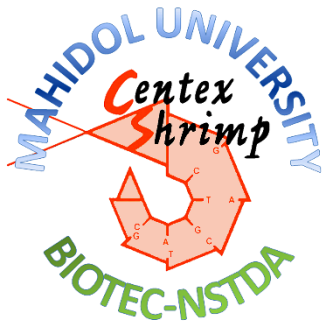
Emergency response to emerging diseases: TiLV in Tilapia

Saengchan Senapin

Introducing Centex Shrimp



Centex Shrimp (Center of Excellence for Shrimp Molecular Biology and Biotechnology) Since 2001

Introducing Centex Shrimp

BIOTEC
a member of NSTDA

National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology

+

Mahidol University
Faculty of Science

=

MAHIDOL UNIVERSITY
Centex Shrimp
BIOTEC-NSTDA

Centex Shrimp (Center of Excellence for Shrimp Molecular Biology and Biotechnology) Since 2001

Research on fish diseases

Nile tilapia (*Oreochromis niloticus*)
Hybrid red tilapia (*O. mossambicus x O. niloticus*)

- *Flavobacterium columnare*
- *Francisella noatunensis*
- *Streptococcus agalactiae*
- *Aeromonas veronii*
- *Hahella chejuensis*
- Tilapia lake virus (TiLV)
- Iridovirus

Barramundi / Asian Seabass
(*Lates calcarifer*)

- *Vibrio harveyi*
- Scale drop disease virus (SDDV)

Striped catfish
(*Pangasianodon hypophthalmus*)

- *Flavobacterium columnare*
- *Edwardsiella ictaluri*

Siamese fighting fish (*Betta* spp.)

- Skin nodule syndrome
- Big belly syndrome

Research on fish diseases

Nile tilapia (*Oreochromis niloticus*)
Hybrid red tilapia (*O. mossambicus x O. niloticus*)

- *Flavobacterium columnare*
- *Francisella noatunensis*
- *Streptococcus agalactiae*
- *Aeromonas veronii*
- *Hahella chejuensis*
- Tilapia lake virus (TiLV)
- Iridovirus

Barramundi / Asian Seabass (*Lates calcarifer*)

- *Vibrio harveyi*
- Scale drop disease virus (SDDV)

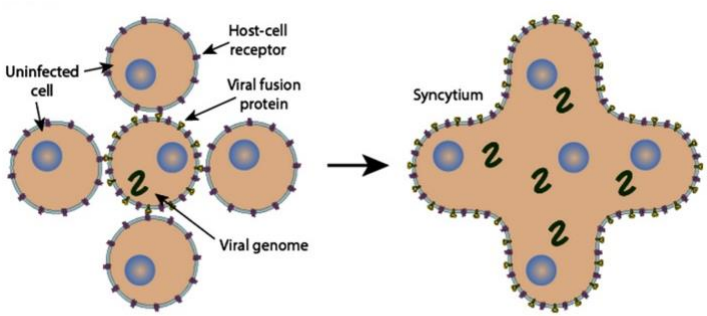
Striped catfish (*Pangasianodon hypophthalmus*)

- *Flavobacterium columnare*
- *Edwardsiella ictaluri*

Siamese fighting fish (*Betta* spp.)

- Skin nodule syndrome
- Big belly syndrome

Tilapia lake virus disease (TiLVD) is a newly emerging disease of tilapia



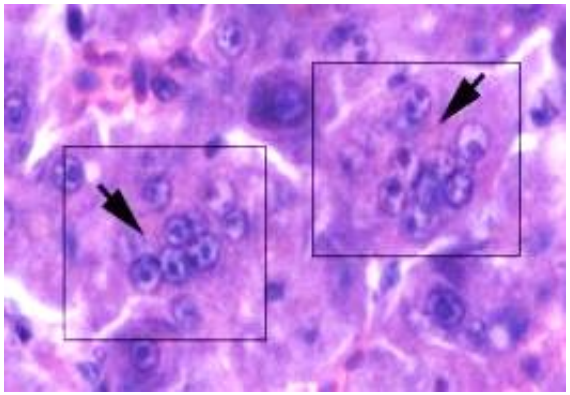
ViralZone:www.expasy.org/viralzone, IB Swiss Institute of Bioinformatics

Synonyms

Syncytial hepatitis of tilapia (SHT)

Summer mortality syndrome (SMS)

Tilapia one month mortality syndrome (TOMS)



Causative agent:

Tilapia lake virus (TiLV) = Orthomyxo-like virus

Tilapinevirus/ Tilapia tilapinevirus

(Bacharach et al. 2016)

April-May 2017

Food and Agriculture Organization of the United Nations

Food Chain Crisis
Early Warning Bulletin

April-June 2017
No.23

Food and Agriculture Organization of the United Nations

GLOBAL INFORMATION AND EARLY WARNING SYSTEM ON FOOD AND AGRICULTURE (GIEWS)

SPECIAL ALERT

No. 338

REGION: Global **DATE: 26 May 2017**

Outbreaks of Tilapia lake virus (TiLV) threatens the livelihoods and food security of millions of people dependent on tilapia farming

---

NACA

DISEASE ADVISORY

Asia Regional Aquatic Animal Health Programme

Tilapia Lake Virus (TiLV) – an Emerging Threat to Farmed Tilapia in the Asia-Pacific Region

Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

---

OIE WORLD ORGANISATION FOR ANIMAL HEALTH
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).

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

---

FACTSHEET

Tilapia lake virus (TiLV): What to know and do?

CGIAR RESEARCH PROGRAM ON Fish

Tilapia lake virus is a newly emerging virus that is associ
mortalities in farmed tilapia. With cases reported across
America, the virus represents a huge risk to the USD 7.5
industry. All countries with a tilapia industry must be vi
investigate cases of mortalities in farms.

Tilapia lake virus – an emerging threat

The tilapia lake virus (TiLV) is a newly emerging virus associated with significant mortalities in farmed tilapia.

Since the first discovery of the virus in Israel in 2014, cases have been reported in Columbia, Ecuador, Egypt and Thailand. Now that screening tools are available and can be accessed by fish disease diagnostic/research labs, the number of reported TiLV cases is expected to rise.

There has been no report of any human health-related issues linked to the consumption of affected tilapia from any of the affected countries since the emergence of TiLV. Looking at fish viruses overall, including TiLV, there is no evidence for a fish virus causing disease in humans.

History of TiLV

2013 Syncytial Hepatitis of Tilapia (SHT)(Ecuador) (Ferguson et al. 2014)

2014 TiLV became known to science (Israel) (Eyngor et al. 2014)

2016 Complete genome of TiLV (Bacharach et al. 2016)
TiLV=unknown virus associated with SHT

2017 TiLV reported in Colombia, Egypt, Thailand, Taiwan, Malaysia, and the Philippines (Kembou Tsofack et al. 2017; Fathi et al. 2017; Nicholson et al. 2017; Surachetpong et al. 2017; Dong et al. 2017; OIE 2017)

- Early warning bulletin (FAO)
  Special Alert (FAO)
  Disease Advisory (NACA)
  Disease card (OIE)
  Fact Sheet & Review (WorldFish/CGIAR)

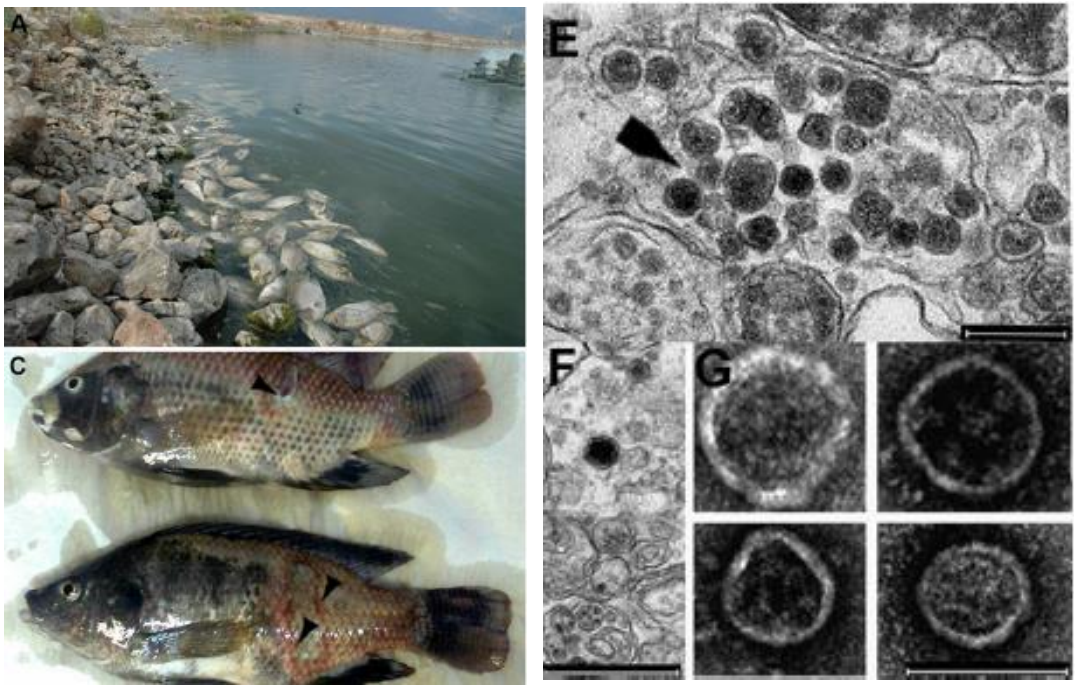
2018 TiLV reported in India, Indonesia, Peru, Tanzania and Uganda (see review in Jansens et al. 2018)

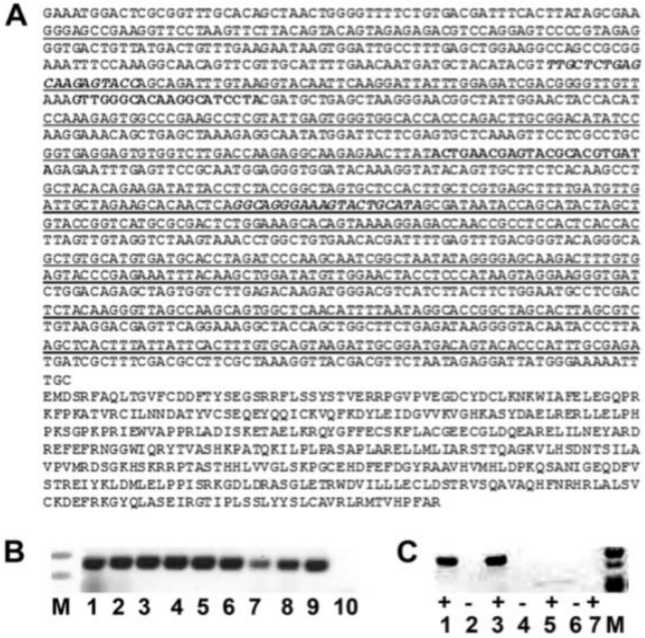
Unexplained mortality in cultivated tilapia in Thailand (2016-2017)

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]

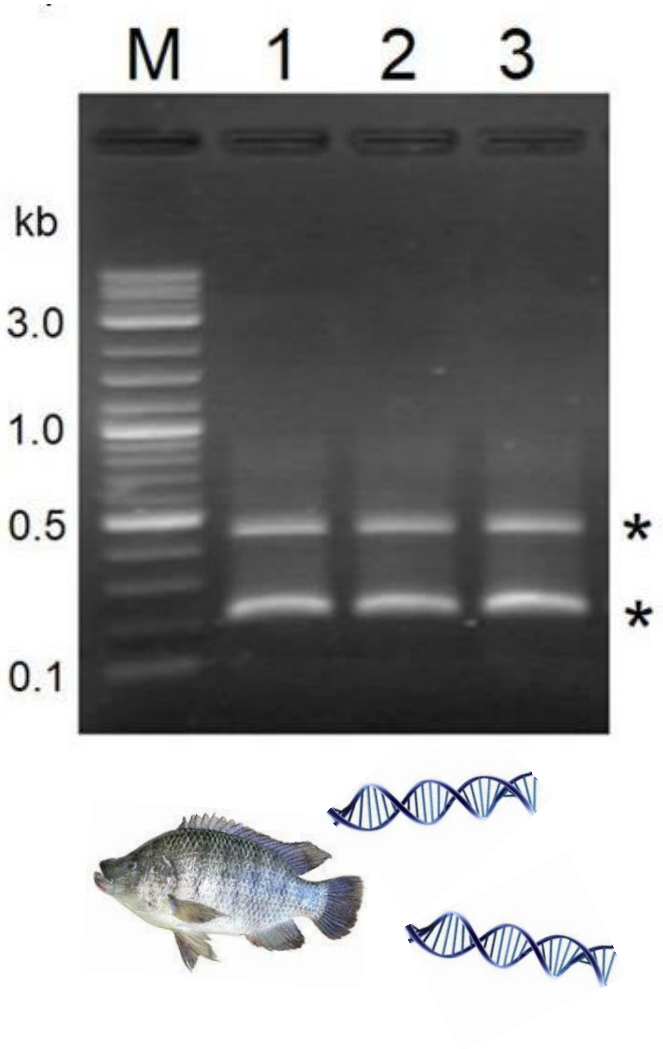




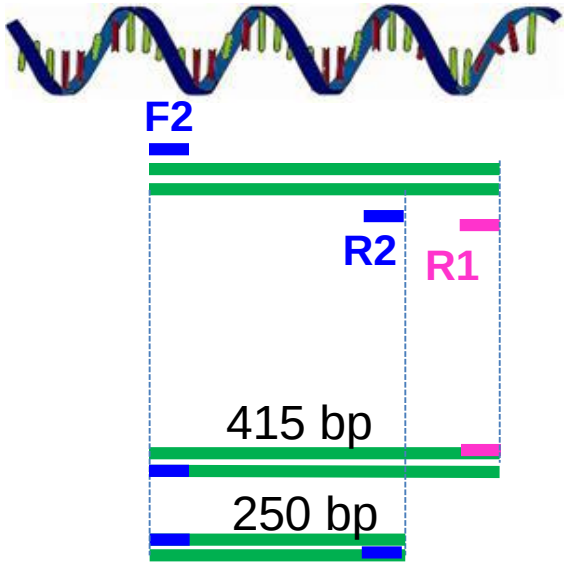
Eyngor et al. 2014 J Clin Microbiol. 52, 4137–4146

Optimization of TiLV detection protocol

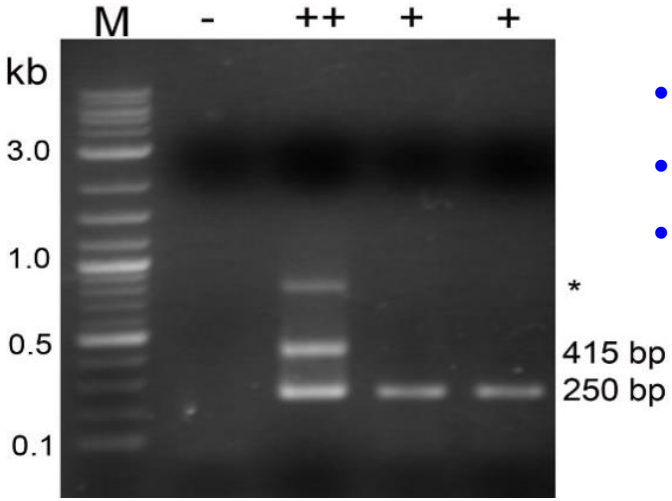
- Primers from Eyngor et al. 2014 (expect 491 and 250 bp)
- Sequencing result revealed false positive
- One primer binds non-specifically to fish gene
- Need to optimize the PCR conditions



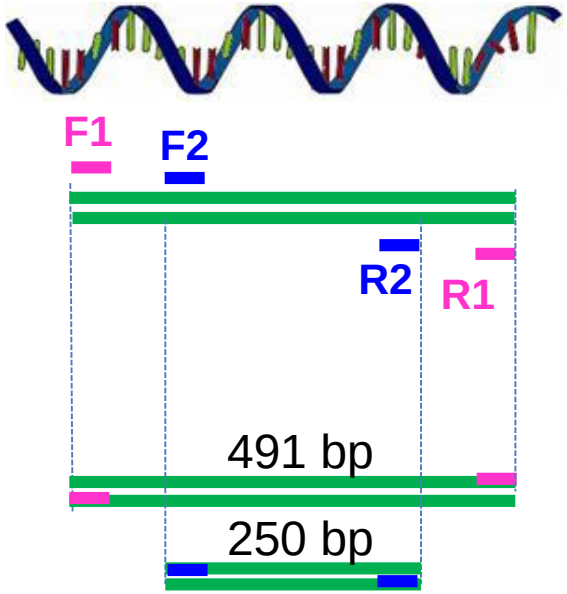
Optimization of TiLV detection protocol



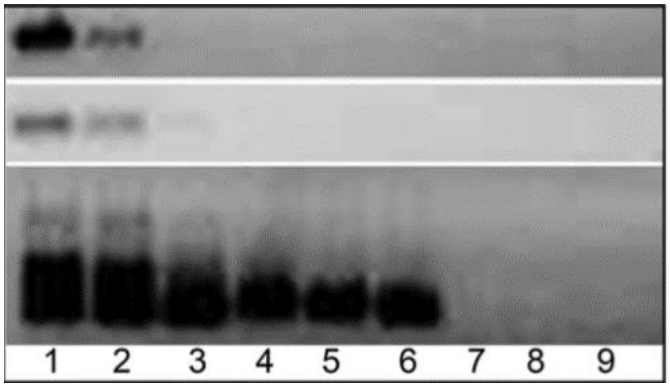
Alternative detection protocol



- Semi-Nested PCR
- Dong et al, 2017a
- 7.5 copies/reaction



Nested RT-PCR

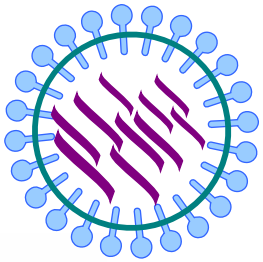


- Kembou Tsofack et al, 2017
- 7 copies/reaction

Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection (Dong et al 2017a)

Year	Farm/ province	Species	Fish stage	Mortality (%)	Number of positive/ number tested
2017	A Pathumthani	Nile tilapia, *Oreochromis niloticus*	Fingerling (2.5-3 cm)	~ 90	**14/14**
2016	CL Phetchaburi	Red and Nile tilapia	Fingerling (3.5-4 cm)	~ 20	**7/7**
	CN Chainat	Red tilapia *Oreochromis* sp.	Juvenile (8.5-9 cm)	~ 90	**6/6**
	Control	Nile tilapia	Juvenile (~ 15 cm)	-	0/2

TiLV was confirmed by sequencing other genome segments



Segment 1

```
AMR44593.1      MWAFQEGVCKGNLLSGPTSMKAPDSAARESLDRASEIMTGKSYNAVHTGDLSKLPNQGES
Seg1_CL         MWAFQEGVCKGNLLSGPTSMKAPDSAARESIDRASEIMTGKSYNAVHTGDLSKLPNQGES
                ********************************:*****************************

AMR44593.1      PLRIVDSDLYSERSCCWVIEKEGRVVCKSTTLTRGMTGLLNTTRCSSPSELICKVLTVES
Seg1_CL         PLRIVDSDLYSERSCCWVIEKEGRVVCKSTTLTRGMTSLLNTTKCSSPSELICKVLTVES
                ************************************.*****:****************

AMR44593.1      LSEKIGDTSVEELLSHGRYFKCALRDQERGKPKSRAIFLSHPFFRLLSSVVETHARSVLS
Seg1_CL         LSEKIGDTSVEELLSHGRYFKCALRDQERGKPKSRAIFLSHPFFRLLSSVVETHARSVLS
                ************************************************************

AMR44593.1      KVSAVYTATASAEQRAMMAAQVVESRKHVLNGDCTKYNEAIDADTLLKVWDAIGMGSIGV
Seg1_CL         KVSAVYTATASAEQRAMMAAQVVESRKHVLNGDCTKYNEAIDADTLLKVWDAIGMGSIGV
                ************************************************************

AMR44593.1      MLAYMVRRKCVLIKDTLVECPGGMLMGMFNATATLALQGTTDRFLSFSDDFITSFNSPAE
Seg1_CL         MLAYMVRRKCVLIKDTLAECPGGMLMGMFNATATLALQGTTDRFLSFSDDFITSFNSPAE
                ****************.*******************************************

AMR44593.1      LREIEDLLFASCHNLSLKKSYISVASLEINSCTLTRDGDLATGLGCTAGVPFRGPLVTLK
Seg1_CL         LREIEDLLFVSCHNLSLKKSYISVASLEINSCTLTRDGDLATGLGCTAGVPFRGPLVTLK
                *********.****************************************************

AMR44593.1      QTAAMLSGAVDSGVMPFHSAERLFQIKQQECAYRYNNPTYTTRNEDFLPTCLGGKTVISF
Seg1_CL         QTAAMLSGAVDSGVMPFHSAERLFQIKQQECAYRYNNPTYTTRNEGFLPTCLGGKTVISF
                ********************************************.***************

AMR44593.1      QSLLTWDCHPFWYQVHPDGPDTIDQKVLSVLASKTRRRRTRLEALSDLDPLVPHRLLVSE
Seg1_CL         QSLLTWDCHPFWYQVHPDGPDTIDQKVLSVLASKTRRRRTRLEALSDLDPLVPHRLLVSE
                ************************************************************

AMR44593.1      SDVSKIRAARQAHLKSLGLEQPTNFNYAIYKAVQPTAGC     519
Seg1_CL         SDVSKIRAARQAHLKSLGLEQPTNFNYAIYKAVQPTAGC     519
                *************************************
```

96.3–97.5% nt identity
97.3–98.8% aa identity
to the prototype strain (Israel)

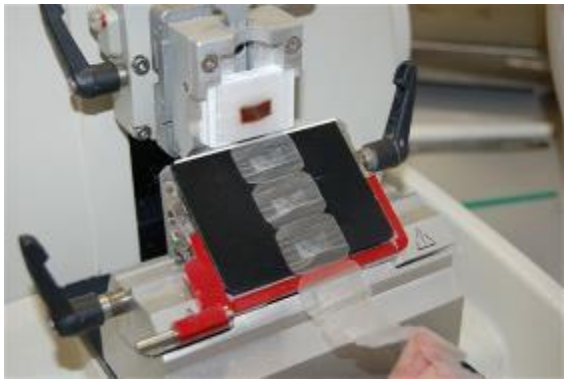
Segment 5

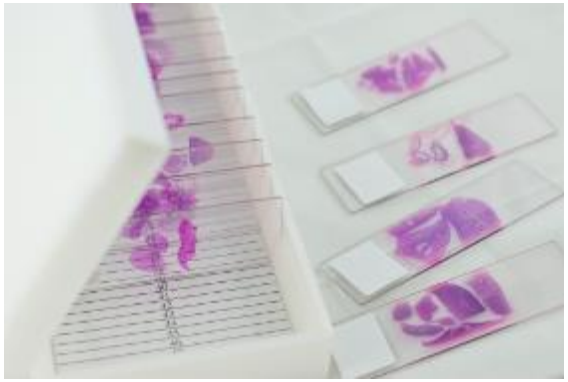
```
AMR44597.1      MFLLSQTPIAMQALVLISCLVCALASDESLRIKRLQSYLNNTYQSREIESEIRRGFASKF
Seg5_CL         ---LSQTPIAMQVLVLTSCLVCALASDESLRIKRLQSYLNNTYQSREIESEIRRGFASKF
                   *********.*** ****************************************

AMR44597.1      RMESCSCTMGVHYIVTPSSGGSFCTGLHAVPNSFPALGYKLPKAGGRGDWRATEVRIDED
Seg5_CL         RMESCSCTMGVHYIVTPSSGGSFCTGLHAVPDSFPALGYKLPKAGGRGDWKATEVRVDED
                ********************************:*****************:*****:***

AMR44597.1      SGVVLYNVSRCSHSSECRDLEVYSTVLPGQCDCTRPTVDDYKTMLASRQPKSFVVAGLII
Seg5_CL         SGVVLYNVSRCSHSTECRDLEVYSTVLPGQCECTRPTVDDYKTMLASRQPKSFVVAGLII
                **************:***************:**************************

AMR44597.1      LCLLASSVAIGMGVYNYAGVIGLADAAQADVSEIWEYLEALTREVTGMTLGEFCSIKSLV
Seg5_CL         LCLLASSVAIGMGVYNYAGVIGLADAAQADVSEIWEYLEALTREVTGMTLGEFCSIKSLV
                ************************************************************

AMR44597.1      CKSDNIGKFKEQFAAFGEAILAIVFGMLEKYKFVYYLVLSLMVLSLLSKLVSLLKQVPFY
Seg5_CL         CKSDNIGKFKEQFAAFGEAILAIVFGMLEKYKFVYYLVLSLMVLSLLSKLISLLKQVPFY
                ************************************************:*********

AMR44597.1      GSIKVLVFRRLRVVCFKTFFYIKKRLKKKSPLEDDEVPLLPLS 343
Seg5_CL         GSIKVLVFRRLRVVCFKTFFEIKKRLKKKGPLEDDEVPLLPLS 340
                ********************:********.*************
```

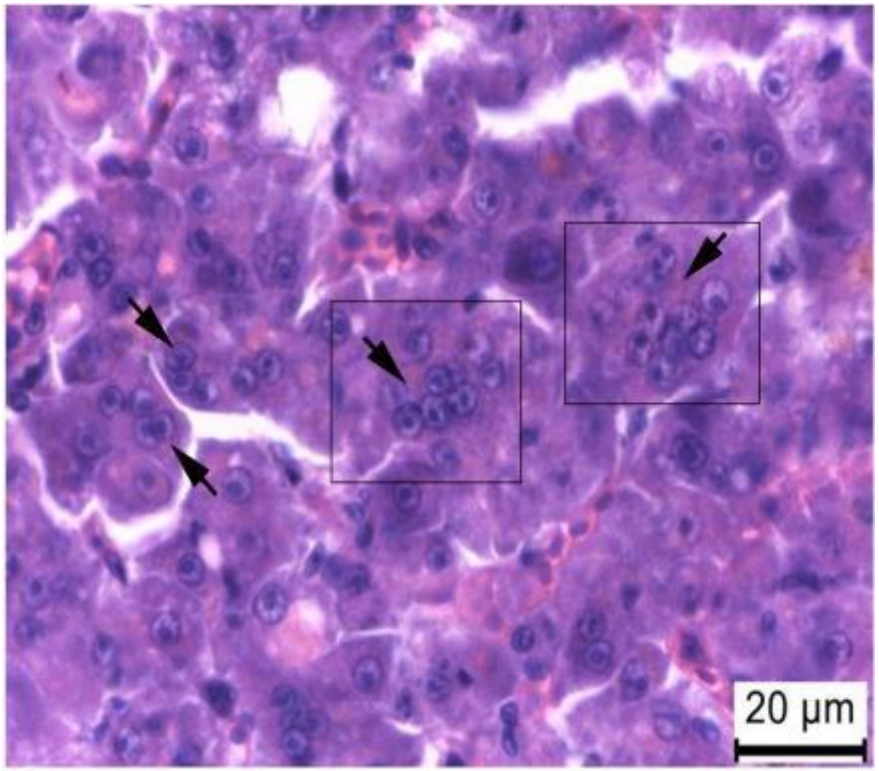
Segment 9

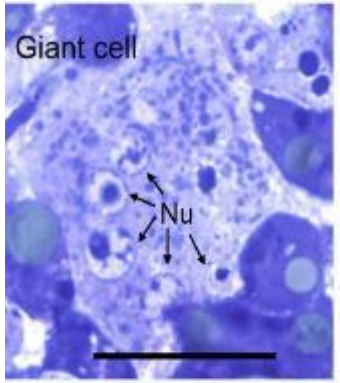
```
AMR44601.1      MVMSRWIENTDSVSVILTEMSRSYVTLCHYPPWSLCCGGKTSQPGQVWILDRKHCLECSE
Seg9_CL         --MSRWIENTDSVSVILTEMSRSYVTLCHYPPWSLCCGGKTSQPGQVWILDRKHCLECSE
Seg9_CN         --MSRWIENTDSVSVILTEMSRSYVILCHYPPWSLCCGGKTSQPGQVWILDRKHCLECSE
                  ********************** ***********************************

AMR44601.1      ISELMTLSQPTWQESAMVIGSWVQVVTEHGILHTSTSERQAPRRVMWRISDMAGDRTL
Seg9_CL         ISELMTLGQPTWQESAMVIGSWVQVVTEHGILHTSTSERQAPRRVMWRISDMAGDKTL
Seg9_CN         ISELMTLGQPTWQESAMVIGSWVQVVTEHGILHTSTSERQAPRRVMWRISDMAGDKTL
                *******.****************************************************:**
```

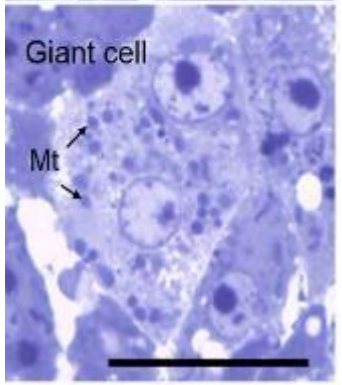
Histopathological findings





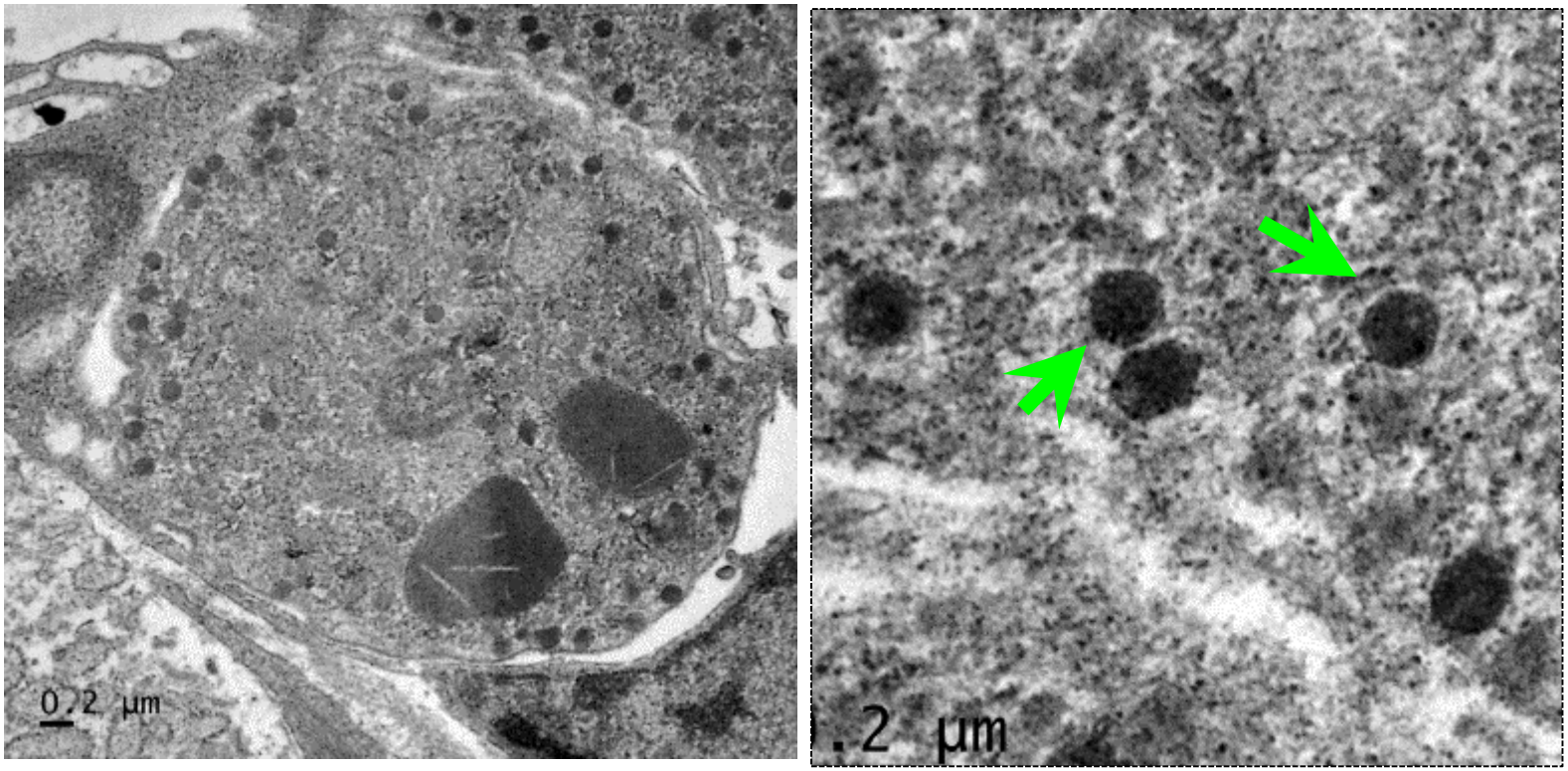






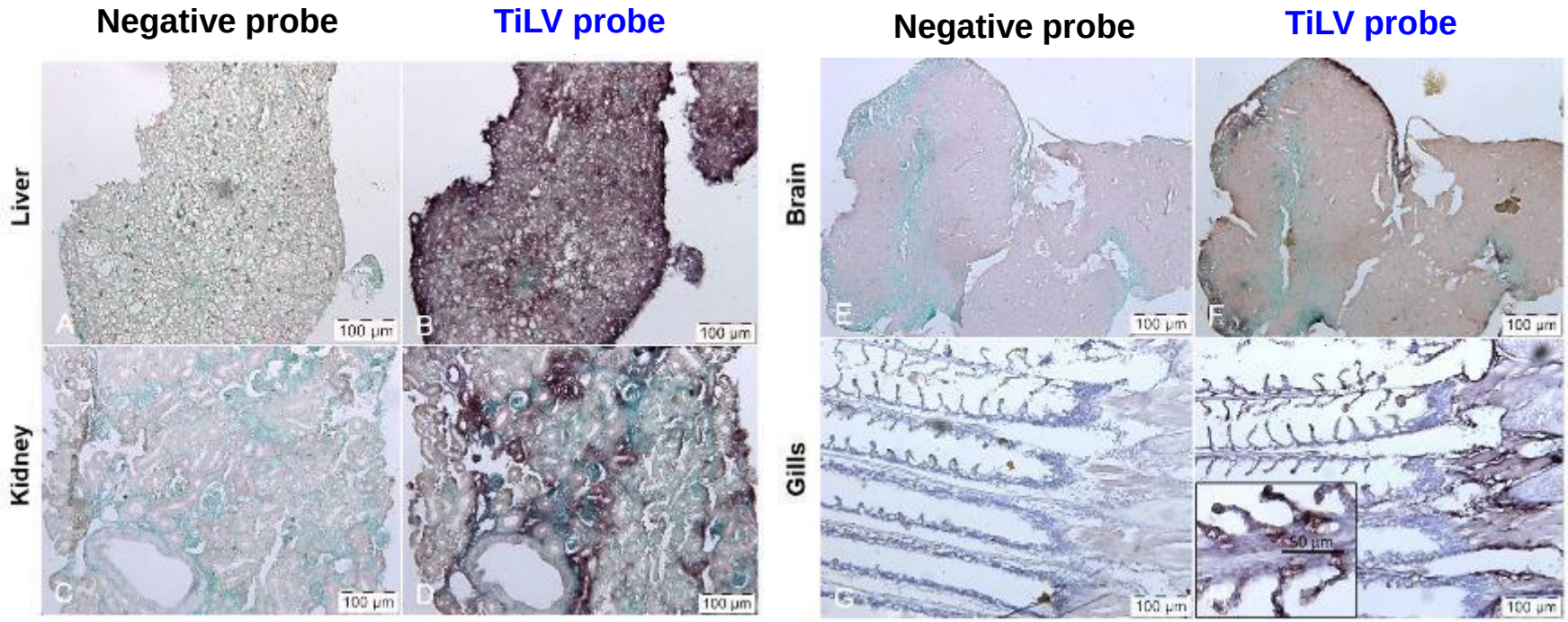
TiLV-infected liver tissue of tilapia revealed typical feature of syncytial hepatitis

Ultrastructure findings



Transmission electron micrographs of intracytoplasmic viral particles of TiLV from liver tissue of the infected tilapia

In situ hybridization revealed multiple tissue tropisms of TiLV



Target tissues: Liver, kidney, brain, gills, spleen, muscle

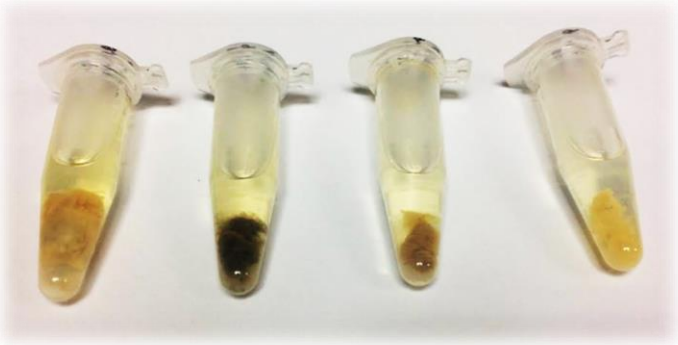
Our responsibilities

- Reported to DOF, Thailand
- Reported to private sectors
- A warning & an improved detection method to NACA
- Provide free positive control plasmid for non-commercial organizations (42 requests from 24 countries)
- Published full-work in Aquaculture
- Training upon request

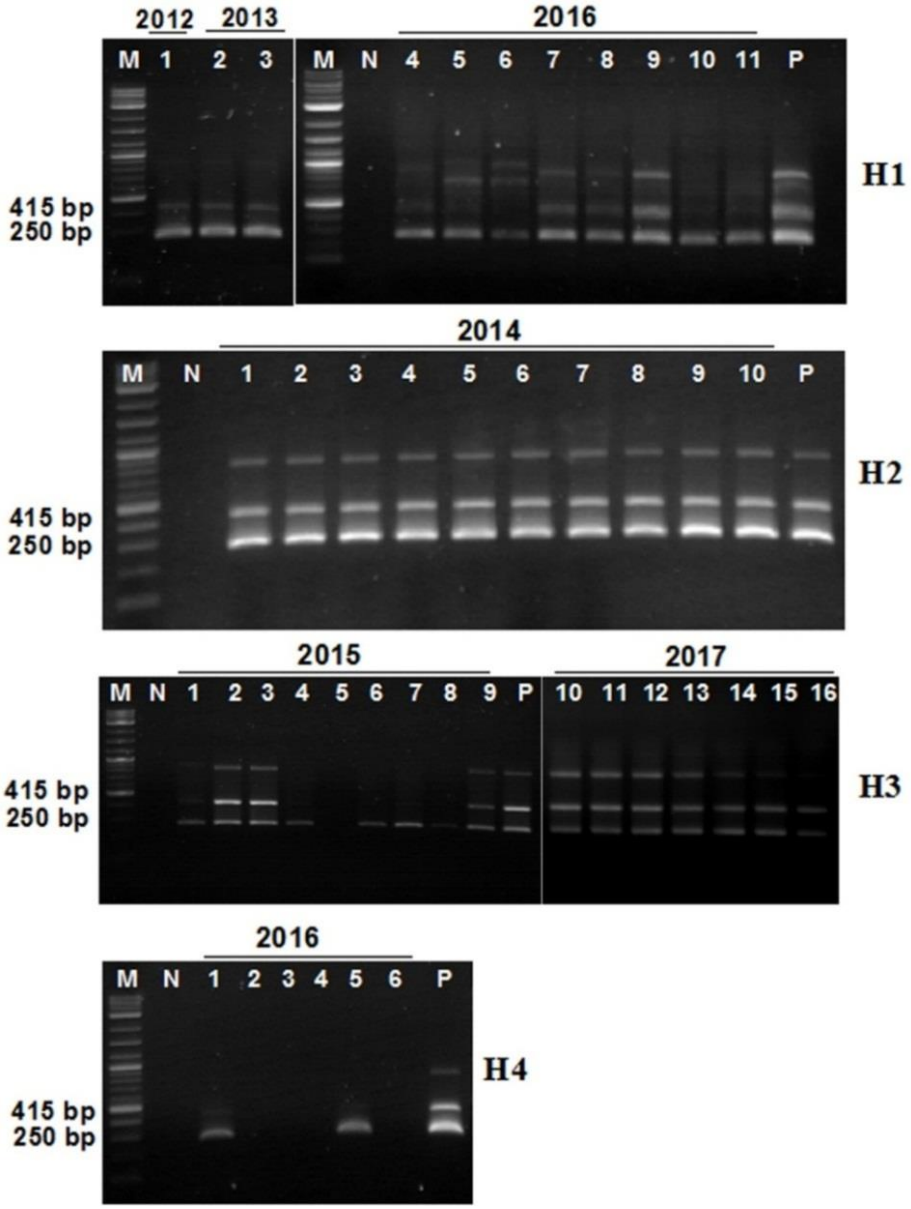
The End is a New Beginning

How long had TiLV been present in Thailand?

- Archived samples kept in -20°C from unexplained mortality in 2012-2017



Testing results of samples from 2012-2017



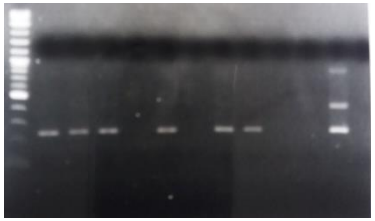
Before TiLV become known to science in 2014…

- TiLV had been present in the country
- We were faultlessly unaware of TiLV contamination
- A brief article to NACA to warn countries involved in live tilapia trade for aquaculture to quickly initiate monitoring for TiLV
- Published in Aquaculture
- Complete genome sequence of all archived samples are being anlayzed

Mortality due to genotypes??

- Israel and Ecuador: 80%
- Thailand: 20-90%
- Egypt: 5-15%
- Taiwan: 6.4%
- Malaysia: 0.71-15%
- India: 80-90%

Mortality due to genotypes??



Aquaculture 487 (2018) 51–55

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

Inapparent infection cases of tilapia lake virus (TiLV) in farmed tilapia

Saengchan Senapin[a,b], K.U. Shyam[a], Watcharachai Meemetta[a], Triwit Rattanarojpong[c], Ha Thanh Dong[c,*]

[a] Fish Health Platform, Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, 272 Rama VI Road, Bangkok 10400, Thailand

[b] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathumthani 12120, Thailand

[c] Aquaculture Vaccine Platform, Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand

TiLV genotype specific detection

Fact: 0.71-90% mortality of TiLV positive tilapia.

Hypothesis: There is a TiLV genotype complex and not all genotypes associated with disease.

TiLV genotype specific detection

Fact: 0.71-90% mortality of TiLV positive tilapia.

Hypothesis: There is a TiLV genotype complex and not all genotypes associated with disease.

An example of shrimp virus:

YHV (yellow head virus) complex in shrimp composes of 8 genotypes. Some genotypes were detected in healthy shrimp. Only genotype 1 has been recently listed by OIE.

Nucleotide sequence identity among the complex: 80-93%

TiLV genotype specific detection

3 OIE PCR protocols for YHV (yellow head virus) complex in shrimp

Protocol 1: RT-PCR for specific detection of YHV1 in diseased shrimp

Protocol 2: Nested RT-PCR for differential detection of YHV1 and GAV in healthy or diseased shrimp

Protocol 3: Nested RT-PCR for detection of all currently characterised genotypes in the yellow head complex YHV1 to YHV7

TiLV genotype specific detection

3 OIE PCR protocols for YHV (yellow head virus) complex in shrimp

Protocol 1: RT-PCR for specific detection of YHV1 in diseased shrimp

Protocol 2: Nested RT-PCR for differential detection of YHV1 and GAV in healthy or diseased shrimp

Protocol 3: Nested RT-PCR for detection of all currently characterised genotypes in the yellow head complex YHV1 to YHV7

What about TiLV??

Lesson learned from TiLV

- There are huge knowledge gaps for TiLV
- Needs strong collaboration between private sectors, researchers and competent authority
- Always ready for emerging diseases (e.g. pathogen discovery and rapid diagnosis platform)
- Emergency funding mechanism
- Dealing with known pathogens is cheaper and easier than unknown
- Transparency is a pain but gain

Acknowledgements

Centex Shrimp/BIOTEC/ Mahidol University

Prof. Tim W. Flegel
Prof. Boonsirm Withyachumnarnkul
Assist. Prof. Rapeepun Vanichviriyakit
Dr. Pakkakul Sangsuriya
Mr. Warachin Gangnonngiw
Ms. Kornsunee Phiwsaiya

KMUTT

Assist. Prof. Triwit Rattanarojpong
Assist. Prof. Pongsak Khunrae
Dr. Ha Thanh Dong

Chulalongkorn University

Assist. Prof. Dr. Channarong Rodkhum
Assoc. Prof. Dr. Nopadon Pirarat
Mr. Vuong Viet Nguyen

Private companies & Non-profit organizations

Thank you