Monday September 3rd – Archibald / Campbell
Tilapia Health 2
Moderators – Win Surachetpong (Kasetsart University) **Paola Barato** (Copavet – Colombia)

3:15 PM	**Tilapia Health 2**	Sanguinetti - Identification of Tilapia Lake Virus In Fish Farms of The Rainforest Region of Peru
3:30 PM		Montufar - Epidemiologic Assessment and DNA Sequencing of Tilv From Colombian Tilapia Farms Using Motif Fingerprints
3:45 PM		**Liamnimitr - Mucus as a Source of Horizontal Transmission and Non-Lethal Sampling For Tilapia Lake Virus Detection**
4:00 PM		Soto - Tilapia Lake Virus Susceptibility to Iodine and Chlorine Disinfectants

Identification of Tilapia Lake Virus in Fish Farms of the Rainforest Region of Peru

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Tilapia (*Oreochromis sp.*) are an increasingly important protein source worldwide due to their omnivorous diet, high-stress tolerance and disease resistance. In recent years, the emergence of Tilapia lake virus (TiLV) disease, caused by a novel Orthomyxo-like virus that affects *Oreochromis sp*., has been reported in Israel, Egypt, Thailand, India, Colombia and Ecuador producing high mortalities and important economic losses for tilapia aquaculture. By the end of 2017 several outbreaks of high mortality have been observed in wild and farmed tilapias in the north of Peru. Our first report confirmed the presence of TILV in Piura region. Two months later, high mortalities were reported in the Peruvian northeast, in the rainforest area of San Martin region. For this study samples from two affected tilapia farms in San Martin region were taken, consisting of 210 fish from different productive stages. Samples of liver, brain and intraocular fluid were collected from juvenile and adult tilapia and processed in pools of all organs every 3 fish. In the case of younger productive stages (fry), whole fish were processed in pool of 100 individuals approximately. In order to diagnose the etiological agent, molecular and histopathological assays were performed. Pools of tissue samples were processed for ARN extraction and analyzed through RT-PCR nested technique to amplify segment 3 of TiLV genome. PCR products were sequenced and compared with Israeli isolate of TiLV (KU751816.1) to determine identity between all strains. Results showed, for the second time, the presence of TiLV in Peruvian tilapia, evidencing high nucleotide identity (96.9-97.1%) to Israeli strain at the sequence analysis and the presence of typical syncytial giant cells in liver observed in histopathology. This results evidence the distribution capability of TiLV through South America as an important emerging disease for tilapia aquaculture, and its rapid dissemination inside Peruvian territory. Tilapia culture is a raising economic activity in Peru and its production is principally distributed in the north and northeast regions of the country. Our results must offer important information in order to stablish and improve control and prevention of dissemination strategies in Peru.

Conference Session Designation: (Emergent Disease or Tilapia Disease)
Presentation Format: (Oral)

Epidemiologic Assessment and DNA Sequencing of Tilv from Colombian Tilapia Farms Using Motif Fingerprints

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The Tilapia Lake Virus (TiLV) is a novel orthomyxo-like virus which causes high mortalities and economical losses among tilapia (*Oreochromis* sp.) producers worldwide. While TiLV was reported in May 2015 from a Colombian tilapia fish farm, little is known of the virus distribution within the country. To determine the specific frequency of TiLV disease, several fish farmers and Corpavet, began to collect tilapia samples with lesions associated TiLV infection. From June 2016 to March 2018 we evaluated 463 cases from 23 (72%) of 32 Departments of Colombia. Necropsy evaluations at several developmental stages were performed using 5 to 10 tilapias per case (50 cases of larvae, 310 of alevins, 18 of pre-growth-out, 28 of growth-out fish and 57 of adults for reproduction). Four hundred thirty-three (433) cases had mortality history and 30 did not report clinical signs. Spleen, liver, brain and eyes from five fish of each batch were pooled aseptically, homogenized and frozen in PBS or RNA later at -40ºC to latter RT-PCR for TiLV described by Eyngor et al., (2014). Brain, eyes, gills, heart, liver, spleen, stomach, intestine, kidney and skin were also processed for histopathological analysis. Molecular assays showed that 109 cases (23%) yield positive amplification of the segment 3 of TiLV genome. On site metagenomic sequencing of three positive samples was performed using the MinION portable DNA sequencing device. This approach yielded more than > 3 million reads that were scanned against a library of 8.5 billion motif fingerprints covering all know organisms. This approach mapped thousands of reads to different segments of the TiLV genome. Six of 109 cases had not mortality or clinical signs, they came from regular health monitoring. Ninety-eight (98) cases positive to TiLV for RT-PCR were alevins, 3 were larvae, 3 adults for reproduction, 3 were pre-growth-out fish and 2 in growth-out phase. Alevins with mortality history and RT-PCR positive (92 cases) had histopathological lesions compatible with TiLV infection (syncytial hepatitis, encephalitis and/or keratitis). This is the first epidemiological assessment and on site DNA sequencing-based confirmation of the presence of TiLV in several regions of Colombia. This information will be very useful to propose a plan to minimize the impact of TilV in the country, improve the epidemiological biosurveillance and optimize the sensitivity of molecular diagnostics.

Conference Session Designation: (Virology / Emergent Disease)
Presentation Format: (Oral)

Mucus as a Source of Horizontal Transmission and Non-Lethal Sampling for Tilapia Lake Virus Detection

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A recent emerging orthomyxo-like virus called Tilapia lake virus (TiLV) has been discovered in wild and farm-raised tilapia in Israel in 2014. Later, multiple outbreaks of TiLV were reported in three continents and eight countries including Ecuador, Colombia, Egypt, Thailand, Malaysia and India. The cumulative mortality rate of TiLV in affected farm are as high as 80-90% depend on the management practices, breed of fish and other environmental factors. Although studies of TiLV receive more attention, little is known about the mode of transmission and how the virus spread in fish population. In this study, we demonstrated that cohabitation of TiLV-infected tilapia and susceptible tilapia led to high mortality of 55.71% within 14 days. Interestingly, TiLV genomic RNA could be detected in liver and mucus of cohabitation challenge fish. The material prepared from TiLV-infected mucus caused CPE formation in E-11 cells within 5-7 days, suggesting that live viruses are present in the infected fish mucus. Our results also revealed that TiLV RNA persists in fish mucus up to 12 days post infection, allowing the possibility of virus to spread in fish population. For the diagnostic purpose, mucus could provide a non-lethal sampling procedure for TiLV detection in valuable broodstocks or large fish. Horizontal transmission via direct contact of carrier or TiLV-infected fish is important route of virus transmission. Taken together, our data provide important insight that could be applied for the control and implementation of biosecurity program for TiLV control.

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

Tilapia Lake Virus Susceptibility to Iodine and Chlorine Disinfectants

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Tilapia Lake Virus (TiLV) is an emergent orthomyxo-like virus affecting the tilapia (*Oreochromis* sp.) aquaculture industry worldwide. Mortality in affected farms typically reach 80-90% in affected systems and there is no current vaccine or therapy against the diseases. The aim of this study was to identify the biocide efficacy of two commonly used disinfectants in aquaculture, namely, house hold bleach (free-chlorine) and Buffered Povidone-Iodine (PVP Iodine) complex against TiLV. Cultured TiLV was grown on endothelial *Oreochromis mossambicus* bulbus arteriosus cell line (TmB) and a stock stored at -80C. Chlorox (The Clorox Company, Oakland, California, USA) and Ovadine (Syndel, Ferndale, Washington, USA) ranging from 10-100 mg/L (ppm) were diluted the day of the assay in autoclaved water collected from an aquaculture facility in California, USA and added to TiLV for 0.5, 1, or 24h. At each timepoint, sodium thiosulphate was added to inactivate the available iodine and dilutions in media containing 10% FBS were used to inactivate the free chlorine. All aliquots were then titrated on TmB cells to determine the $TCID_{50}$/ml. Virucidal reductions in titre of >4 $\log_{10}$ $TCID_{50}$/ml after 0.5 and 1h were only obtained at concentrations of ≥20ppm free chlorine and ≥50ppm available iodine. When contact time with disinfectant increased to 24h, virucidal reductions in titre of >4 $\log_{10}$ $TCID_{50}$/ml were obtained at concentrations of ≥10ppm free chlorine and ≥25ppm available iodine. In conclusion, chlorine and iodine were found effective for the disinfection of fish farming equipment at the manufacturer's recommended dose for 30 min duration. This information should be taken into consideration when developing biosecurity protocols in tilapia aquaculture.

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