Food and Agriculture Organization of the United Nations

FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)

Sun Yat Sen University, Guangzhou, China

18-24 June 2018

Session 2

Win Surachetpong DVM, PhD, CertAqV, DTBVP

TiLV diagnosis

Learning objectives

- **Describe diagnostic methods for detecting infection with TiLV**
  - **Clinical signs and gross pathology**
  - **Molecular diagnostic methods**

General overview of diagnostic methods in virology

Direct methods

- Electron microscopy
- Light microscopy
- Immunofluorescence
- Molecular techniques

Indirect methods

- Cell culture
- Embryonated egg
- Laboratory animals

Serology

Classical techniques

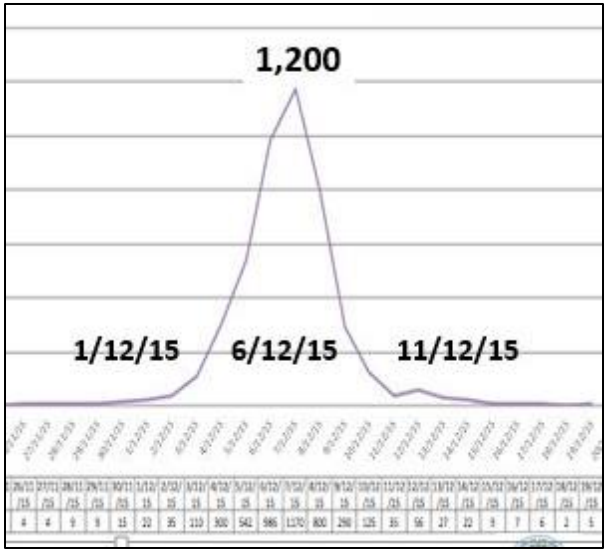
- Complement fixation test
- Haemagglutination inhibition test
- Neutralization test

Advanced techniques

- Immunoassay (ELISA etc.)
- Western blot

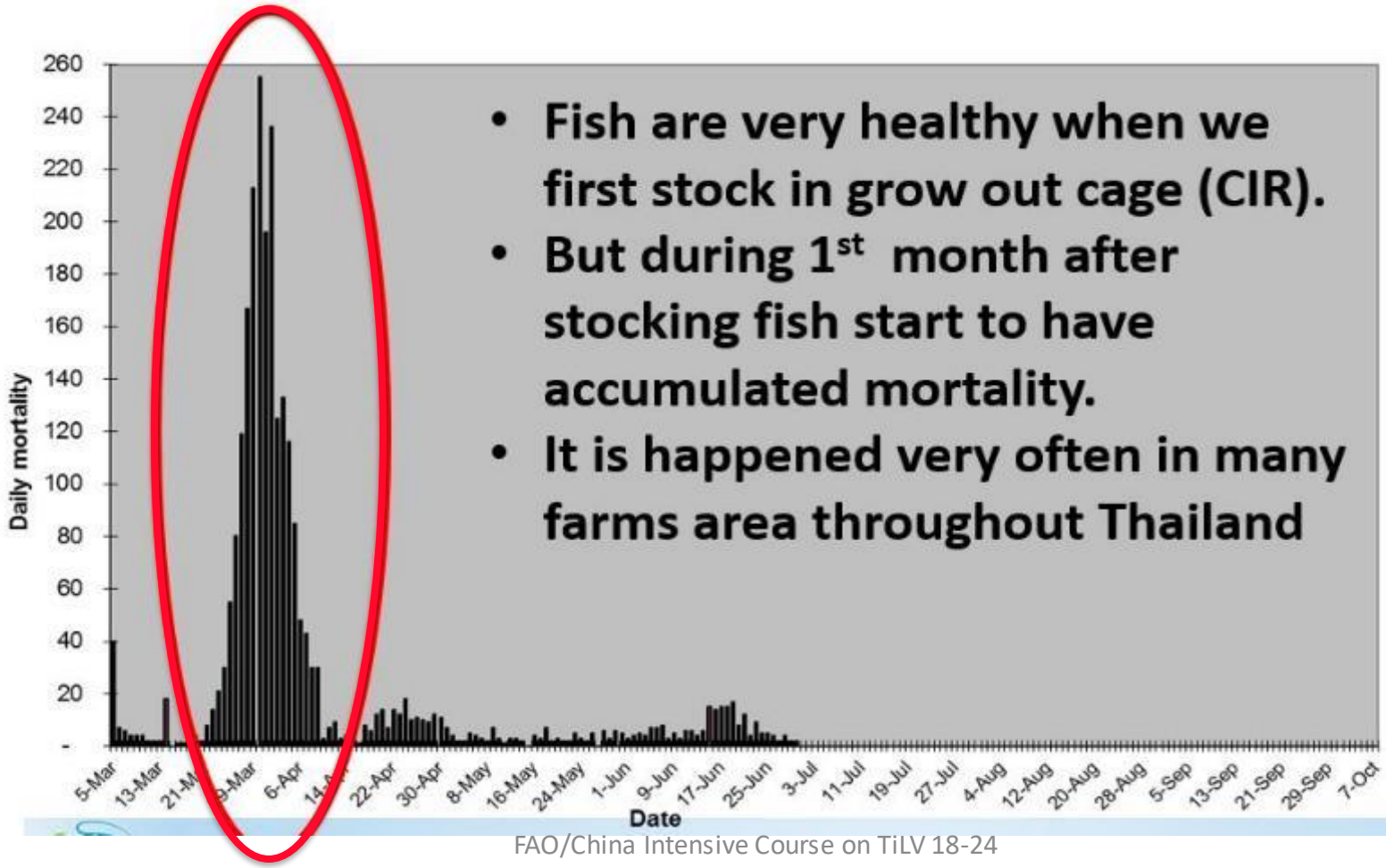
How do we know if TiLV exists in a farm?

- High mortality **20-90%**
- Swimming at water surface
- Skin redness, erosion
- Red tilapia (pale body)
- Exophthalmos, scale protrusion



***Parinda kumchum , MSD ,Bangkok Thailand 2015

1st month post stocking mortality



- **Fish are very healthy when we first stock in grow out cage (CIR).**
- **But during 1st month after stocking fish start to have accumulated mortality.**
- **It is happened very often in many farms area throughout Thailand**

Tilapia One Month Mortality Syndrome (TOMMS)

- **High mortality rates often found within one month of transferring tilapia into rearing sites**
  - **Transportation?**
  - **Acclimatization to new environments?**
  - **Pathogens at rearing site?**

Immunized tilapia fingerings

ปลาผ่านเชื้ออนุบาลในแม่น้ำเมืองกาญครับสนใจติดต่อสอบถามได้ครับ

- **Nursery culture in the river**
- **Survived from massive die offs**
- **Healthy in growout period**

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Journal of Clinical Microbiology p. 4137–4146

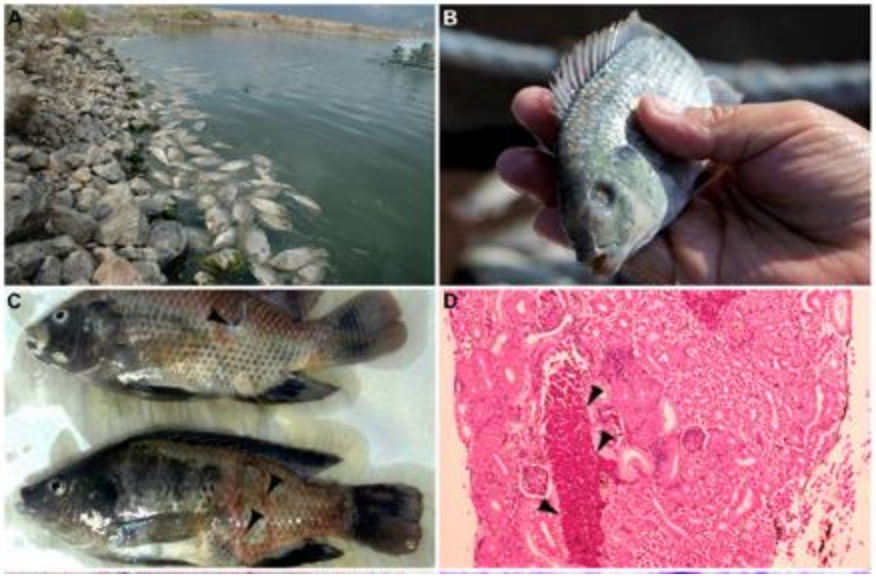
December 2014 Volume 52 Number 12

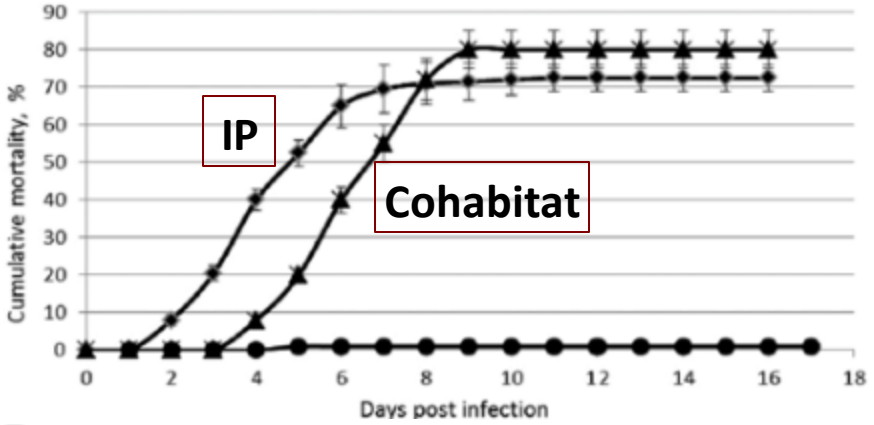
Tilapia Lake Virus: TiLV

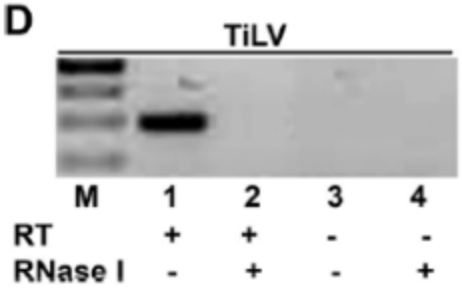
Identification of a Novel RNA Virus Lethal to Tilapia

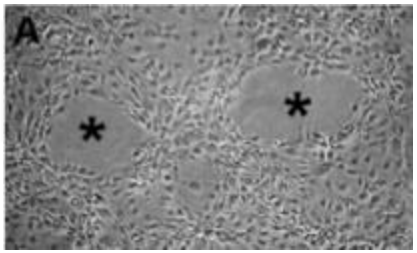
Marina Eyngor,[a] Rachel Zamostiano,[b] Japhette Esther Kembou Tsofack,[b] Asaf Berkowitz,[a] Hillel Bercovier,[c] Simon Tinman,[d] Menachem Lev,[e] Avshalom Hurvitz,[f] Marco Galeotti,[g] Eran Bacharach,[b] Avi Eldar[a]

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]









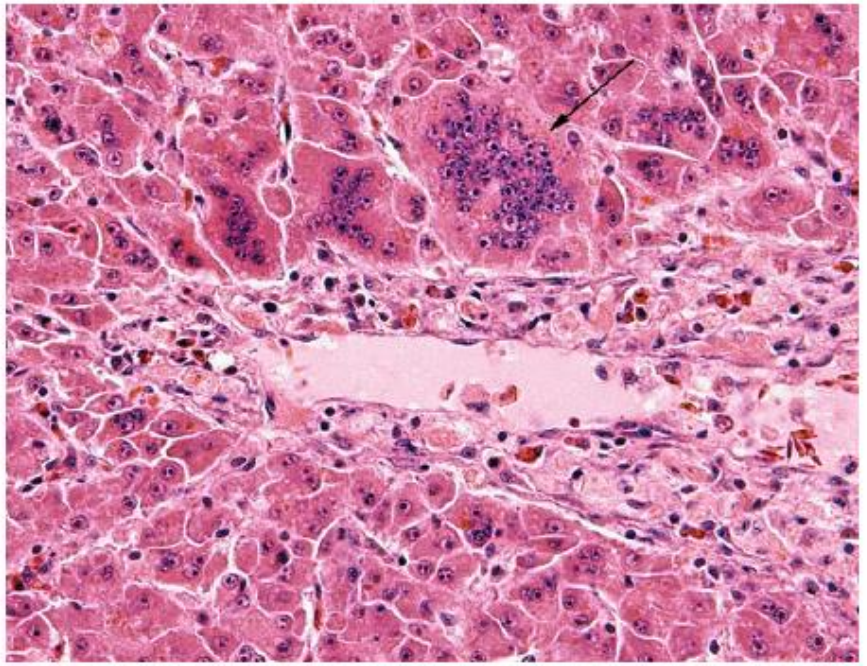
Syncytial hepatitis of farmed tilapia, *Oreochromis niloticus* (L.): a case report

Journal of Fish Diseases 2014, 37, 583–589

H W Ferguson[1], R Kabuusu[1], S Beltran[2], E Reyes[2], J A Lince[2] and J del Pozo[3]

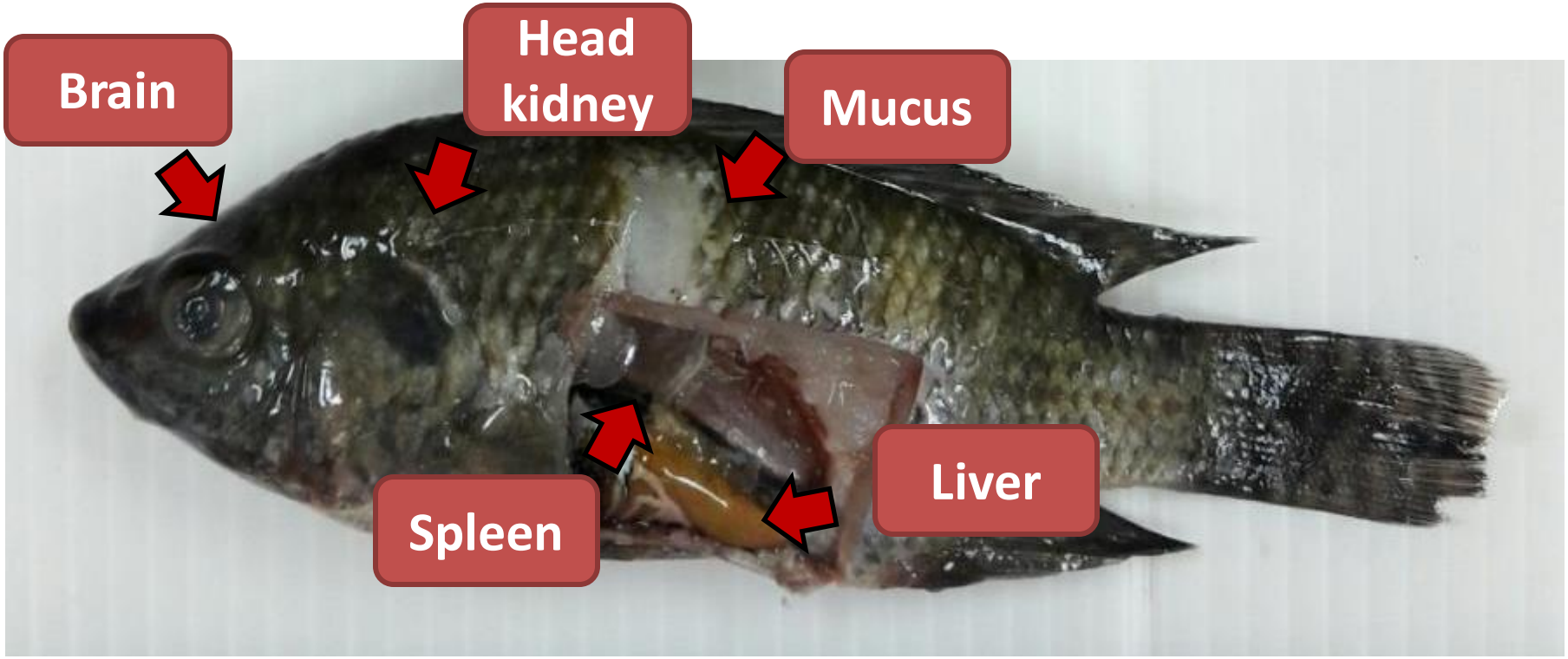
1 Marine Medicine Programme, School of Veterinary Medicine, St George's University, St George, Grenada
2 Produmar S.A., Guayaquil, Ecuador
3 Department of Pathology, Royal (Dick) School of Veterinary Medicine, University of Edinburgh, Edinburgh, Scotland, UK

Ascites



Sample collection for diagnostic procedures

Organs be used for diagnosis



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Classical techniques

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- **Haemagglutination inhibition test**
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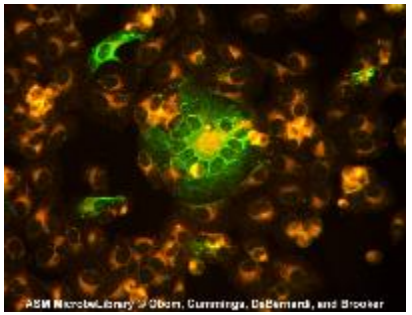
Advanced techniques

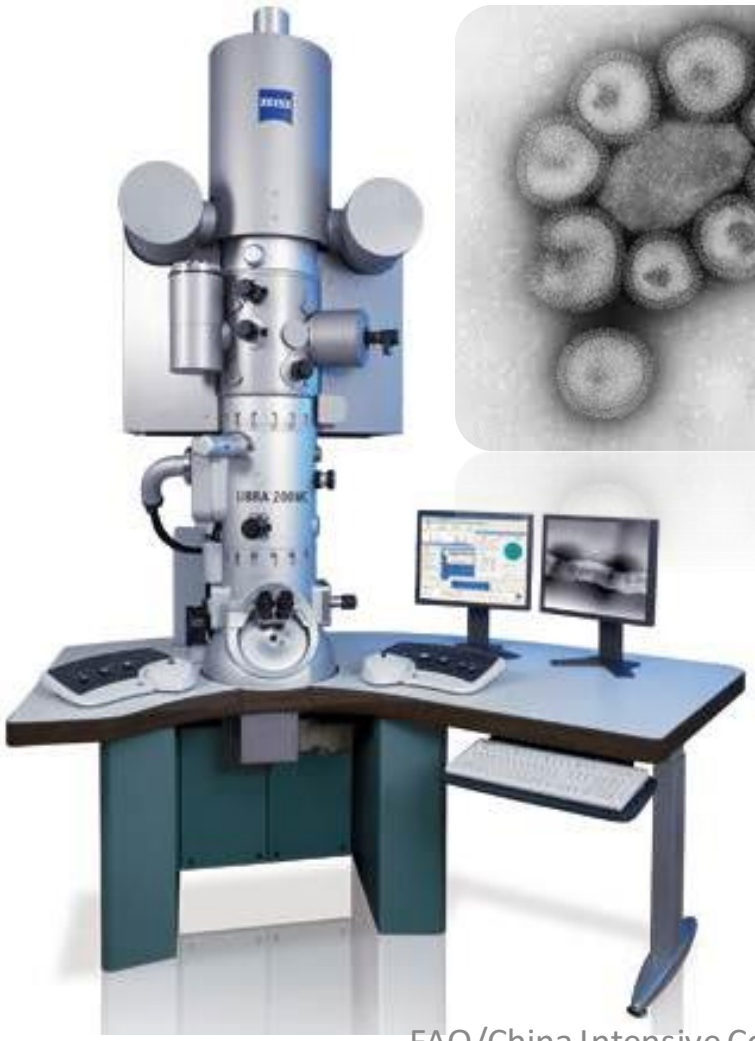
- **Immunoassay (ELISA etc.)**
- **Western blot**

Direct Examination

1. Electron microscopy
2. Light microscopy
3. Immunofluorescence
4. Molecular techniques

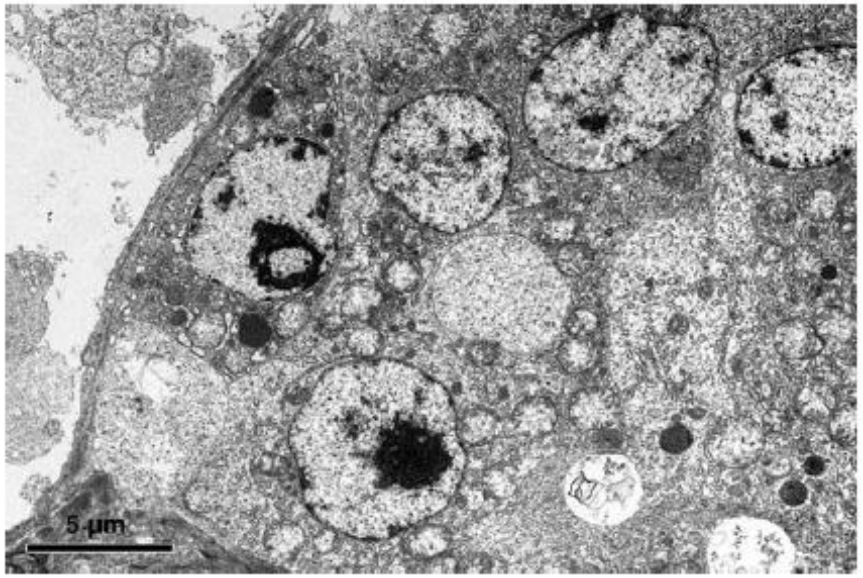


1. Transmission electron microscopy

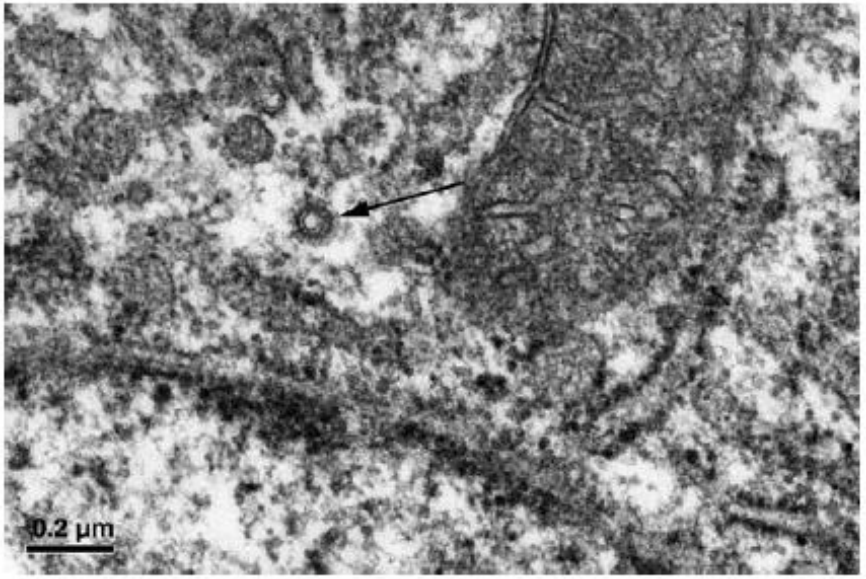


- Examination of virus preparations in which their **morphological identity** is unknown
- A beam of electrons is transmitted through an ultra thin specimen → An image is formed, magnified, and focused in an imaging device

SHV morphology
Transmission electron microscopy



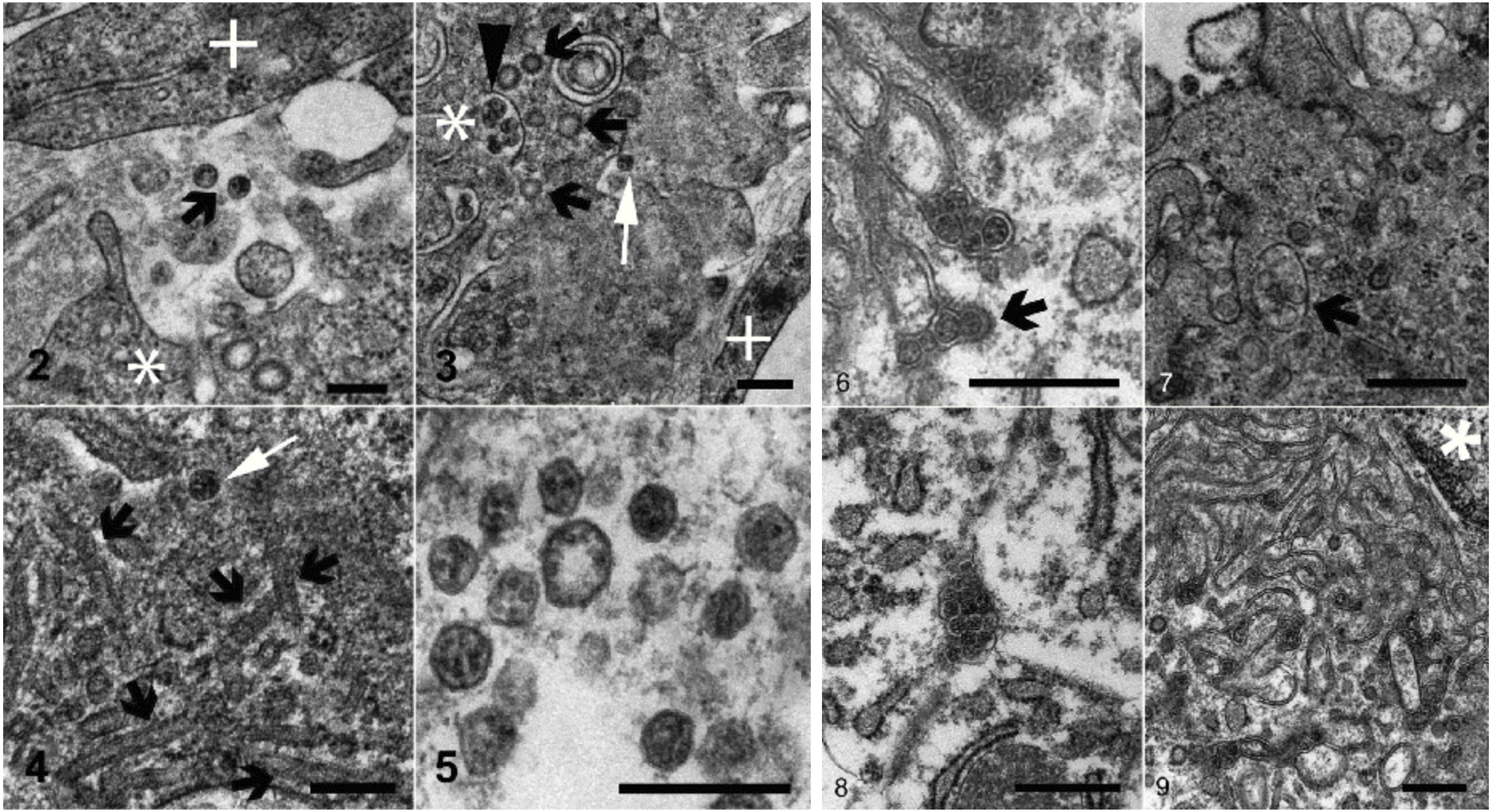
Liver from moribund tilapia showing multinucleated hepatocyte



Electron micrograph of hepatocyte from moribund tilapia showing typical appearance of virus-like particle within cytoplasm (arrow)

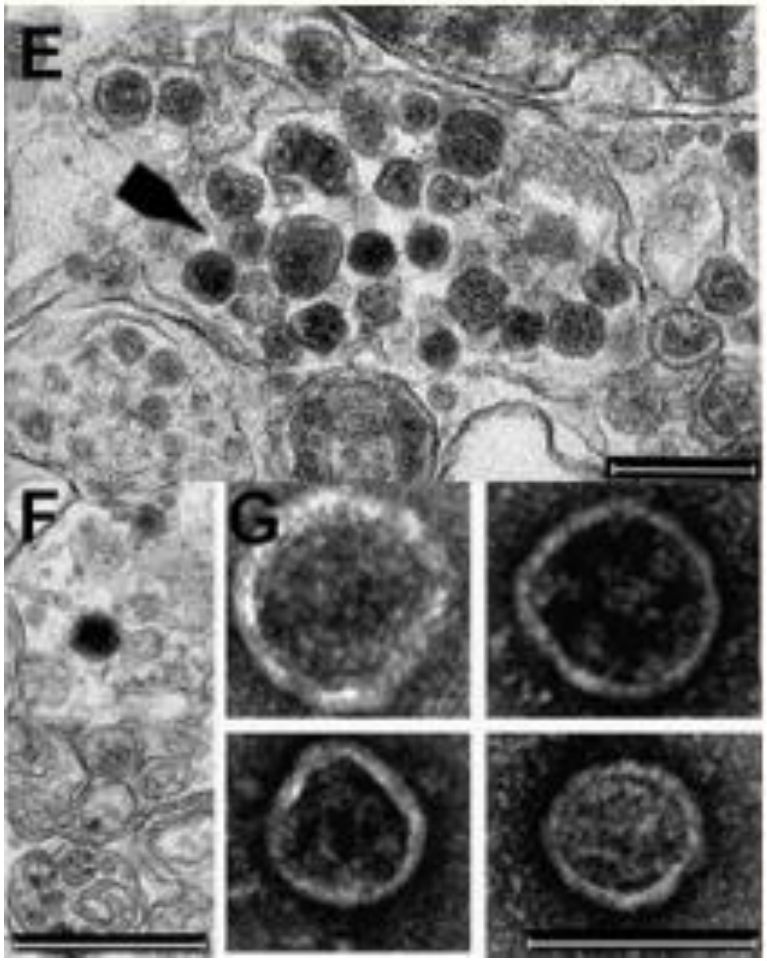
Ferguson et al., 2014 J Fish Dis.

TiLV morphology
Transmission electron microscopy

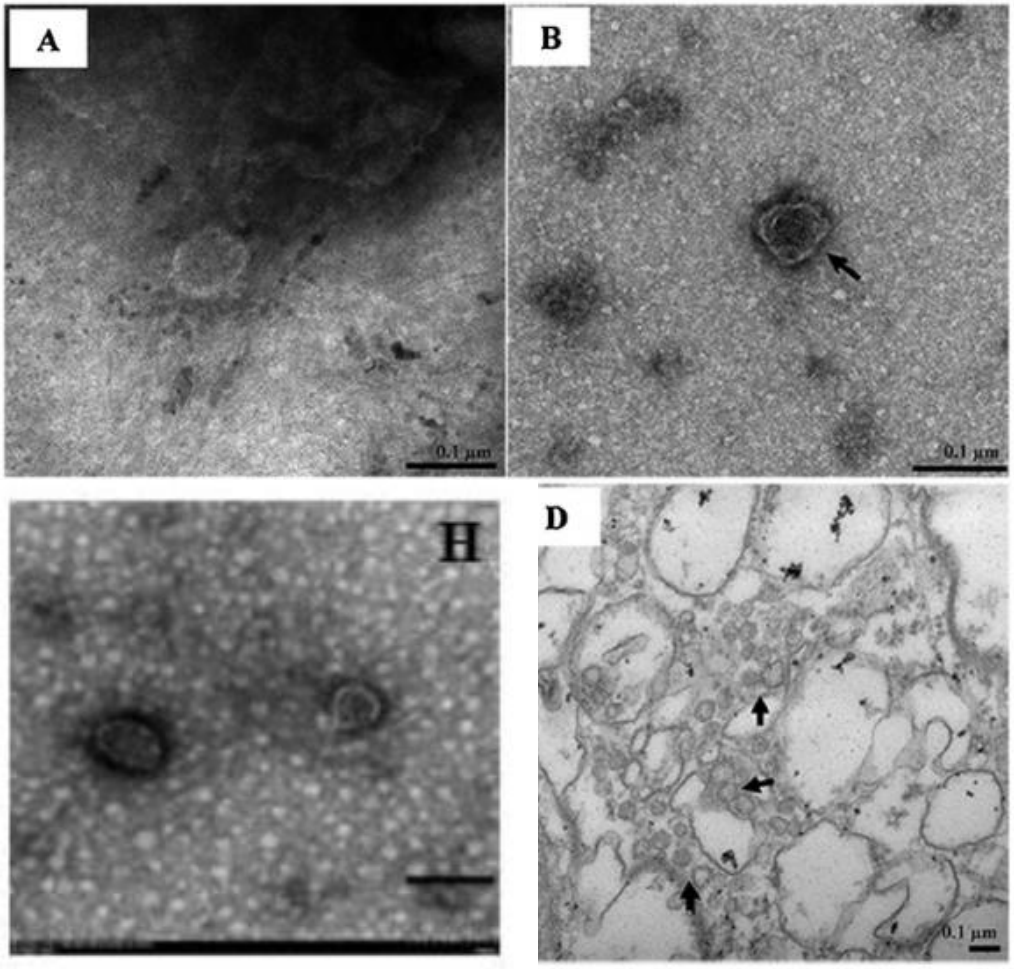


Del-Pozo et al., 2016 Vet. Pathol.

TiLV morphology
Transmission electron microscopy



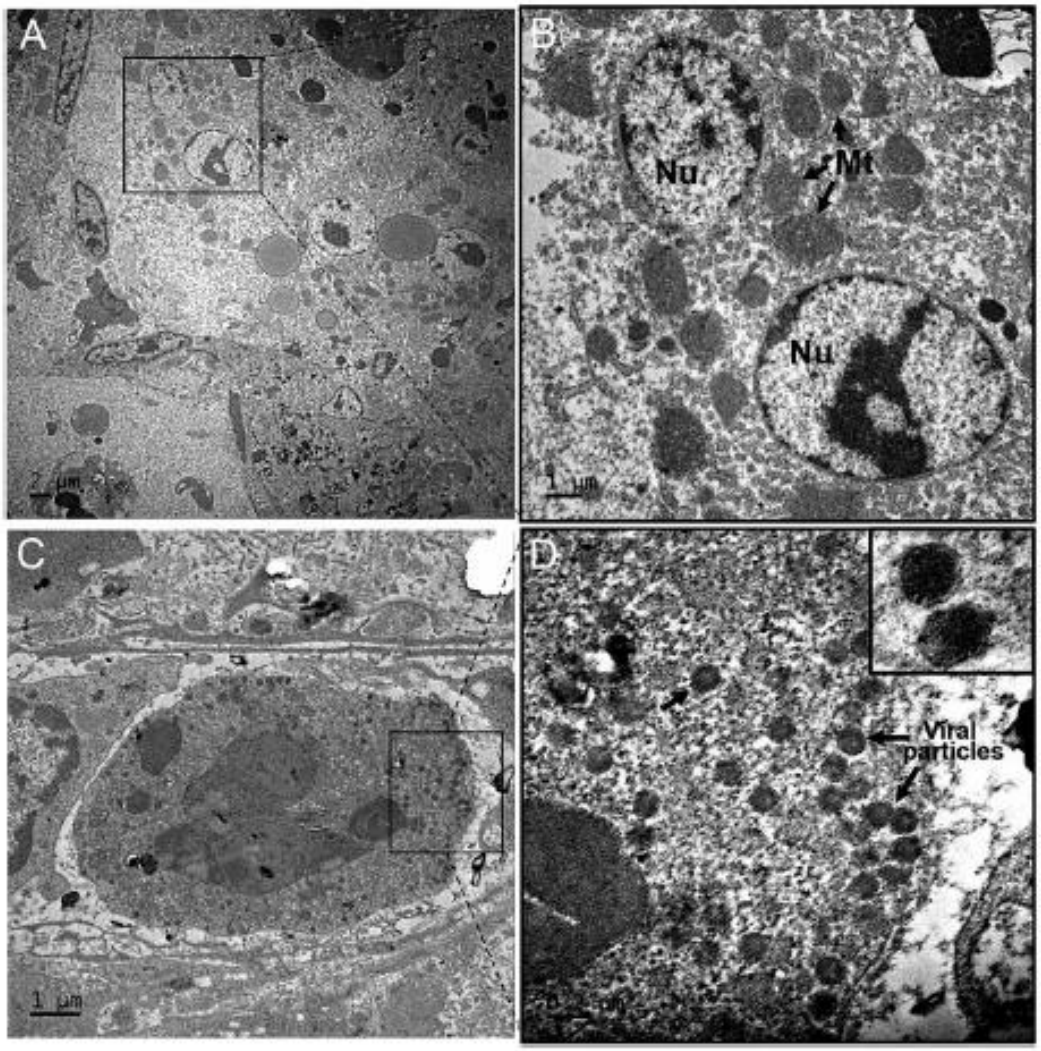
Eyngor et al 2014



Surachetpong et al 2014

Tattiyapong et al 2017

TiLV morphology
Transmission electron microscopy



A Ultrastructure of liver from diseased tilapia showing multinuclear hepatocytes

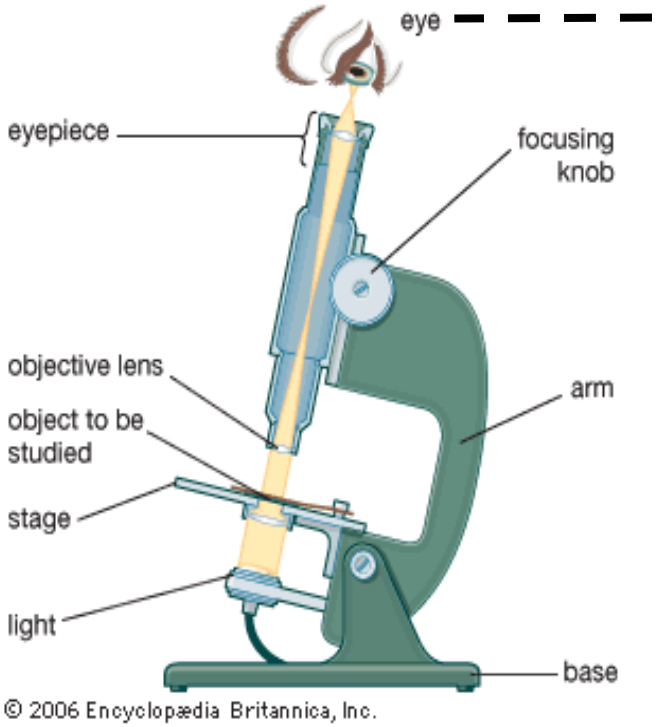
B numerous swollen mitochondria within cytoplasm

C Infected cell contained typical viral particles in cytoplasm

D Intracytoplasmic virions observed showing round-shaped particles with diameter around 100 nm

Dong et al 2017

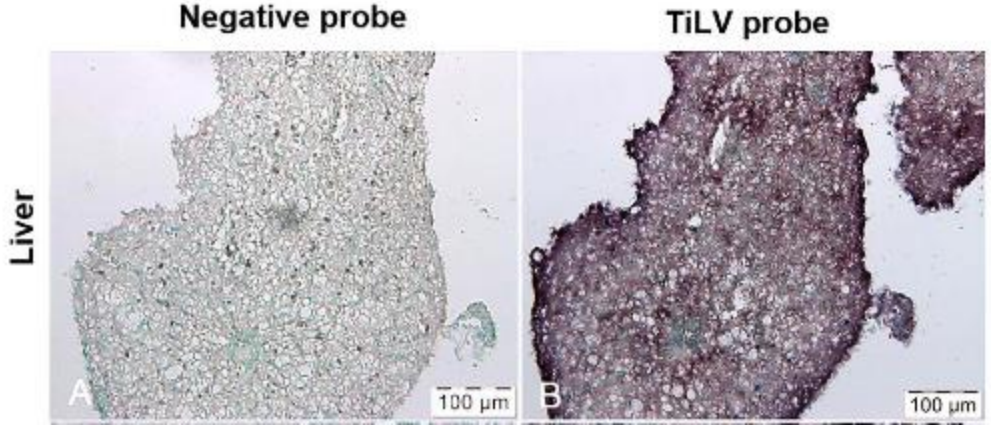
2. Light microscopy



- **Viral detection**
- **Microscopic pathological examination**

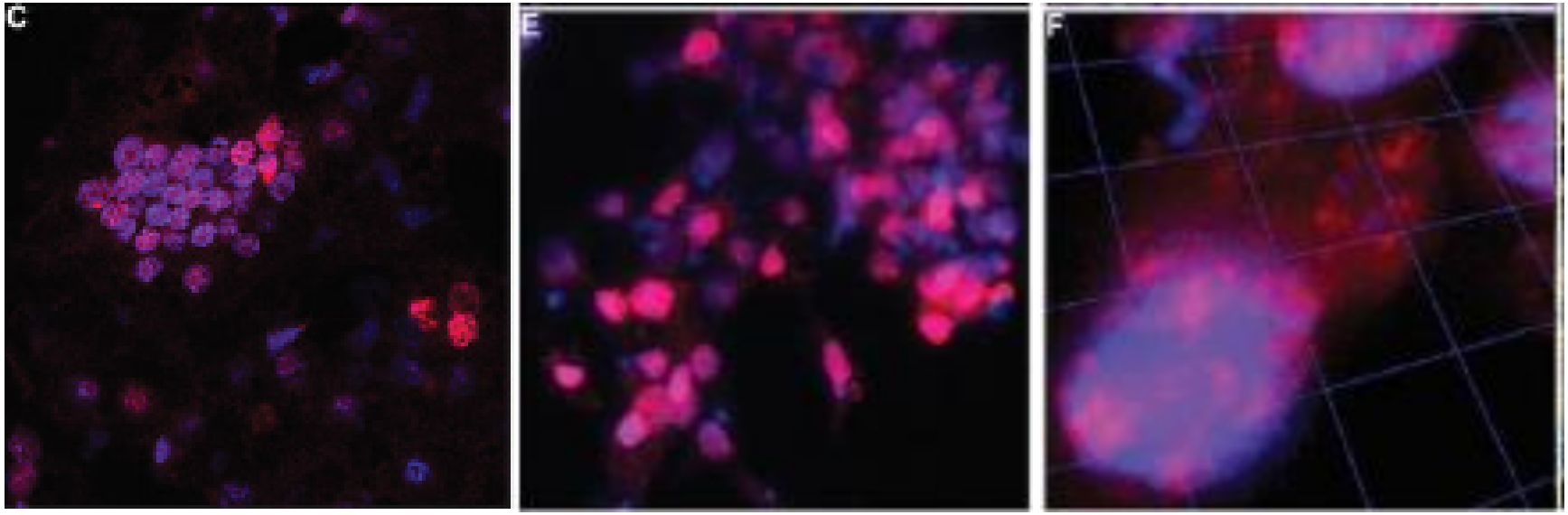
In situ hybridization

Bacharach et al 2016



Dong et al 2017

In situ hybridization with fluorescence probes



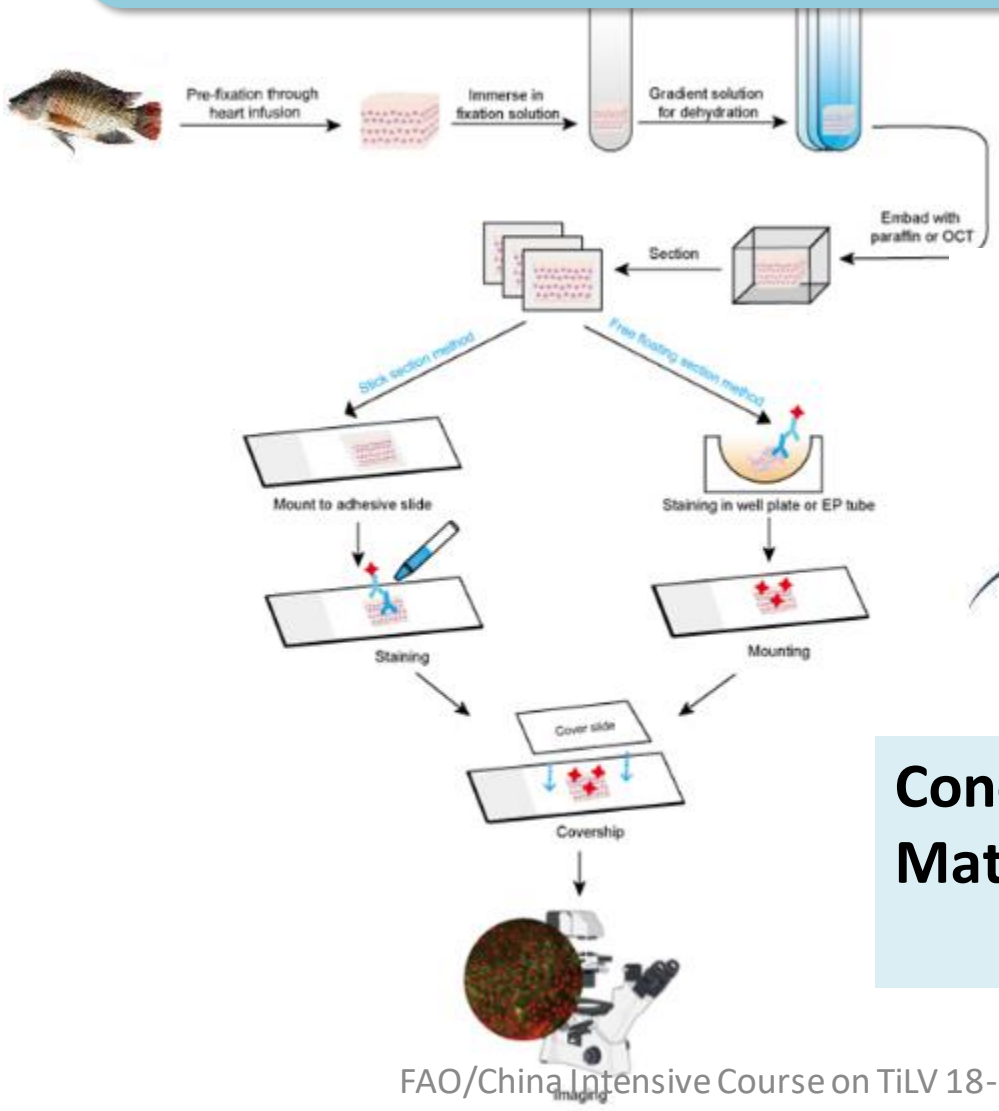
Liver sections
Cy3-conjugated (red) Stellaris probes to segment 3 to detect mRNA
Nuclei are stained with DAPI (blue).

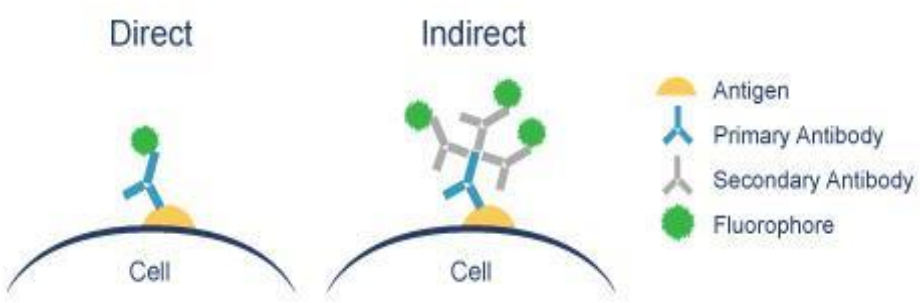
TiLV-infected E-11 cells
Quasar 670-conjugated (red) Stellaris probe to segment 3 to detect TiLV mRNA.
Nuclei are stained with DAPI (blue).

Images of confocal sections of cells in panel E were reconstituted into a 3D image.

Bacharach et al., 2016 MBIO

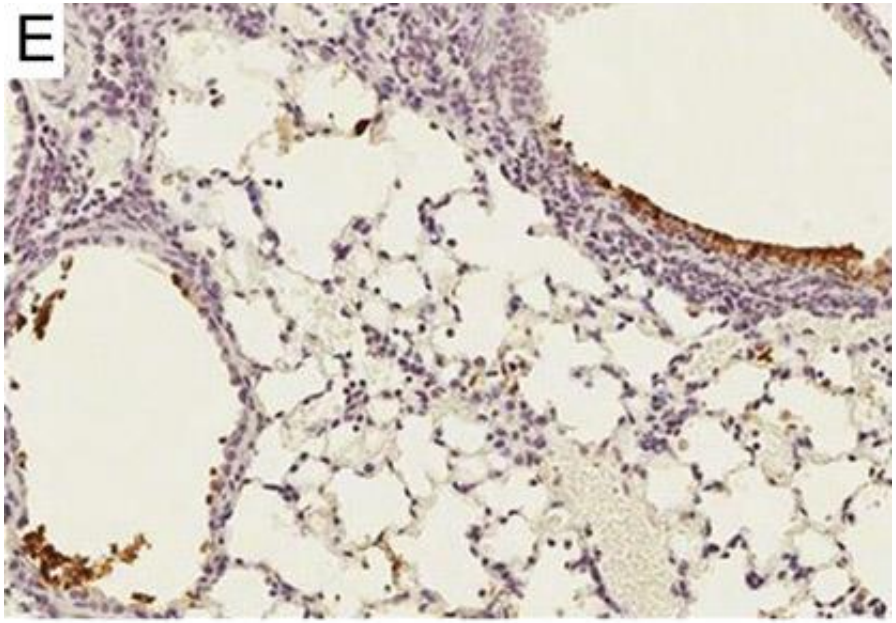
3. Immunofluorescence Immunohistochemistry staining

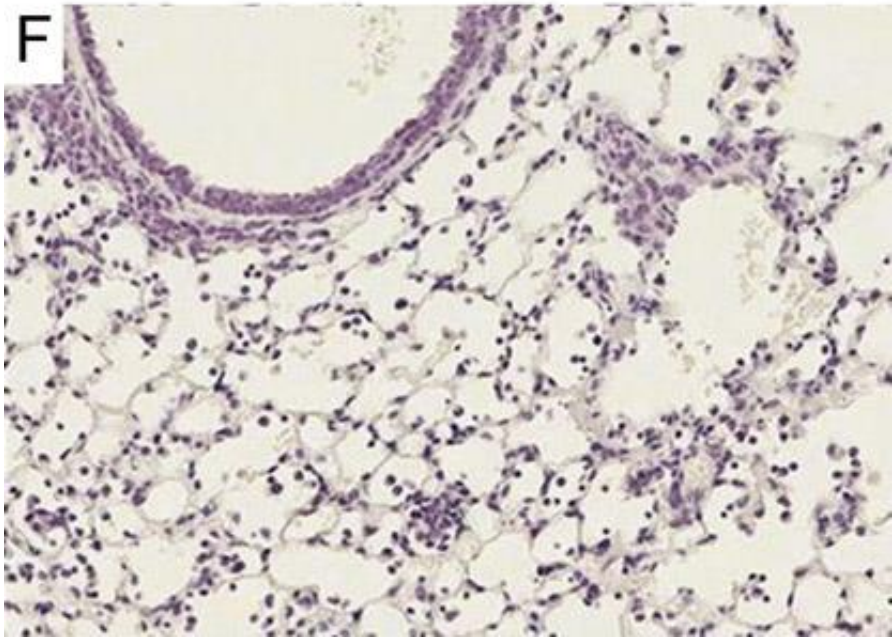




Concept: detection of antigen
Material: frozen tissue, cells (smear) or cultured cells

IHC staining of influenza A virus in mouse lung tissue





Brown staining indicates positive staining in cell's cytoplasm and/or nucleus.

Kash et al., 2013
Free Radic Biol Med.

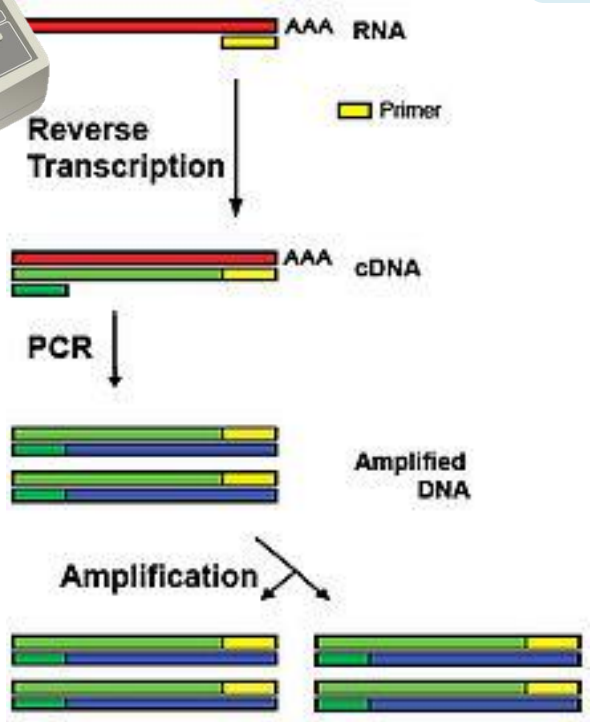
4. Molecular techniques

- **Reverse transcription polymerase chain reaction (RT-PCR)**
- **Quantitative reverse transcription polymerase chain reaction (RT-qPCR)**
- **Insulated isothermal PCR (iiPCR)**

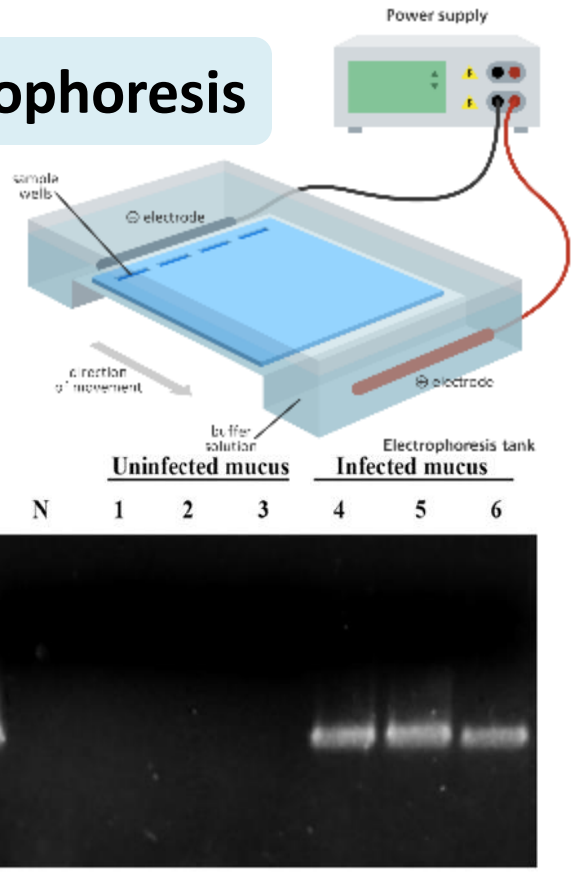
Reverse transcription polymerase chain reaction (RT-PCR)

TiLV = negative sense RNA genome

Thermo cycler



Gel electrophoresis



Reverse transcription polymerase chain reaction (RT-PCR)

- **Several PCR methods have been described for the detection of TiLV including**
  - **RT-PCR, RT-qPCR, *in situ* hybridization**
- **Recently, a SYBR green-based RT-qPCR method targeting the same segment was developed with a reported sensitivity of 2 copies**

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Journal of Clinical Microbiology p. 4137–4146

Identification of a Novel RNA Virus Lethal to Tilapia

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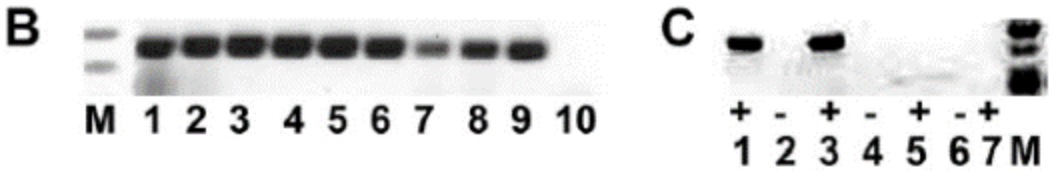
RT-PCR method based on the design of segment 3 of TiLV

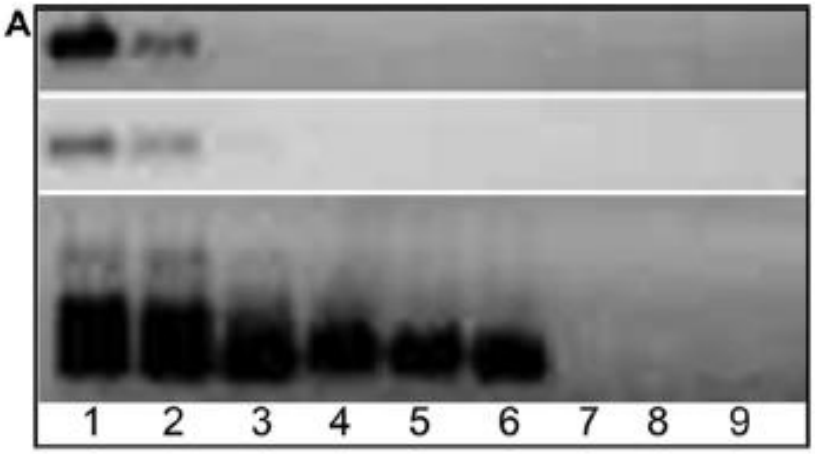


RT-PCR method based on the design of segment 3 of TiLV

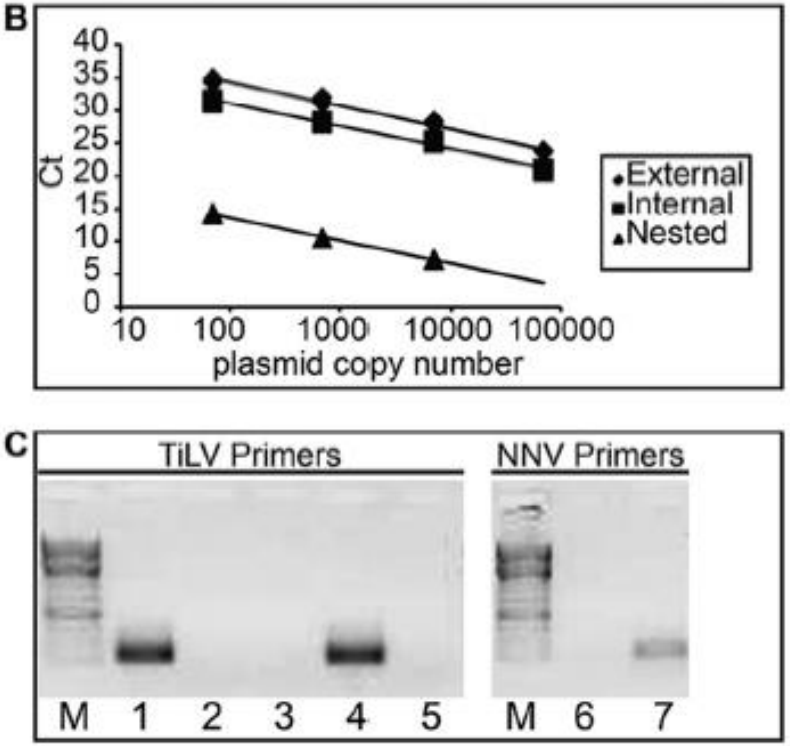
Detection of Tilapia Lake Virus in Clinical Samples by Culturing and Nested Reverse Transcription-PCR

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

Japhette Esther Kembou Tsofack,[a] Rachel Zamostiano,[a] Salsabeel Watted,[b] Asaf Berkowitz,[b] Ezra Rosenbluth,[b] Nischay Mishra,[c] Thomas Briese,[c] W. Ian Lipkin,[c] Richard M. Kabuusu,[d] Hugh Ferguson,[d] Jorge del Pozo,[e] Avi Eldar,[b] Eran Bacharach[a]

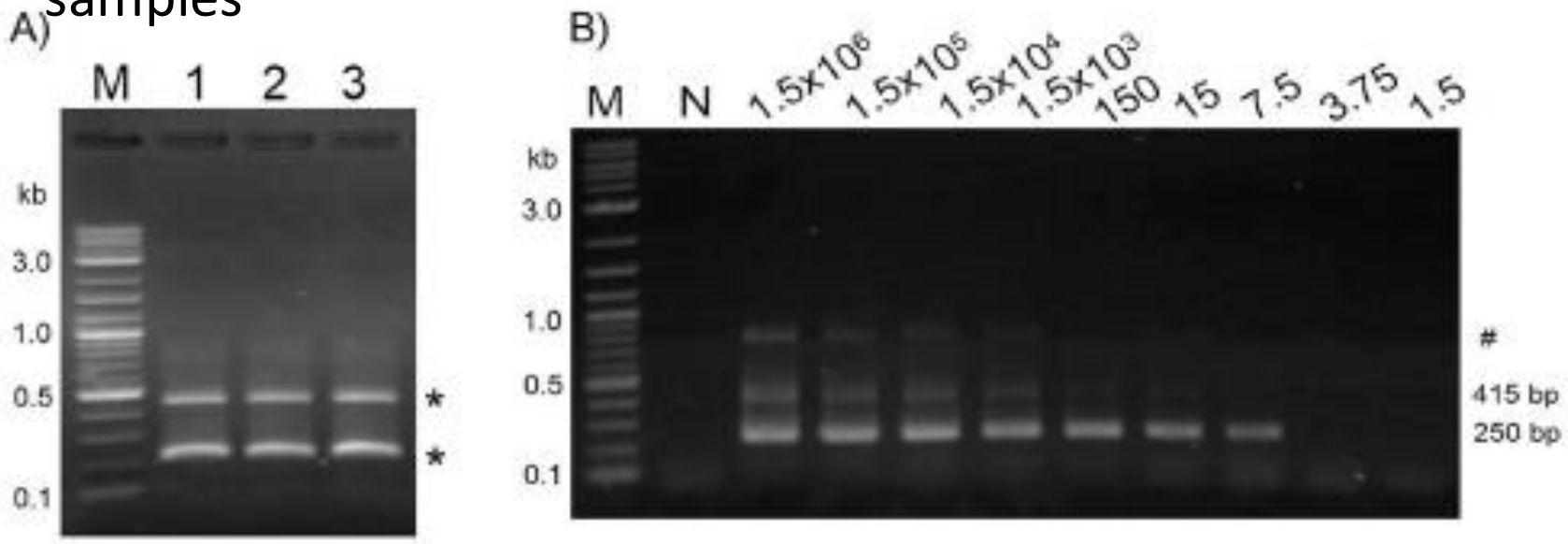


Sensitivity of PCR, nested PCR (491 bp plasmid)



RT-PCR method based on the design of segment 3 of TiLV

Semi-nested RT-PCR for TiLV detection of clinical sick fish samples



Three nested RT-PCR reactions run at an annealing temperature 56 °C non-specifically amplified fish mRNA

The detection sensitivity assay from 3 replicates using newly modified semi-nested RTPCR.

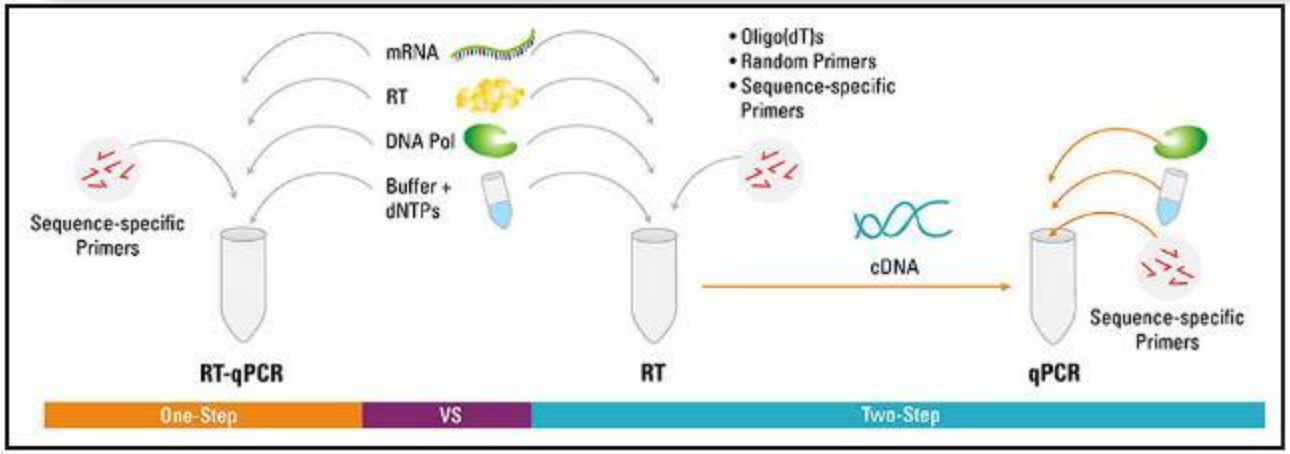
#Marks band probably derived from cross hybridizations of the amplified products. M, DNA marker

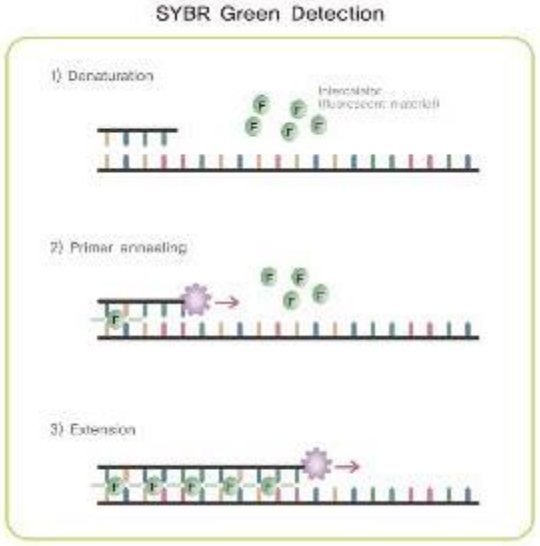
Serial dilutions of positive control plasmid (pGEM-415_bp) are indicated. Expected band sizes of 415 bp and 250 bp represent amplicons from the first and seminested.

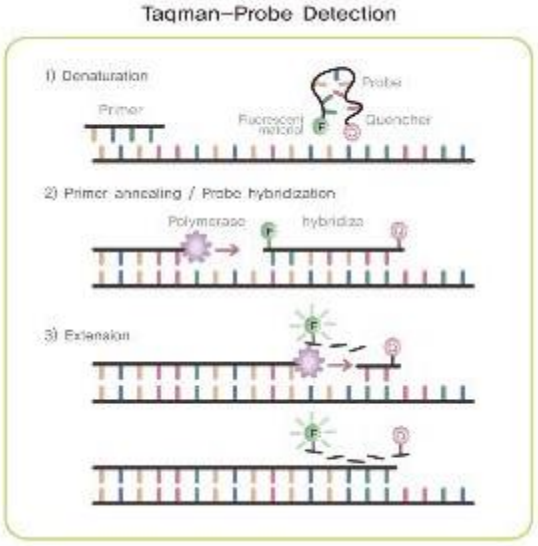
Expected band sizes of 415 bp and 250 bp represent amplicons from the first and seminested PCR

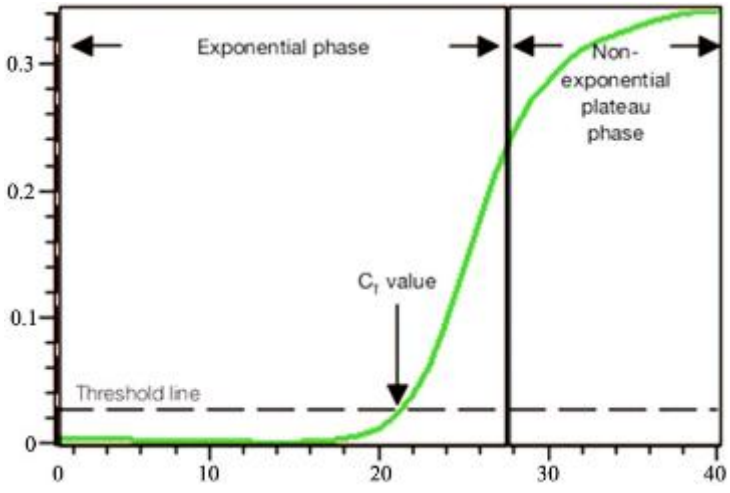
Dong et al 2017

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)







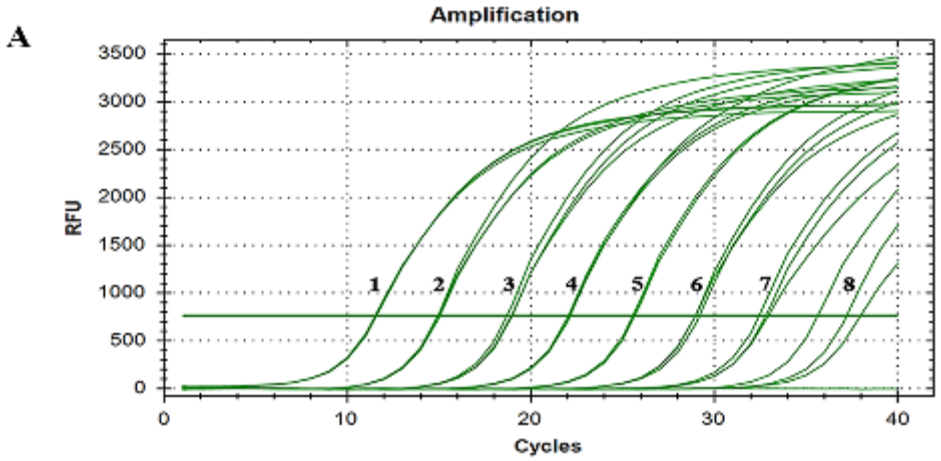


Received: 1 June 2017 | Revised: 20 July 2017 | Accepted: 23 July 2017
DOI: 10.1111/jfd.12708

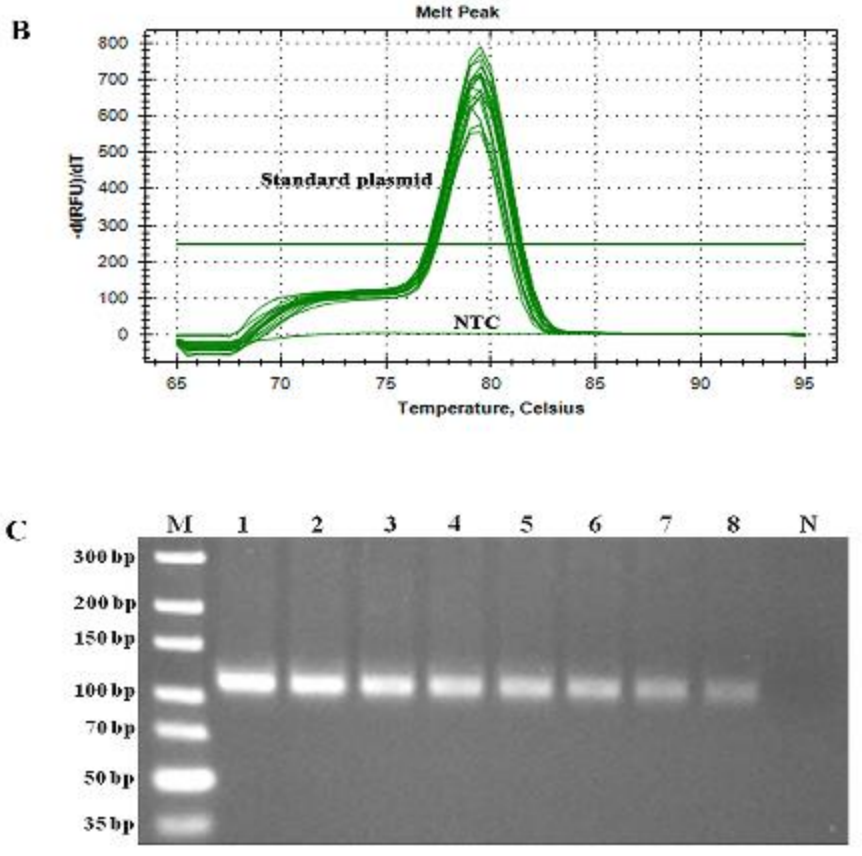
Journal of Fish Diseases

Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

P Tattiyapong[1,2] | K Sirikanchana[3,4] | W Surachetpong[1,2]



Amplification curve of serial ten-fold diltuion



Melt curve and gel electrophoresis

Validation of qPCR assay with field collected samples

Journal of Fish Diseases

Supplementary Table 1 List of field samples and estimated TiLV copies in liver

Sample No.	Collection date	Locations	Mean Ct values	Viral loads[a]
1	30/10/2015	Ang Thong	23.52	1.06×10^{4}
2	11/11/2015	Ang Thong	22.75	1.77×10^{4}
3	05/01/2016	Pathum Thani	24.70	4.75×10^{3}
4	19/01/2016	Ratchaburi	22.37	2.28×10^{4}
5	02/08/2016	Pathum Thani	15.21	2.80×10^{6}
6	05/08/2016	Pathum thani	26.66	1.28×10^{3}
7	16/08/2016	Ratchaburi	13.31	9.95×10^{6}
8	22/08/2016	Ang Thong	18.09	4.00×10^{5}
9	24/08/2016	Nakhon Pathom	13.52	8.67×10^{6}
10	27/08/2016	Nakhon Pathom	28.82	3.00×10^{2}
11	02/09/2016	Suphanburi	25.13	3.60×10^{3}
12	16/09/2016	Pathum Thani	22.30	2.39×10^{4}
13	23/09/2016	Nong Khai	13.66	7.90×10^{6}
14	02/10/2016	Pathum Thani	12.83	1.37×10^{7}
15	05/10/2016	Pathum Thani	19.46	1.61×10^{5}

Supplementary Table 1 (Cont.) List of field samples and estimated TiLV copies in liver

Sample No.	Collection date	Locations	Mean Ct values	Viral loads[a]
16	16/10/2016	Pathum Thani	19.20	1.94×10^{5}
17	25/11/2016	Ang Thong	14.56	4.35×10^{6}
18	26/11/2016	Pathum Thani	13.46	9.08×10^{6}
19	21/12/2016	Petchaburi	17.16	7.55×10^{5}
20	14/11/2016	Khon Kaen	19.45	1.62×10^{5}
21	01/01/2017	Pathum Thani	21.11	5.35×10^{4}
22	14/02/2017	Nakhon Sawan	16.72	1.01×10^{6}
23	20/02/2017	Uthai Thani	13.26	1.04×10^{7}
24	24/02/2017	Phitsanulok	27.84	5.85×10^{2}
25	24/02/2017	Uttaradit	32.89	1.97×10^{1}
26	25/02/1017	Ratchaburi	25.39	3.01×10^{3}
27	25/02/2017	Nakhon Si Thammarat	19.87	1.22×10^{5}
28	25/02/2017	Prachinburi	16.21	1.43×10^{6}
29	25/02/2017	Nakhon Nayok	14.36	4.98×10^{6}
30	25/02/2017	Ang Thong	18.76	2.55×10^{5}

Standard curve of SYBR green-based RT-qPCR

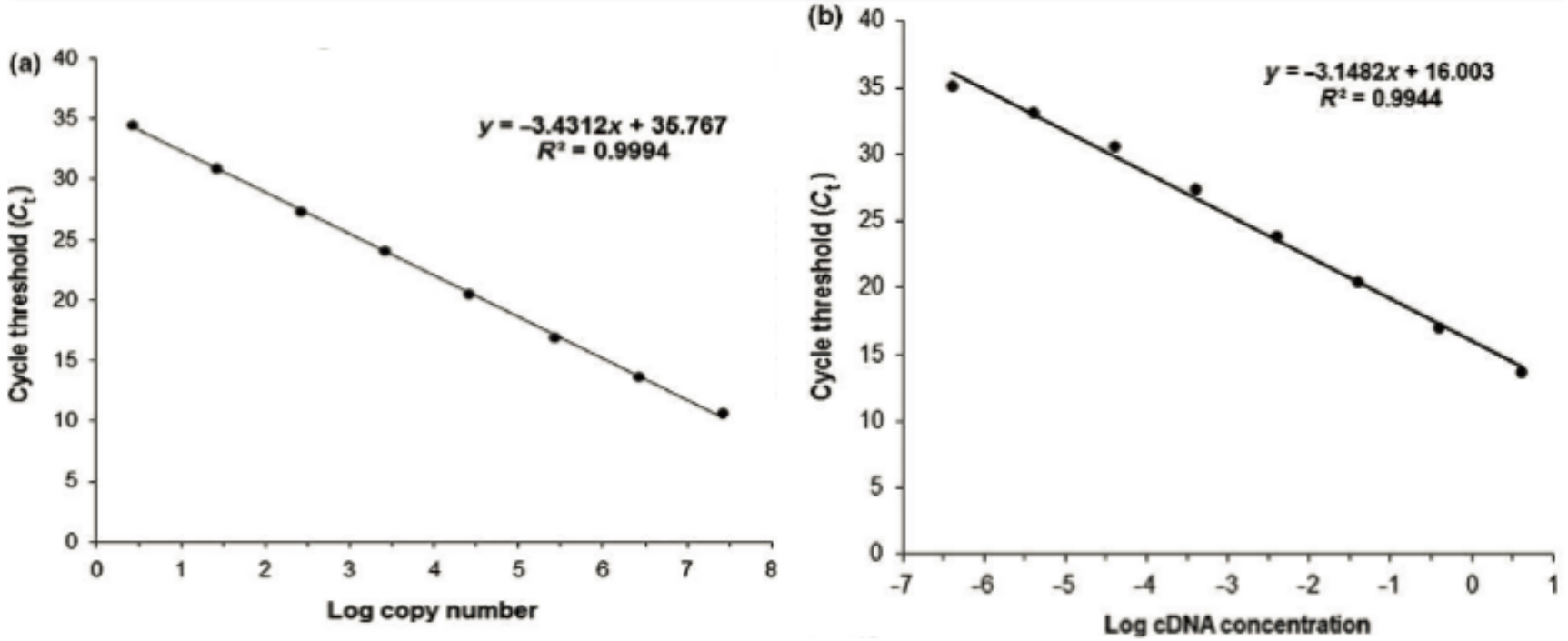


FIGURE 1 Standard curve of SYBR green-based RT-qPCR amplification of plasmid pTiLV containing segment three of TiLV and infected tissue. (a) Standard curve was plotted between mean Ct values obtained from each dilution of standard plasmid pTiLV against calculated log copy number (slope = -3.4312, R^2 = 0.9994). (b) Standard curve of cDNA prepared from TiLV-infected fish tissue showed slopes = -3.1482, R^2 = 0.994

Tattiyapong et al., 2018 J. Fish Dis.

Received: 1 June 2017 | Revised: 20 July 2017 | Accepted: 23 July 2017
DOI: 10.1111/jfd.12708

Journal of Fish Diseases

Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

P Tattiyapong[1,2] | K Sirikanchana[3,4] | W Surachetpong[1,2]

Table 3 Analysis of viral loads in different tissues

Sample No.	Viral loads (copies μg^{-1} of total RNA)					
	Gills	***Liver***	***Brain***	***Heart***	***Anterior kidney***	***Spleen***
1	2.2×10^5	1.7×10^5	3.4×10^5	6.3×10^5	1.6×10^5	2.3×10^4
2	3.1×10^5	6.3×10^3	1.3×10^6	3.9×10^5	3.1×10^5	2.8×10^4

Received: 1 June 2017 | Revised: 20 July 2017 | Accepted: 23 July 2017
DOI: 10.1111/jfd.12708

Journal of Fish Diseases

Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

P Tattiyapong[1,2] | K Sirikanchana[3,4] | W Surachetpong[1,2]

Table 4 Comparison of RT-qPCR, conventional RT-PCR and virus isolation in cell culture

Detection method	Template dilution							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
RT-qPCR	+	+	+	+	+	+	+	-
Conventional RT-PCR	+	+	+	+	+	-	-	-
Virus isolation in cell culture	+	+	+	-	-	-	-	-

Detection of TiLV in clinical samples using RT-qPCR method

Fish samples	Number of samples	TiLV positive (%)	Mean Ct values (range)	Estimated viral loads (Copy numbers)[c]
Clinical samples[a]	30	30/30 (100)	22.86 (12.83 - 32.89)	1.65×10^4 (1.37×10^7-1.97×10^1)
TiLV-challenged fish	10	10/10 (100)	23.65 (20.08 - 27.28)	9.72×10^3 (1.00×10^5-8.50×10^2)
Non-challenged fish	10	0/10 (0)	ND[b]	ND[b]

[a]Clinical samples were collected from 30 field outbreaks with history of massive mortality.
[b]ND = No detection.
[c]Copy numbers of TiLV template per μg of total RNA.

Tattiyapong et al., 2018 J. Fish Dis.

Contents lists available at ScienceDirect

Aquaculture

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

Non-lethal sampling for Tilapia Lake Virus detection by RT-qPCR and cell culture

Pavarit Liamnimitr[a], Worrayanee Thammatorn[a], Sonicha U-thoomporn[a], Puntanat Tattiyapong[b], Win Surachetpong[a,b,*]

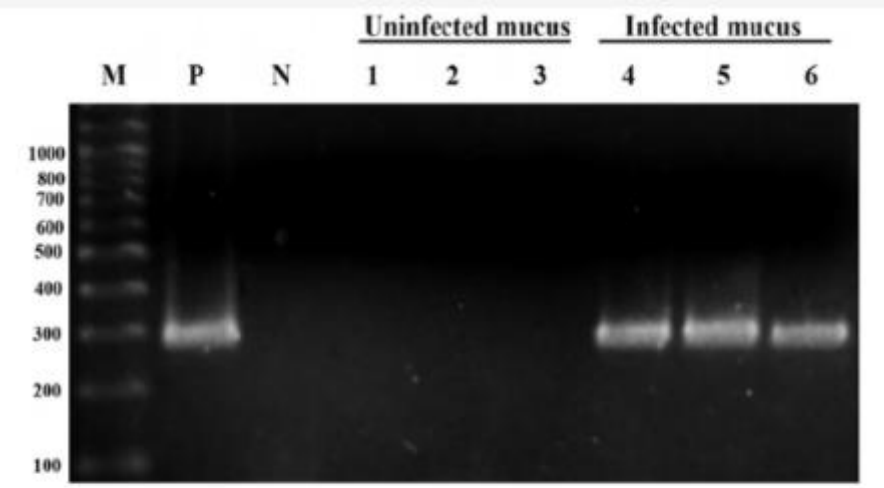
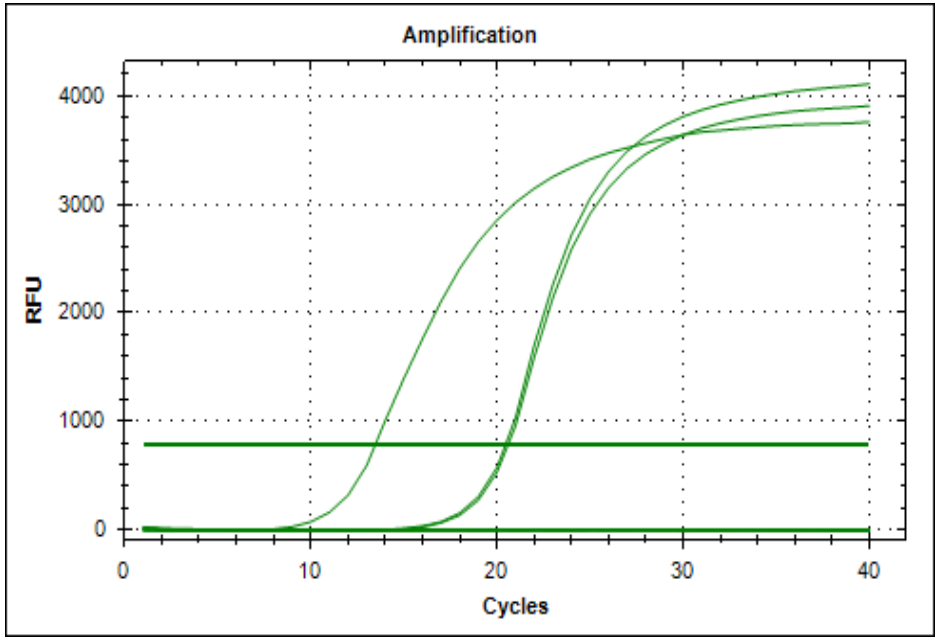


Fig. 2. Amplification of PCR products from E-11 cells inoculated with uninfected or TiLV-infected mucus. (M) 100 bp marker; P = Positive control (pTiLV), N = No template control, Lanes 1–3; E-11 inoculated with mucus from normal fish (uninfected mucus), Lanes 4–6; E-11 inoculated with mucus from TiLV-positive fish (infected mucus). The uninfected and infected mucus analysis was based on three clinical samples representing different geographic locations.



On farm diagnostic PCR for TiLV detection

- **A commercial pond-site TiLV RT-PCR detection assay based on insulated isothermal PCR (iiPCR) is available**
- **POCKIT™ Micro (GeneBeach Biotechnology Corp.)**
- **Assay can be completed in 45 min.**
- **Rapid, inexpensive, sensitive, easy to maintain**

POCKIT™ Micro (GeneBeach Biotechnology Corp.)

Insulated isothermal PCR (iiPCR)

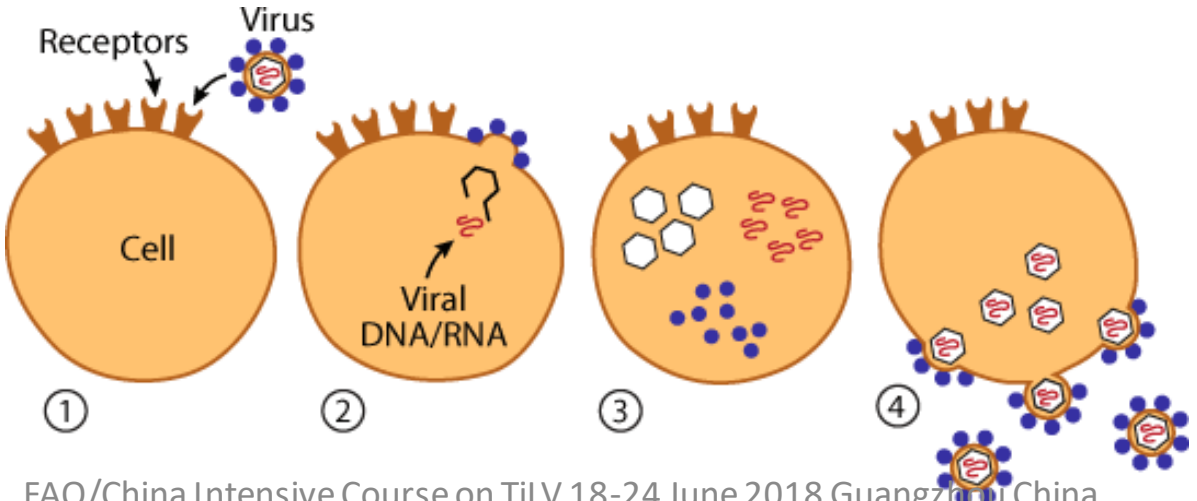
Commercial pond-site TiLV RT-PCR detection

The TiLV RT-PCR has a limit of detection LoD95% of 12 genome

www.genereach.com

Indirect Examination

1. Cell Culture methods
2. Embryonated eggs
3. Laboratory animals

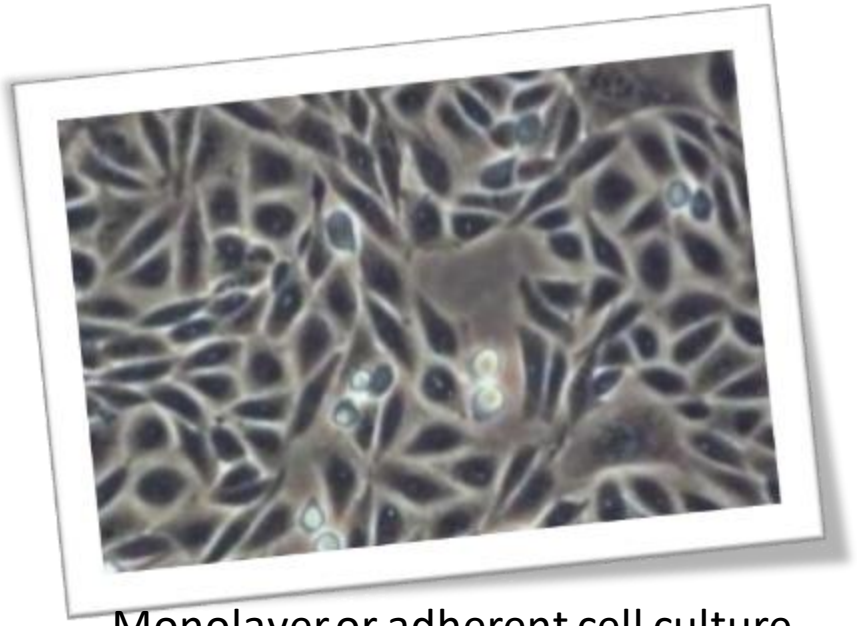


1. Cell culture methods

- Since the discovery by Enders (1949) that polioviruses could be cultured in cells, cell culture has become a very useful and convenient method for isolating viruses *in vitro*
- **Gold standard for virus isolation and identification**

Cell culture methods

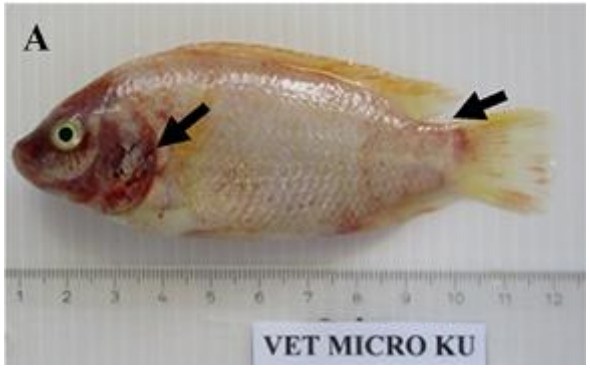
- Mainly propagate viruses in monolayer cell cultures
  - Main advantage is the ease of monitoring of infected cultures microscopically



Monolayer or adherent cell culture

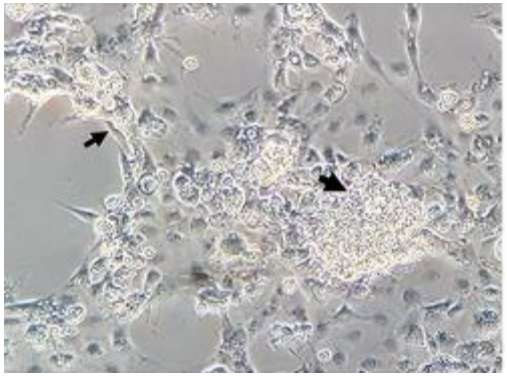
Suspension cell culture

TiLV viral isolation



Clinical specimens
- Organ and tissue
- Mucus

Virion isolation and cultivation in cell culture

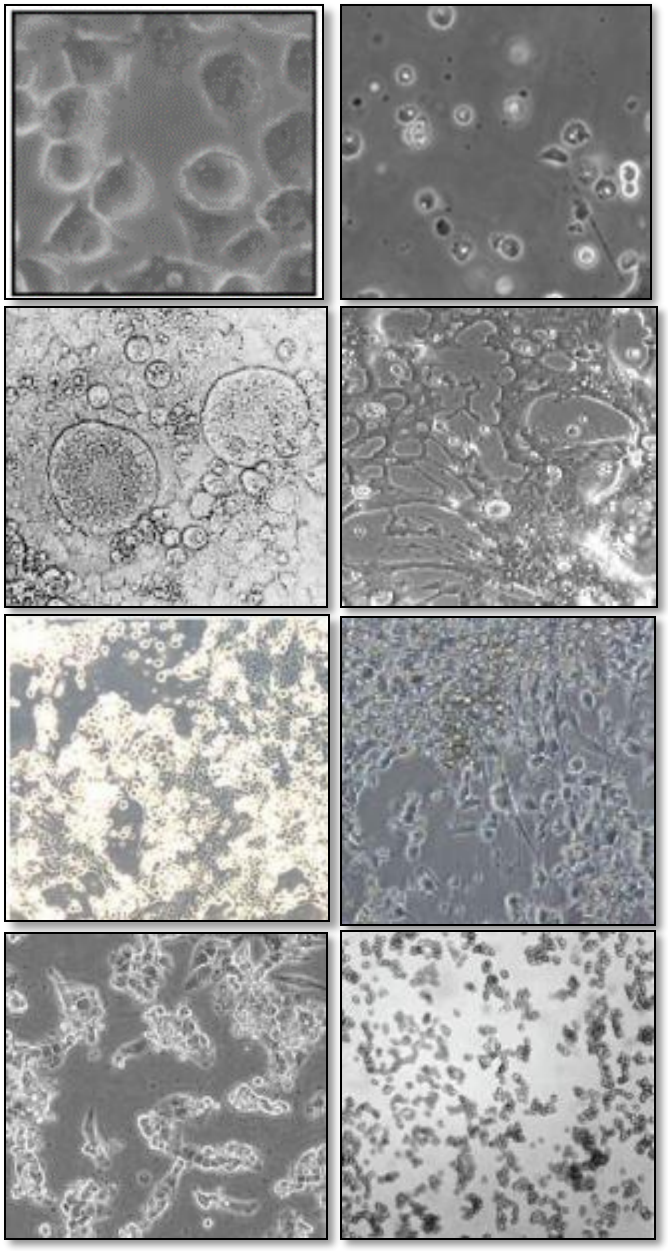


Observe CPE in viral replicated cells

Cytopathic effects (CPE)

Morphological changes occurring in viral infection

1. Rounding
2. Detachment
3. Syncytia or fusion
4. Shrinkage
5. Increased refractivity
6. Aggregation
7. Loss of adherence
8. Cell lysis or death



2. Embryonated eggs

- First used by Goodpasture and Burnet in 1931 for the cultivation of virus
- The process of cultivation depends on the type of egg being used
- Eggs provide a suitable means for
  - Primary isolation and of viruses
  - Maintenance of stock cultures
  - Production of vaccines

3. Laboratory animals

- Play an essential role in the studies of viral pathogenesis
- Routes of viral inoculation
  - Intracerebral
  - Subcutaneous
  - Intraperitoneal
  - Intranasal
- After inoculation, the animal is:
  - Observed for signs of disease or visible lesions
  - Euthanized so that infected tissues can be examined




Veterinary Microbiology 207 (2017) 170–177

Contents lists available at ScienceDirect

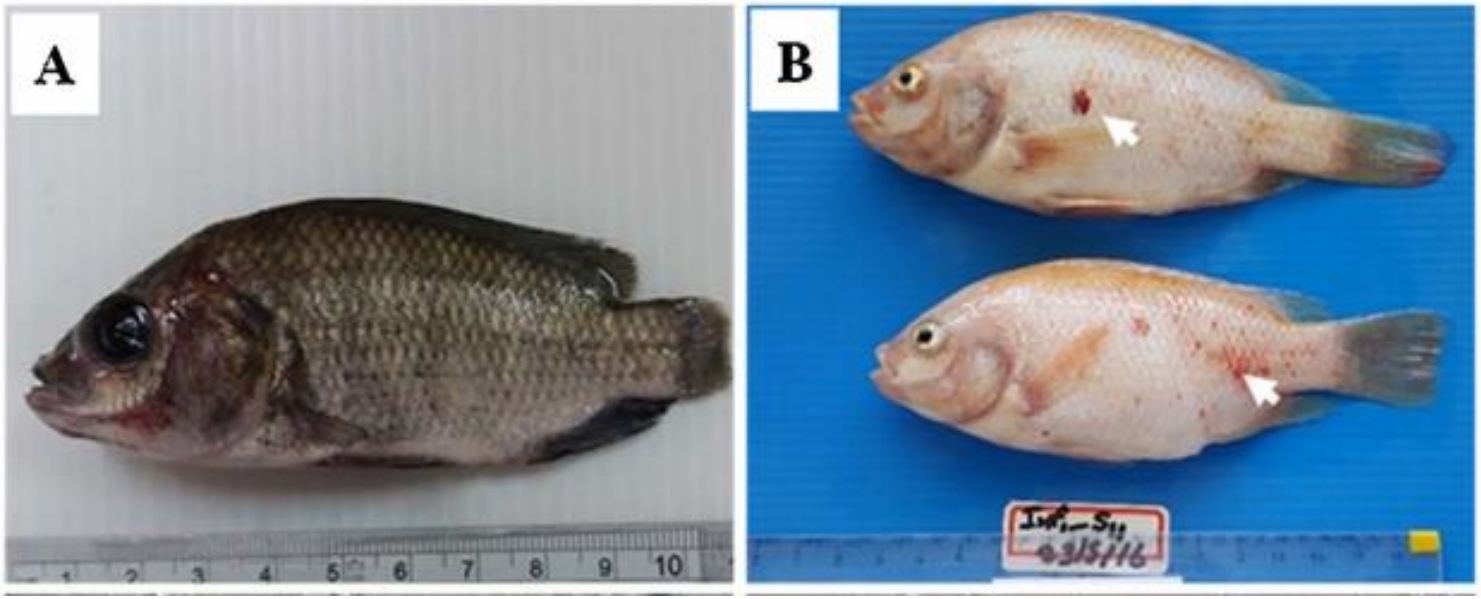
Veterinary Microbiology

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

Experimental infection of Tilapia Lake Virus (TiLV) in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis* spp.)

Puntanat Tattiyapong[a,b], Worawan Dachavichitlead[a,b], Win Surachetpong[a,b,*]

[a] Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand
[b] Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand



Serology

Classical techniques

- Complement fixation test
- Haemagglutination inhibition test
- Neutralization test

Advanced techniques

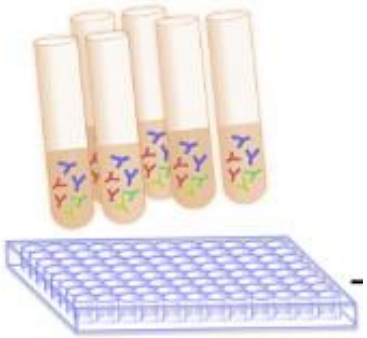
- Immunoassay (ELISA etc.)
- Western blot

Classical techniques

- **Neutralization test**
  - Detects the presence of viral neutralizing antibodies
  - Complete blockage of viral activity → No cell infection (no CPE)

Antibody detection

Patient serum (dilution)	0	0	1/1000	1/100	1/10	1
Virus concentration	0	5000 pfu	5000 pfu	5000 pfu	5000 pfu	5000 pfu
Virus concentration						
CELL CULTURE serum/virus mixture	 No virus	 CPE	 CPE	 No CPE	 No CPE	 No CPE
		Infection		Neutralization		



2-fold serial dilutions of sera

JOURNAL OF
FISH DISEASES

Original Article

Validation of a serum neutralization test for detection of antibodies specific to cyprinid herpesvirus 3 in infected common and koi carp (*Cyprinus carpio*)

J Cabon, L Louboutin, J Castric, S Bergmann, G Bovo, M Matras, O Haenen, N J Olesen, T Morin

- Neutralizing antibodies were steadily detected in infected carp subjected to restrictive or permissive temperature variations over more than 25 months post-infection. The results suggest that this non-lethal diagnostic test could be used in the future to improve the epidemiological surveillance and control of CyHV-3 disease.

CVI
Journals.ASM.org

Development and Evaluation of a Blocking Enzyme-Linked Immunosorbent Assay and Virus Neutralization Assay To Detect Antibodies to Viral Hemorrhagic Septicemia Virus

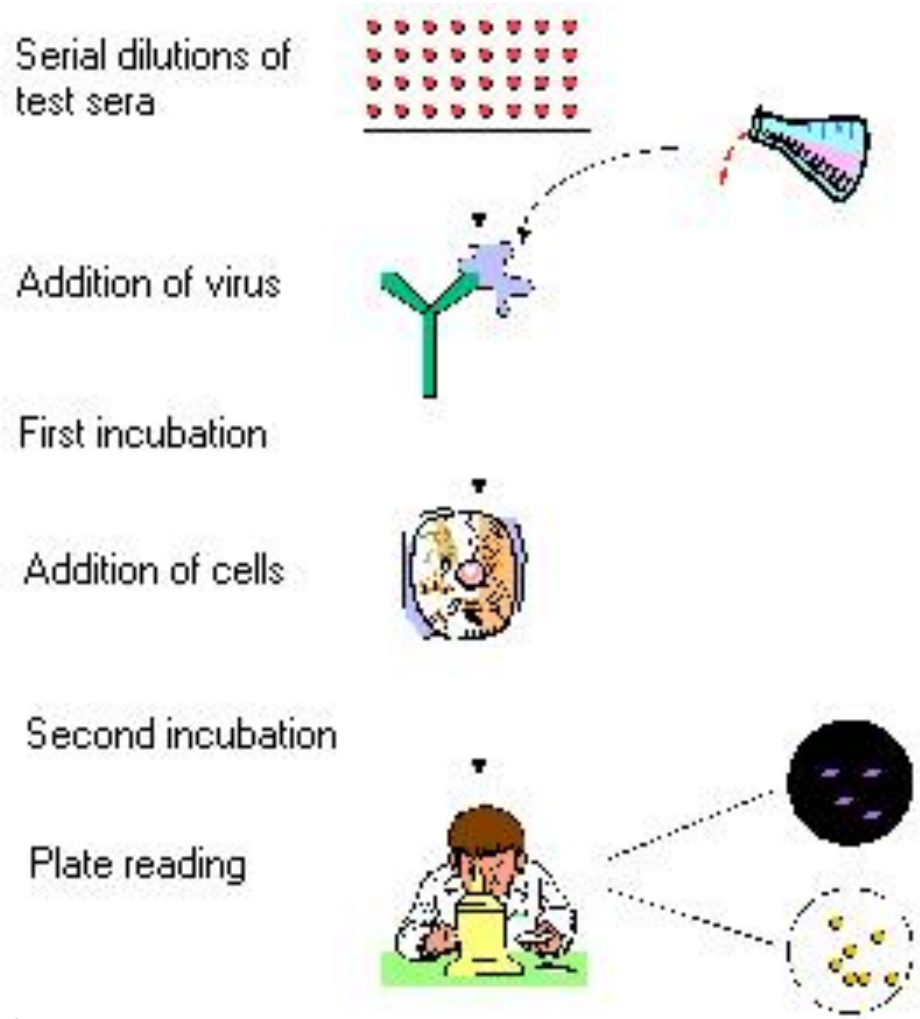
Anna Wilson,[a] Tony Goldberg,[a] Susan Marcquenski,[b] Wendy Olson,[c] Frederick Goetz,[d] Paul Hershberger,[e] Lucas Hart,[e] Kathy Toohey-Kurth[a,f]

TABLE 4 Results of VN assay[a]

VHS infection status	No. of known positives ($n = 28$)	No. of known negatives ($n = 34$)
Positive	12	0
Negative	16	34

[a] $n = 62$. The sensitivity is 42.9% and the specificity is 100%, both calculated from fish in the VHS-negative and VHS-positive groups.

Classical techniques



Neutralization test (limitation)

- The test was not very sensitive
  - Fish has predominant IgM subtype in serum (low specificity)
  - Absence of affinity maturation in B-lymphocytes

ELISA

- **Immunoassay (ELISA)**
  - Uses antibodies and color change to identify a substance (antigen)
  - Adsorb certain components onto an immobilized solid phase
  - Color development by the product of an enzymatic reaction

ELISA

Sandwich ELISA

*A detecting antibody and an enzyme-linked secondary antibody may also be used

Plate is coated with a capture antibody

Sample is added, and any antigen present binds to capture antibody

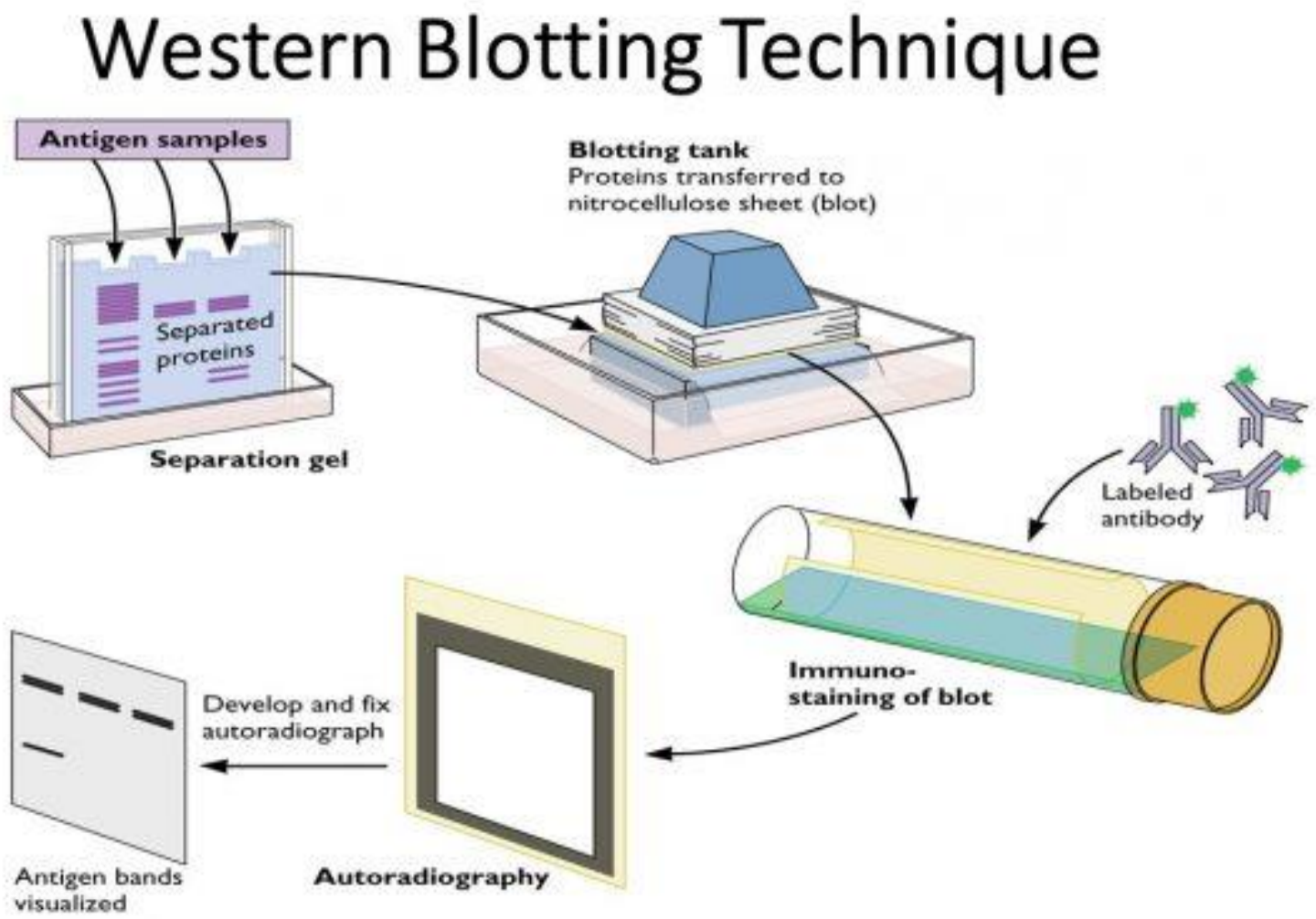
Enzyme-linked detecting antibody is added, and binds to antigen

Substrate is added, and is converted by enzyme to detectable form

Advanced techniques

- **Western blot**
  - Identification of particular protein from a sample
  - Uses antibodies to detect target protein/antigen (Immunoblotting)

Advanced techniques



Rabbit or Duck?

You see what you want to see!

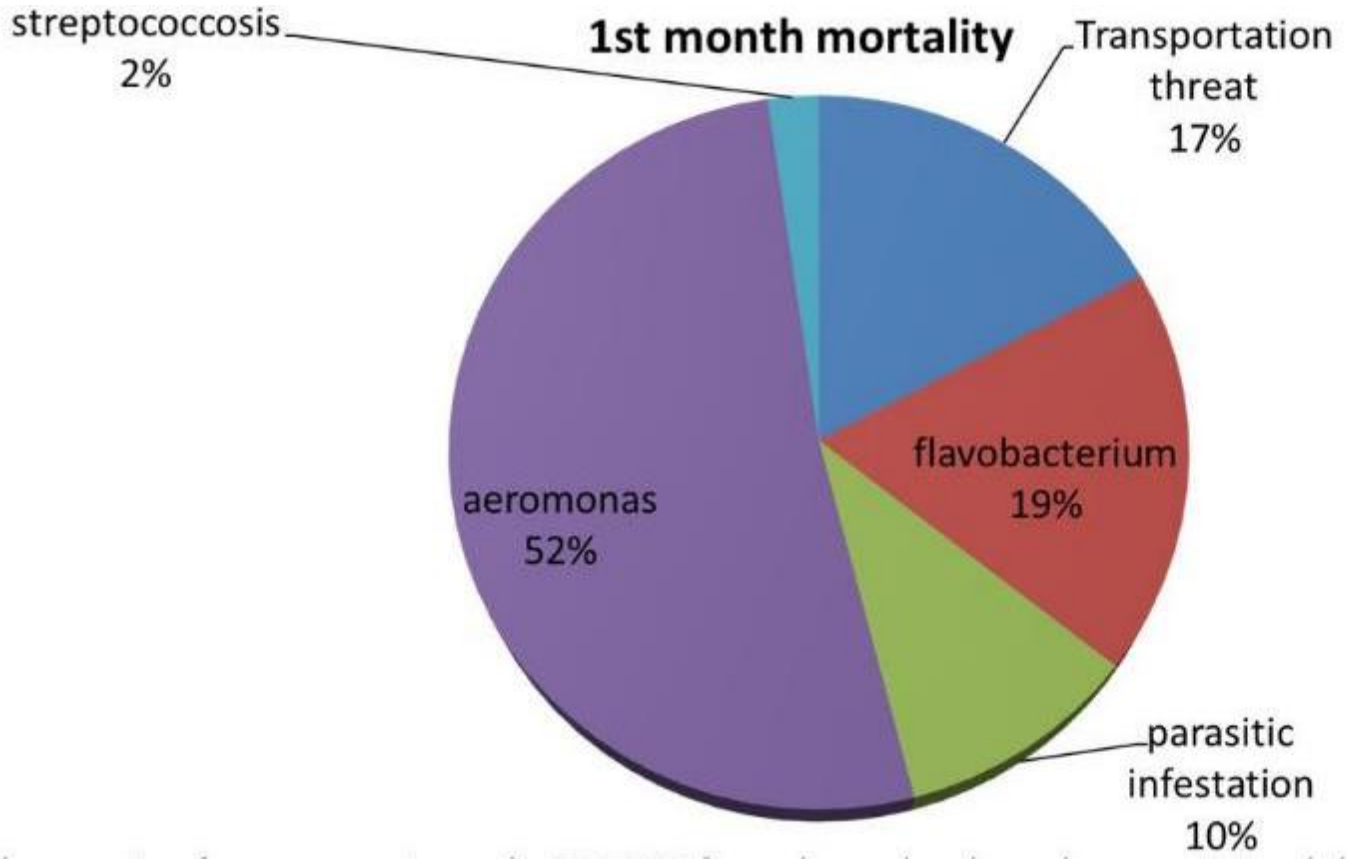
TiLV + Bacteria + Parasites

Technical Appendix Table 1. Description of TiLV outbreaks in Thailand*

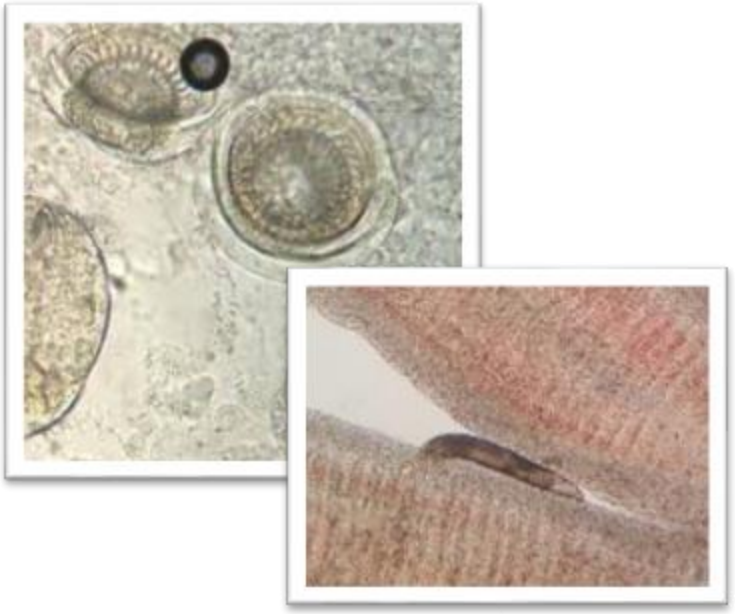
Outbreak	Date	Location	Species	Laboratory diagnosis		
				Ectoparasite†	Bacteria identification‡	TiLV Identification§
1	15/10/2015	Ang Thong	RT	ND	ND	+
2	30/10/2015	Ang Thong	RT	ND	ND	+
3	11/11/2015	Ang Thong	RT	ND	ND	+
4	29/12/2015	Kanchanaburi	RT	ND	No growth	–
5	29/12/2015	Chai Nat	RT	ND	*Flavobacterium*	+
6	29/12/2015	Kanchanaburi	RT	ND	*Flavobacterium, Aeromonas*	+ (TV2)
7	29/12/2015	Chai Nat	RT	ND	*Flavobacterium*	–
8	05/01/2016	Nakhon Ratchasima	RT	1+	*Flavobacterium*	+ (TV3)
9	05/01/2016	Pathum Thani	RT	ND	No growth	+
10	15/01/2016	Pathum Thani	RT	2+	*Aeromonas*	+
11	15/01/2016	Chachoengsao	T	3+	*Aeromonas*	+ (TV4)
12	15/01/2016	Pathum Thani	RT	ND	ND	–
13	19/01/2016	Ratchaburi	RT	1+	*Aeromonas*	+ (TV5)
14	04/02/2016	Pathum Thani	RT	0	*Aeromonas*	+
15	05/02/2016	Kanchanaburi	RT	ND	*Aeromonas*	+
16	09/02/2016	Kanchanaburi	RT	1+	*Aeromonas*	+
17	16/02/2016	Samut Songkhram	RT	2+	ND	–
18	16/02/2016	Samut Songkhram	RT	3+	*Aeromonas*	+
19	18/02/2016	Pathum Thani	RT	3+	*Aeromonas*	–
20	26/02/2016	Pathum Thani	RT	2+	*Flavobacterium, Aeromonas*	+ (TV1)¶
21	27/02/2016	Samut Songkhram	RT	1+	No growth	+
22	30/03/2016	Pathum Thani	RT	ND	*Aeromonas*	+
23	28/04/2016	Nakhon Ratchasima	RT	ND	ND	+
24	28/04/2016	Pathum Thani	RT	ND	ND	+
25	06/05/2016	Pathum Thani	RT	2+	*Aeromonas*	+
26	06/05/2016	Prachin buri	T	0	*Streptococcus*	–
27	10/05/2016	Pathum Thani	T	1+	ND	–
28	13/05/2016	Nong Khai	T	3+	ND	–
29	20/05/2016	Phitsanulok	RT	0	*Aeromonas*	+ (TV6)
30	20/05/2016	Phitsanulok	T	0	*Streptococcus, Aeromonas*	–
31	23/05/2016	Chai Nat	RT	0	*Aeromonas*	–
32	24/					

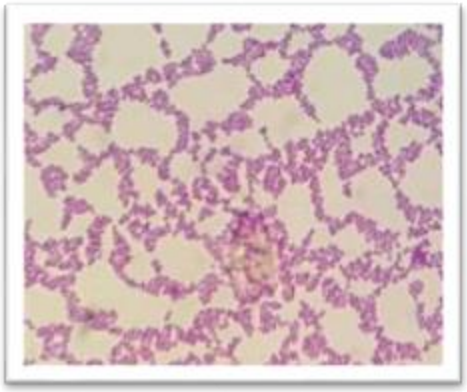
Bacterial culture results of TOMMS

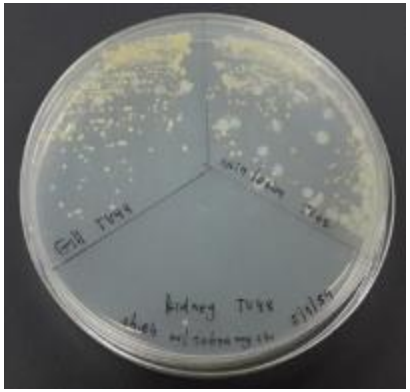
Data between June and December 2015 in a hatchery farm (600,000 fingerlings)

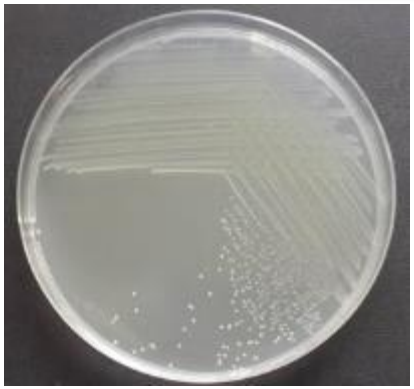


Screening for external parasites and other bacterial infection









SHORT COMMUNICATION

WILEY Journal of Fish Diseases

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]

Aeromonas **were isolated from TiLV-infected fish**

Farm ID	Diseased fish/total fish sampled Morbidity rate (%)[a]	TiLV detected	*Aeromonas* species detected
1	7/13 (54%)	–	*A. veronii* *A. hydrophilia*
2	14/26 (54%)	–	*A. veronii*
3	13/24 (54%)	+	*A. veronii*
4	13/30 (43%)	–	*A. veronii* *A. ichthiosmia* *A. enteropelogenes*
5	21/40 (53%)	+	*A. veronii*
6	14/20 (70%)	–	*A. veronii* *A. enteropelogenes* *A. jandaei*
7	8/24 (33%)	+	*A. veronii* *A. ichthiosmia*
8	10/10 (100%)	+	*A. enteropelogenes* *A. hydrophilia*
WF	0/20 (0%)	–	*A. veronii*

Summary: diagnostic methods for TiLV

Direct methods

- Electron microscopy
- Light microscopy
- Immunofluorescence
- Molecular techniques

Indirect methods

- Cell culture
- Embryonated egg
- Laboratory animal

Serology

Classical techniques

- **Complement fixation test**
- **Haemagglutination inhibition test**
- **Neutralization test**

Advanced techniques

- **Immunoassay (ELISA etc.)**
- **Western blot**

Thank you