World Organisation for Animal Health

Launch of the Regional AquaticAnimal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa

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UNION AFRICAINE
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FishGov 2 Project

World Organisation for Animal Health

Network of WOAH Reference Laboratories in aquatic animal health

Dr Nick Moody

CSIRO ACDP Fish Diseases Laboratory

Australia

- Research Group Leader – ACDP Fish Diseases Laboratory (AFDL)
  - Australia's National Reference Laboratory
  - Diagnosis (and research) into emerging and exotic pathogens of aquatic animals
- WOAH Designated Expert:
  - Infection with yellow head virus genotype 1
  - Infection with epizootic haematopoietic necrosis virus
  - Infection with Ranavirus
  - (Infection with abalone herpesvirus)
  - WOAH *ad hoc* Group on the *Aquatic Manual*
  - WOAH electronic *ad hoc* Group on tilapia lake virus
- FRDC Aquatic Animal Health and Biosecurity Coordination Program
  - Leader
- Sub-committee for Aquatic Animal Health (SCAAH) – Member
  - Sub-committee for Animal Health Laboratory Standards (SCAHLS) – Observer
- Aquatic Consultative Committee on Emergency Animal Disease (aqCCEAD)

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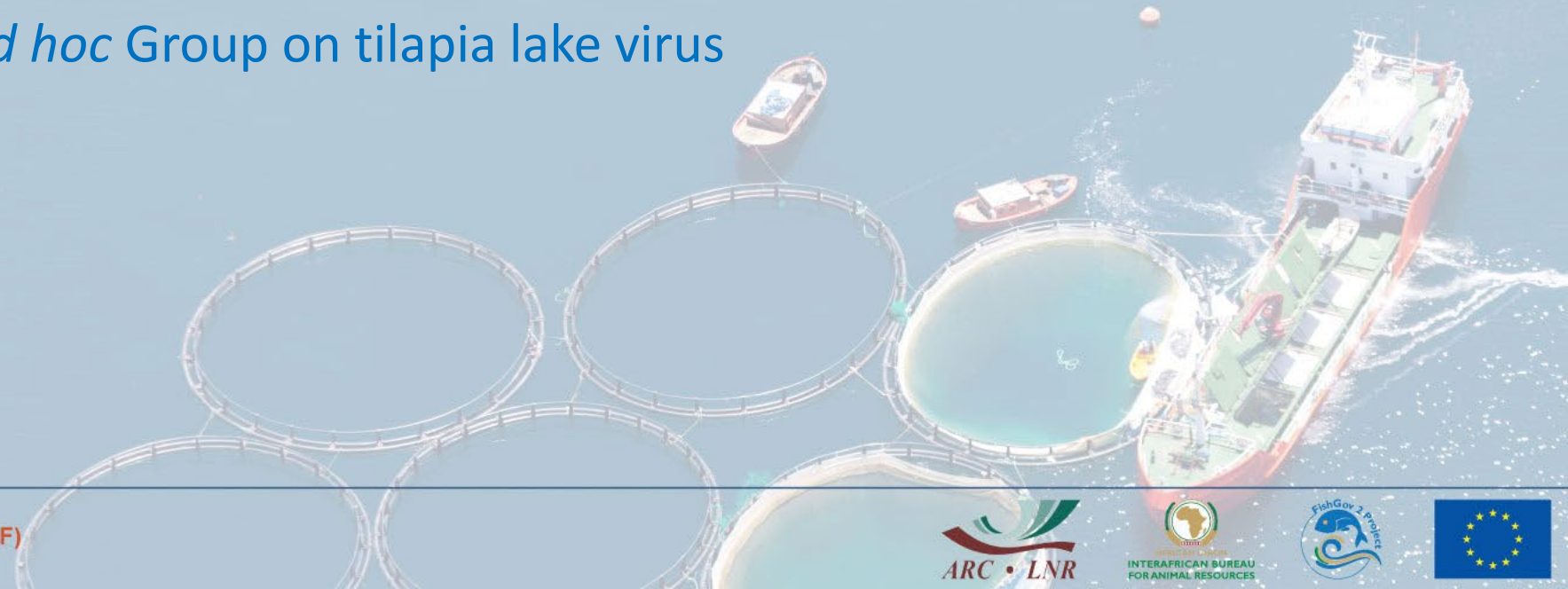
INTERAFRICAN BUREAU FOR ANIMAL RESOURCES

Presentation Overview

- Steering Committee for Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific
- WOAH electronic *ad hoc* Group on tilapia lake virus



- Steering Committee for Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific

- OIE Regional expert consultation on aquatic animal disease diagnosis and control, 15-16 November 2018, Bangkok, Thailand
  - Recommendation to build an OIE Regional Collaboration Framework on aquatic animal diseases in Asia and the Pacific

- OIE Global Conference on Aquatic Animal Health “Collaboration, Sustainability: our Future”, Santiago, Chile, 2 - 4 April 2019
  - Asia-Pacific Regional Side Meeting on aquatic animal health “OIE Regional Collaboration Framework”
  - Draft plan was endorsed at the OIE Regional Commission meeting in September
  - Secretariat: OIE Regional Representation of Asia and the Pacific, Dr Hirofumi Kugita

- Regional Collaboration Framework on Aquatic Animal Diseases in Asia and the Pacific

- Establishment of the Steering Committee;
  - A representative of OIE Collaborating Centres:
    - Diagnostic Test Validation Science in the Asia-Pacific Region
    - New and Emerging Diseases
  - Two representatives of OIE Reference Laboratories in the region
  - Representatives of OIE National Focal Points for aquatic animals
  - Representative from the OIE Aquatic Animal Health Standards Commission
  - Regional partners:
    - FAO; NACA; SEAFDEC
  - Secretariat:
    - OIE Regional representation of Asia and the Pacific

- 1st meeting of *ad hoc* Steering Committee of the OIE Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific 20-21 November 2019, Bangkok, Thailand.
  - Session 1: Mapping of existing initiative and expertise in the region
  - Session 2: Finalizing the Terms of References
  - Session 3: Developing roadmap for the Framework
  - Session 4: Identification of communication mechanism
- 2nd meeting; online, 3-4 December 2020, 3-5pm Tokyo time
  - Updates from Ref Labs, NACA, OIE, general discussion
- 3rd meeting: online, 6-7 December 2021, 3-5pm Tokyo time
  - Updates from Ref Labs, NACA, OIE, general discussion
- 4th meeting: Busan, Republic of Korea, June 29, 2023
  - After the 3 day Regional Workshop for WOAH Focal Points for Aquatic Animals

- Ongoing activities
  - Causative agents of AHPND
  - Appropriateness of using WGS
  - EHP epidemiology and surveillance
  - Comparison of WSSV pen-side tests

- Prioritisation exercise
  1. Support for early disease response
  2. Develop guidelines for collaborative emerging disease response
  3. Formalise coordinated approach to emergency disease response
  4. Provide practical AMR guidance

  → Improving Aquatic Animal Disease Reporting in Asia and the Pacific
  → Concept paper on regional coordination for emergency response

- Really important collaborative network that is still developing

Presentation Overview

- Steering Committee for Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific

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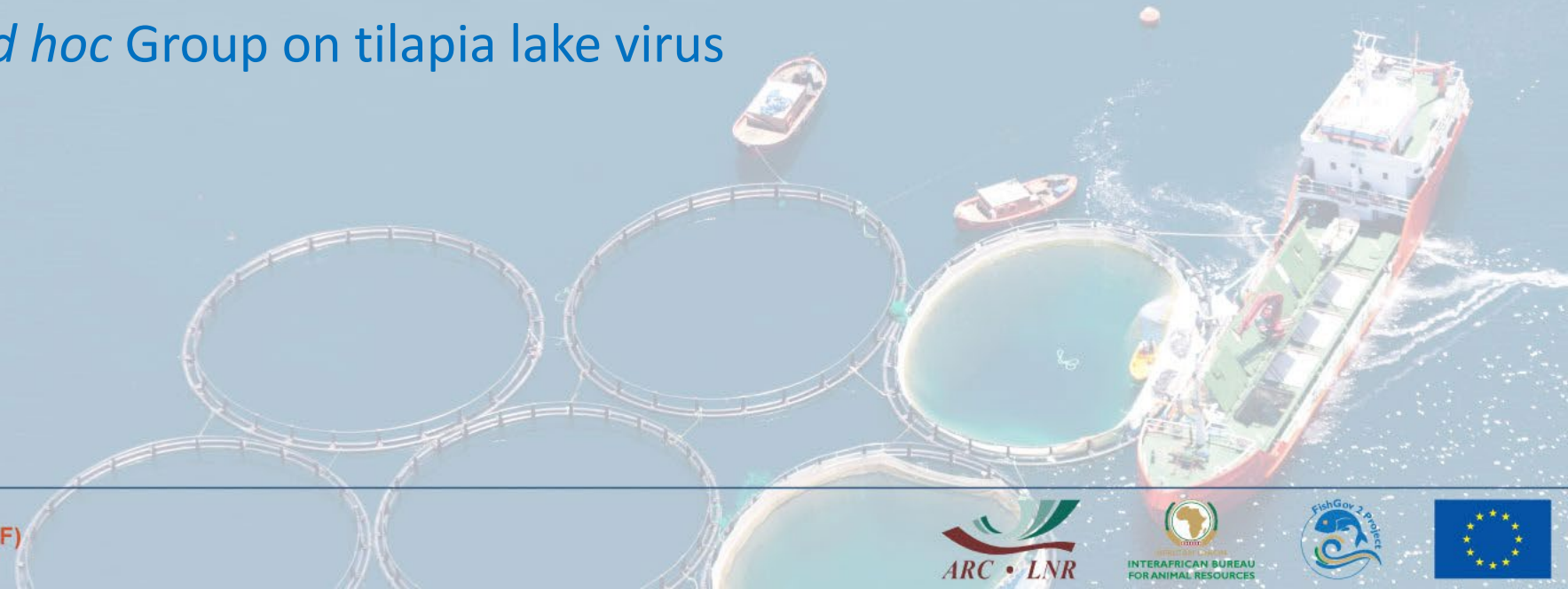

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Identification of a Novel RNA Virus Lethal to Tilapia

Marina Eyngor,[a] Rachel Zamostiano,[b] Japhette Esther Kembou Tsofack,[b] Asaf Berkowitz,[a] Hillel Bercovier,[c] Simon Tinman,[d] Menachem Lev,[e] Avshalom Hurvitz,[f] Marco Galeotti,[g] Eran Bacharach,[b] Avi Eldar[a]

Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel[a]; Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel[b]; The Hebrew University-Hadassah Medical School, Jerusalem, Israel[c]; Department of Animal Facility, Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel[d]; Ein Gev Fisheries, Kibbutz Ein Gev, Israel[e]; Dan Fish Farms, Kibbutz Dan, Upper Galilee, Israel[f]; Department of Food Science, Section of Veterinary Pathology, University of Udine, Udine, Italy[g]

Detection of tilapia lake virus (TiLV) infection by PCR in farmed and wild Nile tilapia (*Oreochromis niloticus*) from Lake Victoria

K K Mugimba[1,2*] | A A Chengula[1,3*] | S Wamala[1,2] | E D Mwega[1,3] | C J Kasanga[3] | D K Byarugaba[2] | R H Mdegela[3] | S Tal[4] | B Bornstein[4] | A Dishon[4] | S Mutoloki[1] | L David[5] | Ø Evensen[1] | H M Munang'andu[1]

Two-year surveillance of tilapia lake virus (TiLV) reveals its wide circulation in tilapia farms and hatcheries from multiple districts of Bangladesh

Partho Pratim Debnath[1,2,3] | Jerome Delamare-Debouteville[4] | Mona Dverdal Jansen[5] | Komsunee Phiwsaiya[6,7] | Afsana Dalia[2] | Md. Abir Hasan[2] | Saengchan Senapin[6,7] | Chadag Vishnumurthy Mohan[4] | Ha Thanh Dong[4] | Channarong Rodkhum[1,3]

Characterization of a Novel Orthomyxo-like Virus Causing Mass Die-Offs of Tilapia

Eran Bacharach,[a] Nischay Mishra,[b] Thomas Briese,[b] Michael C. Zody,[c] Japhette Esther Kembou Tsofack,[a] Rachel Zamostiano,[a] Asaf Berkowitz,[d] James Ng,[b] Adam Nitido,[b] André Corvelo,[c] Nora C. Toussaint,[c] Sandra Cathrine Abel Nielsen,[b*] Mady Hornig,[b] Jorge Del Pozo,[e] Toby Bloom,[c] Hugh Ferguson,[f] Avi Eldar,[d] W. Ian Lipkin[b]

Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel[a]; Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, USA[b]; New York Genome Center, New York, New York, USA[c]; Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel[d]; Easter Bush Pathology, The Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Midlothian, Scotland[e]; Marine Medicine Program, Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, West Indies[f]

A case of natural co-infection of Tilapia Lake Virus and *Aeromonas veronii* in a Malaysian red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) farm experiencing high mortality

M.N.A. Amal[a,f,*], C.B. Koh[b], M. Nurliyana[a], M. Suhaiba[a], Z. Nor-Amalina[c], S. Santha[c], K.P. Diyana-Nadhirah[c], M.T. Yusof[d], M.Y. Ina-Salwany[c,f], M. Zamri-Saad[e,f]

[a] *Department of Biology, Faculty of Science, Universiti Putra Malaysia, Selangor, Malaysia*
[b] *Cargill Feed Sdn. Bhd., West Port, Klang, Selangor, Malaysia*
[c] *Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia*
[d] *Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Selangor, Malaysia*
[e] *Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, Malaysia*
[f] *Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia*

Susceptibility of ornamental African cichlids *Aulonocara* spp. to experimental infection with Tilapia lake virus

Jidapa Yamkasem[a], Chutchai Piewbang[b,c], Somporn Techangamsuwan[b,c], Felipe Pierezan[d], Esteban Soto[e], Win Surachetpong[a,*]

Detection of Tilapia Lake Virus in Egyptian fish farms experiencing high mortalities in 2015

P Nicholson[1*] | M A Fathi[2,3*] | A Fischer[4] | C Mohan[5] | E Schieck[4] | N Mishra[6] | A Heinimann[7] | J Frey[1] | B Wieland[8] | J Jores[1,4]

Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tilapia *Oreochromis niloticus* (Linnaeus 1758) in India

B.K. Behera[a,*,1], P.K. Pradhan[b,1], T.R. Swaminathan[c,1], N. Sood[b,*], Prasenjit Paria[a], Abhishek Das[a], D.K. Verma[b], R. Kumar[c], M.K. Yadav[b], A.K. Dev[b], P.K. Parida[a], B.K. Das[a], K.K. Lal[b], J.K. Jena[d]

[a] *ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata 700 120, West Bengal, India*
[b] *ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkusha 226002, Lucknow, India*
[c] *PMFGR Centre, ICAR-NBFGR, CMFRI Campus, Kochi 682 018, Kerala, India*
[d] *Division of Fisheries, Indian Council of Agricultural Research, Krishi Anusandhan Bhawan - II, New Delhi 110 012, India*

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NETWORK OF AQUACULTURE CENTRES IN ASIA-PACIFIC

Sixteenth Meeting of the Asia Regional Advisory Group on Aquatic Animal Health

REPORT OF THE MEETING

Anvaya Beach Resort, Bali, Indonesia
26-27 August 2017
Prepared by the NACA Secretariat

Tilapia Lake Virus (TiLV), which was reported in Middle East and South America, became a current concern in the region when its presence was confirmed in cultured tilapias in Thailand. NACA, in collaboration with key researchers in Thailand who worked on this disease for the past few years, has published several news articles online including: A waring and an improved PCR method for TiLV disease in Thai tilapia farms; OIE technical disease card: Tilapia lake virus – a novel Orthomyxo-like virus; and, Urgent update on possible worldwide spread of TiLV. In May 2107, NACA also released a Disease Advisory which was disseminated widely to all NACA member countries and partner institutions, and published online at NACA website. NACA also collaborated with WorldFish in the preparation of TiLV Fact Sheet and Literature Review. To address this current disease problem in the region, NACA also approached several donor agencies for holding of an emergency regional consultation, and the Ministry of Agriculture of the People's Republic of China responded positively and agreed to fund the consultation, which was scheduled to be held in Guangzhou, China in September 2017.

4.4. LISTING OF TILAPIA LAKE VIRUS (TILV) IN QAAD-AP

Dr. Eduardo Leaño presented the assessment of TiLV for listing in QAAD in 2018. The assessment was based on the listing criteria of OIE which include Consequences, Spread and Diagnosis. TiLV satisfied one of the criteria for Consequences by causing significant losses among cultured tilapias in Israel, Egypt and Thailand which were affected by the disease, thus it can be considered for listing. For Spread, TiLV satisfied the criteria for Infectious etiology, as the main causative agent has already been identified as Orthomyxo-like virus. It also satisfied two other criteria: international spread is likely; and, several countries may be declared free of the disease. As such, TiLV fully met the criteria for Spread. For Diagnosis, TiLV satisfied the criterion for repeatable and robust means of detection through RT-PCR, Nested and semi-nested RT-PCR, cell culture, and histopathology.

RECOMMENDATIONS

- Since the report of IMNV in India and possibly Malaysia, AG recommended that vigilance for possible translocation of IMNV to other countries in Asia where it is currently not reported be increased. It is also recommended that wild, *P. monodon* captured near Indonesia and used as broodstock in Asian countries be monitored as possible grossly normal carriers of IMNV that might be able to transmit it to their cultivated *P. vannamei*.
- AG recommended that research on mutants and variants or AHPND bacteria be continued so that the basis of virulence, nature of genetic exchange and epidemiology can be better understood.
- AG recommended that research on identification of natural reservoir carriers infected with EHP be given high priority, especially with respect to those commonly used a live broodstock shrimp feeds and larval/PL feeds.
- AG recommended that research on TiLV be continued to understand its variation in virulence (genetic and environmental factors including interaction with other microbes), its global distribution, its global economic impact and the feasibility of developing vaccines.
- AG recommended that TiLV be included in the QAAD list of diseases for 2018.

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Protecting animals, preserving our future

TILAPIA LAKE VIRUS (TiLV)–A NOVEL ORTHOMYXO-LIKE VIRUS

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Virus.

1.2. Disease name and synonyms

Tilapia lake virus (TiLV) disease.

1.3. Pathogen common names and synonyms

Tilapia lake virus (TiLV).

1.4. Taxonomic affiliation

The taxonomic affiliation has not been definitively concluded; however, TiLV has been described as a novel virus in the Family *Orthomyxoviridae* (Eyngor *et al.*, 2014).

1.5. Authority (first scientific description, reference)

The virus was first described by Eyngor *et al.* (2014).

1.6. Pathogen environment (fresh, brackish, marine waters)

Fresh and brackish water.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)

Co-habitation studies have demonstrated that direct horizontal transmission is an important route of transmission. There is no evidence of vertical transmission. The biophysical characteristics of the virus are not well characterised so it is difficult to determine the significance of indirect transmission by fomites.

2.2. Reservoir

Infected populations of fish, both farmed and wild, are the only established reservoirs of infection. The original source of TiLV is not known.

2.3 Risk factors (temperature, salinity, etc.)

Disease has been associated with transfer between ponds and thus may be associated with stress (Ferguson *et al.*, 2014, Dong *et al*, 2017). No other risk factors (temperature, salinity, etc.) have been identified as potential risk factors.

3. HOST RANGE

3.1. Susceptible species

Mortalities attributed to TiLV have been observed in wild tilapia *Sarotherodon (Tilapia) galilaeus*, farmed tilapia *Oreochromis niloticus* and commercial hybrid tilapia (*O. niloticus* X *O. aureus*) (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Eyngor *et al.*, 2014). To date only tilapines have been shown to be susceptible. It is possible that other species will be found to be susceptible.

3.2. Affected life stage

In the outbreak reported by Ferguson *et al.* (2014) and Dong *et al.* (2017) fingerlings were mainly affected. Dong *et al.* (2017) reported approximately 90% mortality in red tilapia fingerlings within one months of stocking into cages. Mortality just over 9% in medium to large sized Nile tilapia was noted by Fathi *et al.* (2017). Other reports have not commented on different levels of mortality by life stage (Eyngor *et al.*, 2014),

3.3. Additional comments

There is some evidence that certain genetic strains of tilapia are resistant. Ferguson *et al.* (2014) noted that one strain of tilapia (genetically male tilapia) incurred a significantly lower level of mortality (10-20%) compared with other strains.

4. GEOGRAPHICAL DISTRIBUTION

TiLV has been reported in Colombia, Ecuador and Israel (Bacharach *et al.*, 2016; Ferguson *et al.*, 2014; Tsofack *et al.*, 2016), and most recently, Egypt (Fathi *et al.*, 2017) Thailand (Dong *et al.*, 2017) India (Behera *et al.*, 2018), Malaysia (Amal *et al.*, 2018) and the Philippines (OIE, 2017). However, a lack of thorough investigation of all mortality incidents means that the geographic distribution of TiLV may be wider than currently. For example, reports of mortality in tilapia in Ghana and Zambia in 2016 have not been attributed to TiLV but the available information does not indicate that the presence of the virus has been investigated. A partial genome from Thailand showed relatively high variation to strains from Israel (around 97% nucleotide identity) (Dong *et al.*, 2017).

- TiLV - Characterisation Bacharach et al 2016 mBio.pdf
- TiLV - characterisation Eyngor et al 2014-4137-46.pdf
- TiLV - co-infection in Malaysia Amal et al 2018 Aquaculture 485.12.pdf
- TiLV - effect of pooling on DSe for TiLV Yamkasem et al 2020 Trans Emerg Dis tbed.13957.pdf
- TiLV - experimental infection Thailand Tattiyapong et al 2017 Vet Micro 207 170.pdf
- TiLV - First detection in Malaysia Abdullah_et_al-2018 J_Fish_Dis.12843.pdf
- TiLV - Genetic diversity Seg 1 2011 to 2019 Taengphu et al 2020 Aquaculture 735423.pdf
- TiLV - Genome characterisation USA Ahasan et al 2020 Microbiology 9.4.e01368-19.pdf
- TiLV - heat and formalin killed vaccines Mai et al 2021 J Fish Dis jfd.13523.pdf
- TiLV - high mortalties Egypt Nicholson_et_al-2017-J_Fish_Dis 12650.pdf
- TiLV - IG and IP challenge models Pierezan et al 2019 J_Fish_Dis 13052.pdf
- TiLV - inapparent infection Senapin et al 2018 Aquaculture 487.51.pdf
- TiLV - India Behera et al 2018 Aquaculture 484.168.pdf
- TiLV - infection dynamics probiotics Yang et al 2022 J Fish Dis 45.1117.pdf
- TiLV - ISH of the brain Dinh-Hung et al 2021 J Fish Dis 13367.pdf
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- TiLV - Malaysian tilapia Amla et al 2018 Aquaculture 485.12.pdf
- TiLV - mortalities in India Behera et al 2018 Aquaculture 484.168.pdf
- TiLV - Non-lethal sampling and detection by RT-qPCR and cell culture Liamnimitr et al 2017 Aquacu...
- TiLV - non-lethal sampling comparison Chiamkunakorn et al 2019 J Fish Dis 42.1629.pdf
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- TiLV - RT-LAMP Yin_et_al-2019-J_Fish_Dis 12983.pdf
- TiLV - RT-nPCR Kembou Tsofack et al 2017 J Clin Micro 55.3.759.zjm759.pdf
- TiLV - RT-qPCR Waiyamitra et al 2018 Aquaculture 497.184.pdf
- TiLV - surveillance Banlgadesh Pratim Debnath et al 2020 J Fish Dis.13235.pdf
- TiLV - surveillance in Bangladesh debnath et al 2020 J Fish Dis 13235.pdf
- TiLV - susceptibility of African cichlids Yamkasem et al 2021 Aquaculture 736920.pdf
- TiLV - SYBR Green RT-qPCR Tattiyapong_et_al-2017-J_Fish_Dis12708.pdf
- TiLV - Thailand from 2012 to 2017 Dong et al 2017 Aquaculture 479 579.pdf
- TiLV - Thailand WGS Surachetpong et al 2017 EID 23.6.1031.pdf
- TiLV - Thailland and new RT-nPCR Dong et al 2017 Aquaculure 476.111.pdf
- TiLV - tilapia brain cell line detection Wang_et_al-2018-J_Fish_Dis.12889.pdf
- TiLV - vaccine protection Mai et al 2022 vaccines-10-00167-v3.pdf
- TiLV - VI and PCR Kebmou Tsofack et al 2017 J Clin Microbiol 759-67.pdf
- TiLV - viability in frozen tilapia fillets Thammatorn_et_al-2019-J_Fish_Dis 12924.pdf
- TiLV - Vietnam pathogenicity and genetics Tran et al 2022 J Fish Dis 45.1389.pdf
- TiLV - warm water fish susceptibility Jaemwimol et al 2018 Aquaculture 497.462.pdf
- TiLV - weight dependent susceptibility Roy et al 2021 peerj-11738.pdf
- TiLV - WGS Ecuador Subramaniam et al 2019 Micro 8.18.1.pdf

Tilapia Lake Virus (TiLV)

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WAHIS

			Jul-Dec-2011	Jan-Jun-2012	Jul-Dec-2012	Jan-Jun-2013	Jul-Dec-2013	Jan-Jun-2014	Jul-Dec-2014	Jan-Jun-2015	Jul-Dec-2015	Jan-Jun-2016	Jul-Dec-2016	Jan-Jun-2017	Jul-Dec-2017	Jan-Jun-2018	Jul-Dec-2018	Jan-Jun-2019	Jul-Dec-2019	Jan-Jun-2020
Tilapia lake virus (TiLV) disease	Chinese Taipei	Domes...	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-
	Colombia	Domes...	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	India	Domes...	-	-	-	-	-	-	-	-	-	-								
	Israel	Domes...	-																	
		Wild																		
	Malaysia	Domes...	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-
		Wild	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-
	Mexico	Domes...	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-
	Peru	Domes...	-	-	-	-	-	-	-	-	-	-	-	-						
		Wild	-	-	-	-	-	-	-	-	-	-	-	-						
	Philippines	Domes...	-	-	-	-	-	-	-	-	-	-	-					-	-	-
	Thailand	Domes...	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-
	United States of America	Domes...	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-

- No data available
9 No information
8 Never reported
7 Absent
6 Suspected limited zones
5 Suspected
4 Inf./Infest. limited zones
3 Inf./Infest.
2 Present limited zones
1 Present

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Tilapia Lake Virus (TiLV)

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WOAH *Aquatic Code*: Chapter 1.2

CRITERIA FOR LISTING AQUATIC ANIMAL DISEASES

Article 1.2.2.

The criteria for the inclusion of a *disease* in the OIE list are as follows:

1) International spread of the *pathogenic agent* (via *aquatic animals*, *aquatic animal products*, *vectors* or fomites) is likely.

AND

2) At least one country may demonstrate country or *zone* freedom from the *disease* in susceptible *aquatic animals*, based on provisions of Chapter 1.4.

AND

3) A precise *case definition* is available and a reliable means of detection and *diagnosis* exists.

AND

4)

a) Natural transmission to humans has been proven, and human infection is associated with severe consequences.

OR

b) The *disease* has been shown to affect the health of cultured *aquatic animals* at the level of a country or a *zone* resulting in significant consequences e.g. production losses, morbidity or mortality at a *zone* or country level.

OR

c) The *disease* has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

WOAH e*ad Hoc* Group for TILV

At the last Aquatic Animals Commission meeting the Commission reviewed the assessment of tilapia lake virus (TiLV) against the new criteria in Chapter 1.2. Criteria for listing aquatic animal diseases, noting that revised criteria had been adopted at the 2017 OIE General Session. New scientific information published since their last meeting in February 2017 was considered to find out if there was enough evidence to meet the third criterion for listing a disease by the OIE: "a precise case definition is available and a reliable means of detection and diagnosis exists".

The Commission agreed that with this additional information the criterion is still not met because of insufficient information concerning analytical and diagnostic specificity and sensitivity of the assay.

Terms of Reference

1. Critically review the available literature regarding detection methods for TiLV and any unpublished methods that may also be available.
2. Provide recommendations on additional method development requirements.
3. Provide recommendations on method validation requirements.
4. Determine sources of well-characterised viable and non-viable positive control material for use in method evaluation and implementation in laboratories.
5. Develop a work plan for inter-laboratory comparability studies.
6. *Ad hoc* Group members should be familiar with the criteria for listing aquatic animal diseases, and use the glossary definitions in Chapter 1.2. of the *Aquatic Code*, in their work. They should also be familiar with the principles and methods of validation of diagnostic essays for infectious diseases in Chapter 1.1.2. of the *Aquatic Manual*.

WOAH e*ad Hoc* Group for TILV

MEMBERS OF THE *AD HOC* GROUP

Dr Axel Colling (Chair)
OIE Collaborative Centre for Diagnostic Test Validation Science
Po bag 24 Geelong VIC 3220
AUSTRALIA
Tel.: +61 3 5227 5255
Tel.: +61 457 515 014
Axel.Colling@csiro.au

Dr Hong Liu
Director
The National Key laboratory of Aquatic Animal Diseases
Animal and Plant Inspection and Quarantine Technical Centre
Shenzhen Exit & Inspection and Quarantine Bureau
General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) of P. R. China
Room 702 of Inspection and Quarantine Building
1011 of Fuqiang Road, Futian Qu, Shenzhen City, Guangdong Province, 518045
P. R. CHINA
Tel.: 86-755-25588410
Liuhong@szciq.gov.cn
709274714@qq.com

Dr Mona Dverdal Jansen
Veterinarian, Researcher, PhD
Norwegian Veterinary Institute
PO Box 750 Sentrum
NO-0106 Oslo
NORWAY
Tel.: + 47 23 21 64 79
Tel.: + 47 934 99 808
mona-dverdal.jansen@vetinst.no
www.vetinst.no

Dr Sergio Hernan Marshall Gonzalez
Pontificia Universidad Católica de Valparaiso
Av. Brazil 2950
Valparaiso
CHILE
Tel.: +55 32-2273444
sergio.marshall@pucv.cl

Dr Henrique César Pereira Figueiredo
Head
National Reference Laboratory for Aquatic Animal Diseases/MAPA
Federal University of Minas Gerais
BRAZIL
Tel.: +55 31 3409-2077
figueiredoh@yahoo.com

Dr Nadav Davidovich
Veterinary Services and Animal Health
Ministry of Agriculture & Rural Development
P.O. Box 12, Bet Dagan 5025001,
ISRAEL
Tel.: +972-50-6241511
Tel.: Office: +972-3-9681728
Nadavd@moag.gov.il

Dr Nick Moody
Senior Research Scientist
Team Leader – Aquatic Diagnostic Capability
CSIRO AAHL Fish Diseases Laboratory
5 Portarlington Rd, East Geelong VIC 3219
Private Bag 24, Geelong VIC, 3220
AUSTRALIA
Tel.: +61 3 5227 5749
nick.moody@csiro.au

Dr Dong Thanh
Researcher, Department of Microbiology, Faculty of Science, King Mongkut's University of technology Thonburi (KMUTT)
Bangkok 10140
THAILAND
hadongntu@gmail.com

REPRESENTATIVE OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Dr Edmund Peeler
(Vice-President)
Group Manager Aquatic Pest & Pathogens
CEFAS
Barrack Road, Weymouth
Dorset, DT4 8UB UK
UNITED KINGDOM
ed.peeler@cefas.co.uk

OIE HEADQUARTERS

Dr Stian Johnsen
Chargé de mission
Standards Department
s.johnsen@oie.int

OIE
Organisation Mondiale de la Santé Animale | World Organisation for Animal Health | Organización Mundial de Sanidad Animal

Director General

Paris, 8th December 2017

Our Ref.: SJ/CC 60.8533

Dr Nick Moody
Senior Research Scientist
Team Leader – Aquatic Diagnostic Capability
CSIRO AAHL Fish Diseases Laboratory
5 Portarlington Rd, East Geelong VIC 3219
Private Bag 24, Geelong VIC, 3220
AUSTRALIA

Electronic OIE *ad hoc* Group on Tilapia Lake virus (TiLV)

Dear Dr Moody

As one of the outcomes of discussions of the meetings of the Aquatic Animal Health Standard Commission, held in September 2017, the OIE decided to convene an *ad hoc* Group (electronic consultation) on Tilapia Lake virus (TiLV).

This group will evaluate published and unpublished methods for detection of TiLV, describe the level of validation of each method and determine additional validation requirements, recommend any additional assays that may need to be developed and facilitate the sourcing and distribution of well-characterised positive control material for method evaluation, implementation and inter-laboratory comparability studies.

The OIE will be very pleased if you would agree to participate in this group. We kindly ask you to provide a short CV (1-2 pages) identifying your relevant experience, area of expertise and a brief outline of related field of work.

As an expert member of the electronic *ad hoc* Group, you are requested to submit a Declaration of Interests form to the OIE. You are also requested to sign an undertaking on confidentiality of information.

Best regards,

Monique Eloit

Cc.: Dr Mark Schipp (OIE Delegate for Australia)
Enclosures: Declaration of Interest & Confidentiality of Information Forms

World Organisation for Animal Health

WOAH e*ad Hoc* Group for TILV

Reference	Purpose of	Source animals	PCR details	ASe	ASp	DSe	DSp	Comments	Comments from Dong	Comments from Hong
Eyngor et al (2014) Identification of a novel RNA virus lethal to tilapia. J Clin Micro 52(12): 4137	Investigation of disease of unknown aetiology (not specifically for TiLV	25 isolates obtained from suspected outbreaks from 5/2011 to 6/2013	RT-PCR with ME1 (GTTGGGCACAAGGCATCCTA) + clone 7450/150R/ME2 (TATCACGTGCGTACTCGTTCAGT) primers.	N/A	Uninfected cells NEG, NNV NEG	N/A	N/A	RT-PCRs developed for characterization of a pathogen of unknown aetiology. Primer 7450 of unknown identity (shotgun cloning)		It is the first RT PCR set up for testing on TiLV, which is lack of sensitivity evaluation and needed to be optimised. For more research on phylogenetic analyse, the segments which is the gene coding major structure protein and can be used for phylogenetic analysed is preferred for designing RT PCR
Tsofack et al (2017) Detection of Tilapia Lake Virus in clinical samples by culturing and nested reverse transcription-PCR. J Clin Micro 55(3): 759	Develop and RT-PCR and virs isolation for TiLV	Clinical samples collected between 2011 and 2013	Initially used Eyngor et al (2014) primers. Nested RT-PCR using Nested ext-1 + ext-2 then ME1 + 7450/150R/ME2. Developed a RT-PCR then SYBR on amplicons. Different reaction conditions used depending on assay format	RT-PCR: 70,000 nPCR: 7 PCR/SYBR: 700	Uninfected cells NEG, NNV NEG	RT-PCR: 23% nPCR: 92% [n = 13]	N/A	Confusingly written and assays formats not consistent with a general diagnostic laboratory (Ase of conventional assays by SYBR). DSe on CPE-positive samples seems very low.	qPCR sensitivity=70	
Dong et al (2017) Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. Aquaculture 476:111	Targeted surveillance for TiLV in farms experiencing disease outbreaks	Diseased fingerlings from 3 farms in 3 provinces from 2016 and 2017.	Modified procedure of Tsofack et al (2017) then developed an alternative semi-nested RT-PCR: RT-PCR: Nested ext-1 + ME1 nPCR: 7450/150R/ME2 + ME1 Developed primers for Segment 1, 5 and 9 for sequence analysis	RT-nPCR: 7.5	N/A	RT-PCR: 100% [n = 27]	RT-nPCR: 100% [n = 2]	Identified specificity issues with the Eyngor primers. Multiple bands observed in RT-nPCR reactions. Would be interesting to see the sensitivity of the RT-PCR alone (is the nPCR actually required for clinically-affected fish?) Developed in-situ but no known-negative samples tested	The semi-nested RT-PCR protocol has been tested against genomic RNA extracted from the laboratory strains of 15 bacterial species including: Aeromonas veronii, A. caviae, A. hydrophila, A. jandaei, Francisella noatunensis subsp. orientalis, Streptococcus agalactiae, S. iniae, Vibrio cholerae, Hahella chejuensis, Plesiomonas shigelloides, Edwardsiella ictaluri, E. tarda, Vogesella sp., Micrococcus sp. Chryseobacterium sp. and RNA extracted from ISKNV-infected tissue, Betanodavirus-infected tissue. The result showed no cross amplification (unpublished data). Limit detection of RT-PCR is 15,000 copies in our labratory, therefore, using nested PCR for diagnosis is highly recommended to obtain an accurate result	If possible, primers adjusted for future consideration of using the sequences for phylogenetic analysis.
Dong et al (2017) Evidence of TiLV infection in tilapia hatcheries from 2012 to 2017 reveals probable global spread	Investigation of previous unexplained mortalities in archival tilapia tissue	Larve, eggs, fry and fingerlings from 2012 to 2016	Used Used Dong modification of Tsofack et al (2017)	N/A	N/A	N/A	N/A	Difficult to determine DSe as no other case investigation information (histology, virus isolation) is provided		
Tattiyapong et al (2017) Experimental infection of Tilapia Lake Virus (TiLV) in Nile Tilapia (Oreochromis niloticus) and red tilapia (Oreocgromis spp.). Vet Micro 2017: 170	Confirm TiLV was the causative agent of the disease seen	Clinical isolates collected from 3 separate locations in 2016	Primers of Eyngor et al (2014): Nested ext-1 + Nested ext-2 cDNA initially prepared using random primers.	N/A	N/A	RT-PCR: 100% [n = 15]	N/A	Fulfilled Koch's postulates		
Tattiyapong et al (2017) Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish. J Fish Dis 12708	Develop a sensitive and reliable assay for TiLV detection in clinically moribund and asymptomatic fish.	30 field samples with 10 fish per sampling	SYBR assay with forward and reverse primers: TiLV-112F (CTGAGCTAAAGAGGCAATATGGATT) TiLV-112R (CGTGCGTACTCGTTCAGTATAAGTTCT) Tm of 79.50-80.0 Conventional PCR according to Tattiyapong et al (2017)	RT-PCR: 2	Iridovirus and 2 bacteria NEG	RT-PCR: 100% [n = 30 clinical] [n = 10 exp. inf.]	RT-PCR: 100% [n = 10]	Validation limited to positive clinical samples and experimentally-infected fish. Good primitary data for ASe, ASp, DSe and DSp.	2 copies/uL, the authors used 4 ul of cDNA for a reaction. Thus, limit detection should be 2x4=8 copies/reaction? (please double check).	
Nicholson et al (2017) Detection of Tilapia Lake Virus on Egyptian fish farms experiencing high mortalities in 2015. J Fish Dis 12650	Detect potential pathogens associated with high summer mortalities in Egyptian cultured tilapia	13-40 randomly sampled fish from 8 farms	Initially used Eyngor et al (2014) primers. Developed additional primers S4_FAGCAGCAGGAGAAAGAG + S4_R-ACCGTCCTGTTTCTGAATGG) and S9_F-TTGGTGATGTCACGATGGATA + S9_R-AGTTCTATCGGCAGCCATGT)	N/A	N/A	N/A	N/A	Disease investigation/surveillance project		
Amal et al (2018) A case of natural co-infection of Tilapia Lake Virus and Aeromonas veronii in a Malaysian red hybrid tilapia (Oreochromis niloticus x O. mossambicus farm experiencing high mortality. Aquaculture 485:12	Disease investigation	20 diseased red hybird tilapia collected form one farm	Used the Dong et al (2017) primers for segment 3 TiLV-Seg3-F (5'-ACGTCCTTAAAGTCATACTT-3') + TiLV-Seg3-R (5'-ACAAGTCCGATTACTTTTTC-3')	N/A	N/A	N/A	N/A	Disease investigation paper.		
Behera et al (2018) Emergence of Tilapia Lake Virus associated with mortalities of farmed Nile Tiplais Oreochromis niloticus (Lanaeus 1758) in India. Aquaculture 484:168	Disease investigation	Clinically-affected tilapia collected from 3 farms. Polycultures with only tilapia affected	Used the Eyngor et al (2014) primers	N/A	N/A	N/A	N/A	Positive results for TiLV in the first step and in the semi-nested PCR. CFF cells cultures showing CPR were positive as well. Bioassay using culture supernatant resulted in mortalities with samples PCR positive.		
Liamnimitr et al (2018) Non-lethal sampling for Tilpia Lake Virus by RT-qPCR and cell culture. Aquaculture 486:75	Asses if mucous could be a non-lethal sample for detection of TiLV	3 fish per outbreak during Aug 2016 to Jan 2017 from various regions	Used the Tattiyapong et al (2017) SYBR assay and combination of TiLV-112F + Nested ext-1 for conventional RT-PCR	N/A	N/A	N/A	N/A	Positive results from 21/35 field samples tested		
Hong LIU pers comm	Molecular test evaluation	Samples of unknown status	Used Dong modification of Tsofack et al (2017) for the semi-nested RT-nPCR: RT-PCR: Nested ext-1 + ME1 nPCR: ME1 + 7450/150R/ME2 RT-qPCR (probe-based): TiLV-F1: 5'-CGAACTGTTGCCTTTGGAAATT-3' TiLV-R1: 5'-TGAAGAATAAGTGGATTGCCTTTG-	N/A	ISAV, VNNV, GCHV, VHSV, IHNV, SVCV and HRV all NEG	See Comments	See Comments	Comparative performance of primary, nested and real-time assays determined: 1. Real-time 10x more sensitive than semi-nested 2. Real-time and conventional assays have same specificity	Note that Hong LIU used semi-nested RT-PCR (Dong et al. 2017) with some mofifications.	Samples used included the virus isolate supernatatn ,the fish with clinical signs and mortality, the fish in the same pond without clinical signs (but virus can be isolated) and healthy fish without clinical signs.
Senapin et al (2018) Inapparentinfectionscausesoftilapia lakevirus(TiLV) in farmed tilapia	investigation of TiLV in clinically healthy tilapia	Clinically healthy adult and fingerling tilapia	Semi-nested RT-PCR (Dong et al. 2017) combined with sequencing of representatives of PCR product	N/A	N/A	N/A	N/A	Semi-nested RT-PCR can be used for TiLV diagnosis in subclinical infection. Positive results from 2/2 clinically healthy adults (in multiple organs), 9/19 clinically healthy fingerlings.		

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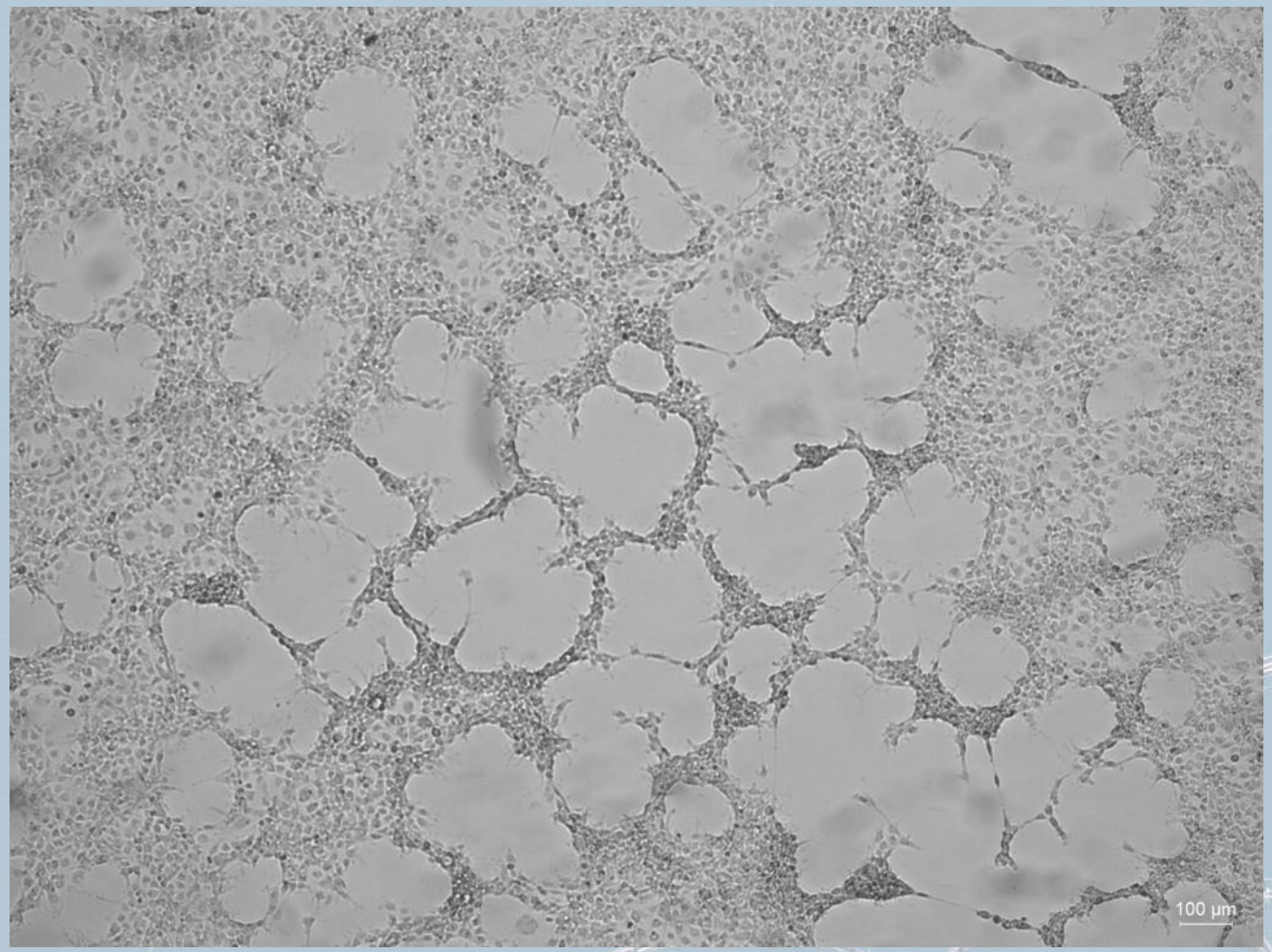
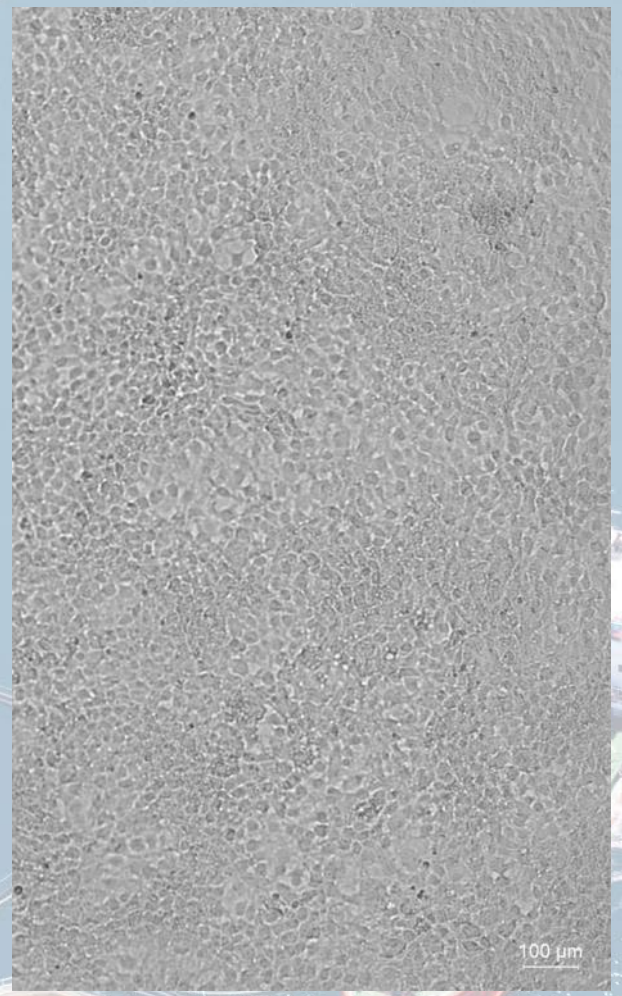
WOAH e*ad Hoc* Group for TILV: Thailand isolate (18-03492)





WOAH e*ad Hoc* Group for TILV: Interlaboratory Comparability (ILC) plan

Inter-laboratory comparability panel

The inter-laboratory comparability panel consisted of 20 positive and 10 negative samples that included:

1. 10-fold dilution series (6 samples) to enable estimates of efficiency of real-time molecular assays;
2. Strong positive (at least 2 samples);
3. Medium positive (at least 2 samples);
4. Weak positive (at least 2 samples);
5. 10-fold dilution of medium and low positive;
6. Positive samples with various viral concentrations to make up the 20 positive samples;
7. Negative samples consisting of supernatant of uninfected cell culture (10 samples).

Assays

Real-time PCR – TaqMan probe based:

1. TiLV Hong RT-qPCR (Hong Liu, China, personal communication to the *ad hoc* group)
2. TiLV Waiyamitra RT-qPCR (Waiyamitra et al. 2018)
3. TiLV CEFAS RT-qPCR (David Stone, CEFAS UK, personal communication to the *ad hoc* Group)

Real-time PCR – SYBR:

4. TiLV Tattiyapong SYBR Green RT-qPCR (Tattiyapong et al. 2017)

Conventional PCR:

5. TiLV Dong RT-nPCR (Dong et al. 2017)
6. TiLV CEFAS RT-nPCR (David Stone, CEFAS UK, personal communication to the *ad hoc* Group)

WOAH e*ad Hoc* Group for TILV: ILC Round 1, June 2019

- Pilot study
  - ACDP Fish Diseases Laboratory, Australia
  - Centre for Environment, Fisheries and Aquaculture Science (CEFAS), United Kingdom

Interim Report of the OIE E-*AD HOC* GROUP ON TILAPIA LAKE VIRUS (TiLV) / Date of report 17.09.2019

TiLV inter-laboratory comparability panel testing results – Tilapia lake virus real-time RT-qPCR assays

Testing of IL was undertaken by AAHL and CEFAS. CEFAS reported results for tests conducted on different days (Test 1 and Test 2). Results were reported as a quantitative C_T value and a qualitative interpretation (positive or negative). The following tables include the compiled test results provided by AAHL and CEFAS for the TiLV Hong RT-qPCR (Table 1), TiLV CEFAS RT-qPCR (Table 2) and TiLV Tattiyapong SYBR Green RT-qPCR (Table 3). AAHL also tested the panel with the TiLV Waiyamitra RT-qPCR (Table 4).

TiLV inter-laboratory comparability panel testing results – Tilapia lake virus conventional nested PCR

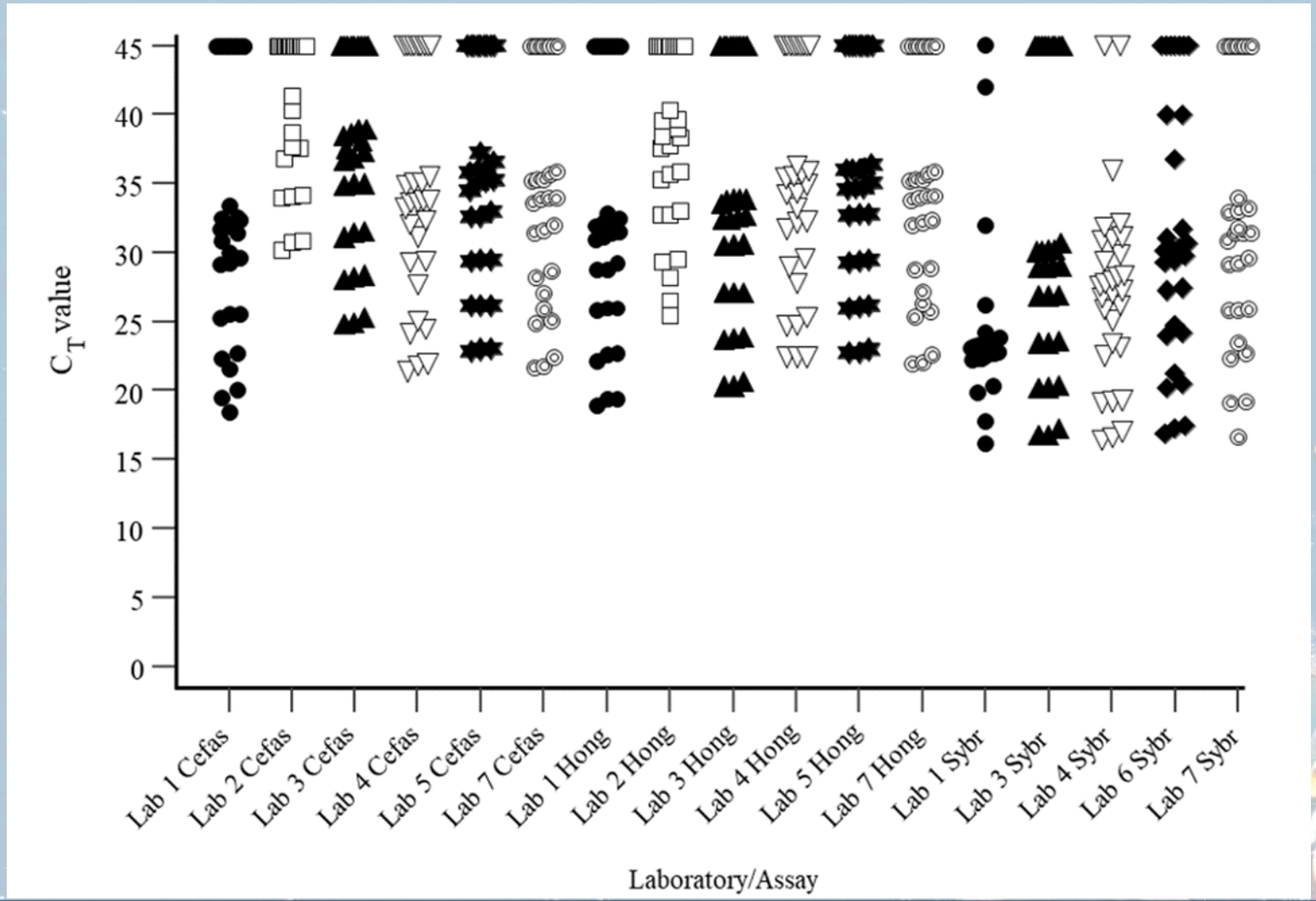
Testing of the TiLV inter-laboratory comparability panel was undertaken by AAHL and CEFAS. Results were reported as a qualitative interpretation (positive or negative). CEFAS reported results for tests conducted on different days (Test 1 and Test 2). compiled test results provided by AAHL and CEFAS for the TiLV Dong RT-nPCR are included in Table 5.

WOAH e*ad Hoc* Group for TILV: ILC Round 2, April 2021

- Delays due to COVID-19 global pandemic
- Laboratories that participated in Round 2:

Australia Peter Mohr ACDP Fish Diseases Laboratory CSIRO Australian Centre for Disease Preparedness Geelong, Victoria, Australia	**Brazil** Marcelo Fernandes Camargos Laboratório Federal de Defesa Agropecuária Ministério da Agricultura, Pecuária e Abastecimento Pedro Leopoldo, Brazil
China (People's Rep. of) Hong Liu The National Key laboratory of Aquatic Animal Diseases, Animal and Plant Inspection and Quarantine Technical Centre, Shenzhen City, Guangdong province, China	**Denmark** Argelia Cuenca EURL for Fish and Crustacean Diseases Technical University of Denmark Kgs. Lyngby, Denmark
South Africa Marco Romito ARC-Onderstepoort Veterinary Institute Onderstepoort, South Africa	**United Kingdom** David Stone CEFAS Barrack Road, Weymouth, Dorset, UK
United States Janet Warg National Veterinary Services Laboratories Ames, Iowa, Unites States	

WOAH e*ad Hoc* Group for TILV: ILC Round 2, April 2021



WOAH e*ad Hoc* Group for TILV: ILC Round 2, April 2021

Based on results provided in this report it is the opinion of the *ad hoc* Group that all tests would allow criterion 3 of Chapter 1.2. "Criteria for listing aquatic animal diseases of the *Aquatic Code*" to be fulfilled.

Report of the meeting of the WOAH Aquatic Animal Health Standards Commission Meeting: Texts to be proposed for adoption at the WOAH 89th General Session in May 2022:

Infection with TiLV clearly meets the criteria for listing (1, 2, 3, 4b and 4c) and is proposed for inclusion in Chapter 1.3. Diseases listed by the WOAH.

The Commission agreed with a comment requesting the WOAH to apply the same approach for future emerging disease events as was applied to infection with TiLV

WOAH e*ad Hoc* Group for TILV

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

The *diseases* in this chapter have been assessed in accordance with Chapter 1.2. and constitute the OIE list of *aquatic animal diseases*.

In case of modifications of this list of *aquatic animal diseases* adopted by the World Assembly of Delegates, the new list comes into force on 1 January of the following year.

Article 1.3.1.

The following *diseases* of fish are listed by the OIE:

- Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
- Infection with epizootic haematopoietic necrosis virus
- Infection with *Gyrodactylus salaris*
- Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
- Infection with infectious haematopoietic necrosis virus
- Infection with koi herpesvirus
- Infection with red sea bream iridovirus
- Infection with salmonid alphavirus
- Infection with spring viraemia of carp virus
- Infection with tilapia lake virus
- Infection with viral haemorrhagic septicaemia virus.

WOAH e*ad Hoc* Group for TILV

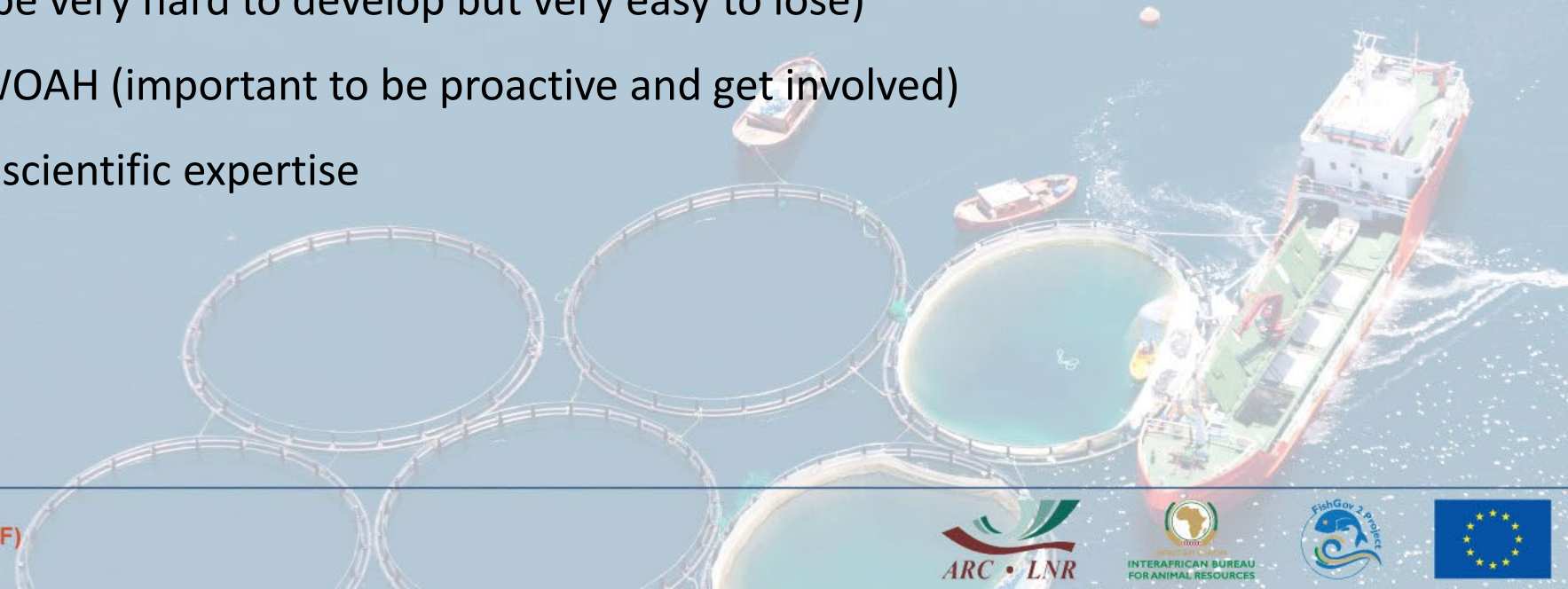
Journal of fish diseases. J Fish Dis.; Research Article
Correspondence: Nick Moody
Funding information: Financial support for the shipment of panels globally and assistance in project management was obtained from the World Organization for Animal Health (WOAH).

International evaluation and comparison of 4 molecular assays for the detection of Tilapia lake virus (TiLV) by interlaboratory rounds.

[1,2]John Hoad | [1,2]Nicholas J. G. Moody | [1,2]Peter Mohr | [3]Henrique César Pereira Figueiredo | [4]Marcelo Fernandes Camargos | [5]Sergio Hernan Marshall Gonzalez | [6]Hong Liu | [7]Argelia Cuenca | [8]Mona Dverdal Jansen | [9]Marco Romito | [10]Dong Thanh | [11]David Stone | [12]Janet Warg | [13]Stian Johnsen | [1,2]Axel Colling |

International Networks

- My networks were initially within aquatic animal health circles within Australia, then terrestrial colleagues as technology developed (whole genome sequencing, bioinformatics, epidemiology) then globally as opportunities came up
- Need to be well considered (don’t want to over-extend)
- Face-to-face communication is really important as opportunities can be opportunistic
- Trust is very important (can be very hard to develop but very easy to lose)
- Opportunities provided by WOAH (important to be proactive and get involved)
- Lot of satisfaction in sharing scientific expertise



Thank you very much for the opportunity to participate in this meeting