiae* is genetically correlated. A total of 144 full and paternal half-sib families were challenged intraperitoneally with *S. iniae* using PIT tagged fish in a common tank. For *S. agalactiae* challenge, 130 full and paternal half-sib families were intramuscularly injected. Cumulative mortality was 46% for *S. iniae* and 68% for *S. agalactiae*. There was a high additive genetic component found for survival in fish injected with *S. iniae* (estimated heritability  $0.52 \pm 0.12$ ) validating our previous results. The estimated heritability for *S. agalactiae* was  $0.38 \pm 0.11$  based on the univariate linear animal model. Positive assortative mating further demonstrated resistance to *S. iniae* was heritable with mean survival of 88% (range 60 – 100%) for families produced on high EBV (*S. iniae* resistant parents) and mean survival of only 10% (range 0 -42%) for families produced using low EBV (*S. iniae* susceptible parents). No genetic correlation was noted amongst resistance to *S. iniae* and *S. agalactiae* Ib. Selective breeding of tilapia to improve survival to *Streptococcus* sp. will require knowledge of the pathogen(s) prevalent in the region so that custom genetic material may be formulated for individual farms.

**Conference Session Designation:**

( Tilapia Disease )

**Presentation Format:**

( Oral )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Phenotyping, Genotyping, and Pathogenicity of *Francisella Noatunensis* subsp. *Orientalis* Isolated from Cultured Tilapia (*Oreochromis* Sp.) in Taiwan

Theeraporn Pulpipat<sup>\*</sup>, Pei-Chi Wang and Shih-Chu Chen

Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Taiwan ROC [pidpudpan@gmail.com](mailto:pidpudpan@gmail.com)  
[pc921003@gmail.com](mailto:pc921003@gmail.com) [scchen@mail.edu.tw](mailto:scchen@mail.edu.tw)

*Francisella noatunensis* subsp. *orientalis* (*Fno*) has been reported as a causative agent of systemic granulomatous disease in tilapia and ornamental cichlids in Taiwan for the past 26 years. However, the phenotypic and genotypic diversities, and also the pathogenicity of Taiwanese *Fno* strains are still poorly understood. In this study, phenotypic and genetic characteristics, as well as pathogenicity of *Fno* isolates obtained from diseased fish in different geographical locations in Taiwan were examined. Bacterial colonies were isolated from Tilapia (n=17) and Green Texas cichlid (*Herichthys cyanoguttatus*) (n=1) on cysteine heart agar supplement with 1% bovine hemoglobin, and identified as *Fno* using polymerase chain reaction with species-specific primers. An assessment of enzymatic profile of *Fno* isolates was carried out under the API ZYM system. Genotypic determination of *Fno* isolates was performed by phylogenetic analysis based on 16S rRNA and housekeeping genes, together with pulsed-field gel electrophoresis (PFGE) using *Xho*I and *Bam*HI restriction enzymes. The phylogenetic tree showed that 16S rRNA and housekeeping genes of Taiwanese isolates possessed a very high nucleotide similarity (99-100%) to that of other *Fno* references from GenBank database. All of the *Fno* isolates in this study revealed identical enzymatic and PFGE profiles which discriminated from *F. philomiragia* isolated from marine fish. The clinical isolates from diseased tilapia were further confirmed for their pathogenicity and virulence by inoculation in cultured tilapia. Systemic granulomatous lesions were presented in spleens and head kidneys, concomitant with high mortalities similar to that observed in a natural outbreak. Based on the cumulative mortalities found at day 21 after challenged with *Fno*, the observed median lethal dose (LD<sub>50</sub>) for the intraperitoneal challenge in tilapia and red tilapia were 9.06 x 10<sup>3</sup> CFU/fish and 2.08 x 10<sup>2</sup> CFU/fish, respectively. Taken together, our data provided a basis for characterization, epidemiology of Taiwanese *Fno* isolates, and future vaccine development.

**Conference Session Designation:** ( Bacteriology or Tilapia Diseases )  
**Presentation Format:** ( Oral )  
**Student Presentation:** ( Yes )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Current Situation of Tilapia Lake Virus: What are the Impact to Tilapia Aquaculture?

Win Surachetpong<sup>1,2\*</sup> Puntanat Tattiyapong<sup>1,2</sup>

<sup>1</sup> Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand

<sup>2</sup> Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand [fvetwsp@ku.ac.th](mailto:fvetwsp@ku.ac.th)  
[been\\_kyo@gmail.com](mailto:been_kyo@gmail.com)

Tilapia is the second most important aquaculture fish species worldwide with the annual production of 5.6 million tonnes. Although tilapia has been recognized as the disease resilient finfish species, mass mortality of an unidentified etiology has been recently observed in major tilapia producing countries. Since 2011, the investigation of mass mortality in wild and farmed tilapia in Israel led to the identification of a novel RNA virus called Tilapia Lake Virus (TiLV). Subsequently, the virus has been detected in tilapia cichlid in Ecuador, Colombia, Egypt, Thailand, Taiwan, Malaysia, and India. In Thailand, the disease has been called “Tilapia One-Month Mortality Syndrome” (TOMMS) as the mortality usually occur during a month period after juvenile tilapia have been transferred from hatchery to the grow-out ponds or cages. The clinical signs of diseased fish included skin redness and erosion, exophthalmos, abdominal distension, scale protrusion, multiple skin hemorrhages, pale and liver contraction. Generally, multiple infections of external parasites and opportunistic bacteria has been found in TiLV-infected fish. The predisposing factors such as inappropriate handling, transportation, poor water quality, and fluctuation of water temperature associate with TiLV outbreaks. Moreover, high stocking density, frequent used of pond, size of fish and genetic background of fish are important risk factors for TiLV epidemic. Currently, the detection of TiLV relies on the molecular methods including RT-PCR, nested RT-PCR, and RT-qPCR together with the clinical signs, histopathology and virus isolation in the susceptible cell culture. To reduce the impact of this emerging viral disease, rapid diagnostic assay, strict biosecurity, and vaccine development could support disease containment and limit virus spreading within the endemic region or to a new geographical area. Furthermore, fundamental research on the pathogenesis, route of disease transmission, susceptible host species and vectors await further investigation. Such knowledges are important to gain better understanding of the TiLV biology that could lead to better control strategies.

**Conference Session Designation:** ( Tilapia Disease )  
**Presentation format:** ( Oral )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



## Tilapia lake virus ( TiLV ) emergency disease in wild Tilapia in Peru

( **WITHDRAWN** )

Muriel Gómez-Sánchez<sup>1\*</sup> and Katherine Zapata<sup>1</sup>

Deputy Direction of Aquaculture health, National Fisheries Agency Health of Peru. Lima Peru  
[muriel.gomez@sanipes.gob.pe](mailto:muriel.gomez@sanipes.gob.pe)

Tilapia lake virus disease (TiLVD) is an emerging disease reported in tilapias farmed and wild, since the first report in Israel (Eyngor et al., 2014). Subsequently the virus has been found in South America, Africa and Asia (Bacharach et al., 2016; Fathi et al., 2017; Surachetpong et al., 2017). The World Organization for Animal and Health (OIE) published a technical card in February 2018 to describe clinical signs of TiLV including ocular alterations, skin erosions, congestion of the spleen and hemorrhages in the leptomeninges. In this study, we described the reports of TiLV outbreak in wild tilapias in Peru. Since November 2017, local fisherman's notified massive mortality of all life stages wild tilapias at four different water sources (reservoirs and lakes) in Piura and San Martín regions. The affected fish had clinical signs with skin erosions and redness, ocular injury and unusual behavior. The PCR diagnosis confirmed TiLV positive in all of the four cases. This is the first report of multiples outbreaks of TiLV in wild tilapias in South America. The emergence of the virus could be related to several factors including illegally movement of fingerlings from positives countries, the spreading of virus in water and potential vectors such as wild fish, birds, crustaceans, others. Meanwhile, ongoing actions are focusing on reduce the virus spread and surveillance tilapias farms and others water sources.

**Conference Session Designation:** ( Tilapia Disease )  
**Presentation Format:** ( Oral )



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada

