FAO/ASTF Project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: Intensive Training Course on TiLV

4-13 December 2018. Kisumu, Kenya

in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Session:

TiLV risk management

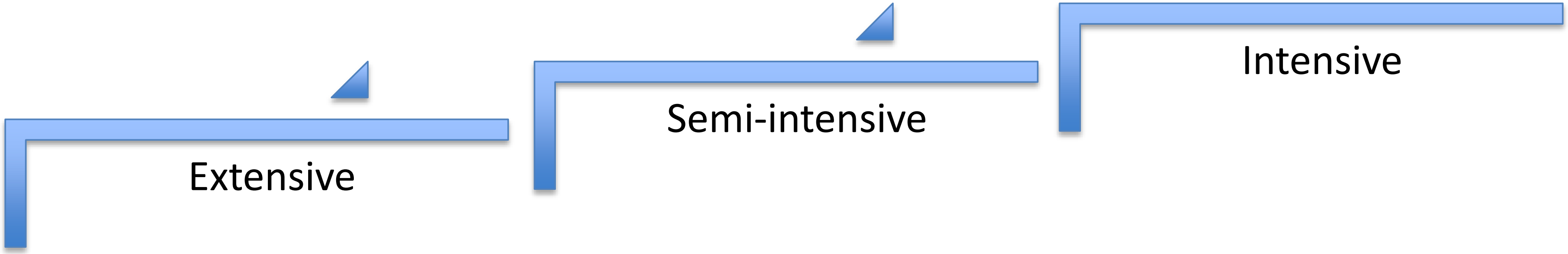
Win Surachetpong (DVM, PhD, DTBVP, CertAqv)

fvetwsp@ku.ac.th

Food and Agriculture Organization of the United Nations

Disease concerns

Level of concerns for disease epidemics in tilapia farming:



What could be the factors?

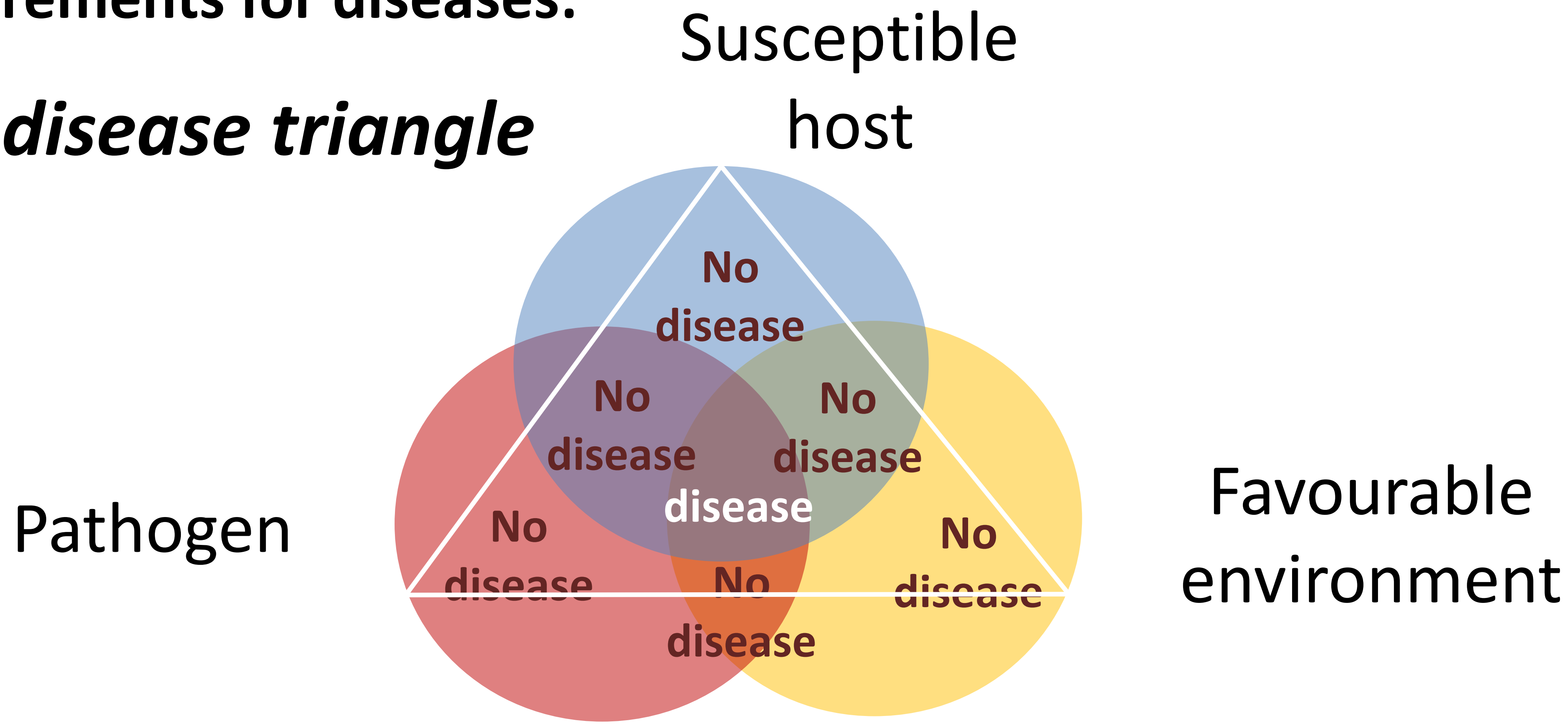
- Crowding
- Competition
  - Food
  - Space
  - Mates

- Introduced pathogens
- Introduced hosts
- Spread to native pop’s
- Competition with native pop’s
- Water condition

TILAPIA HEALTH & DISEASES

Requirements for diseases:

The disease triangle



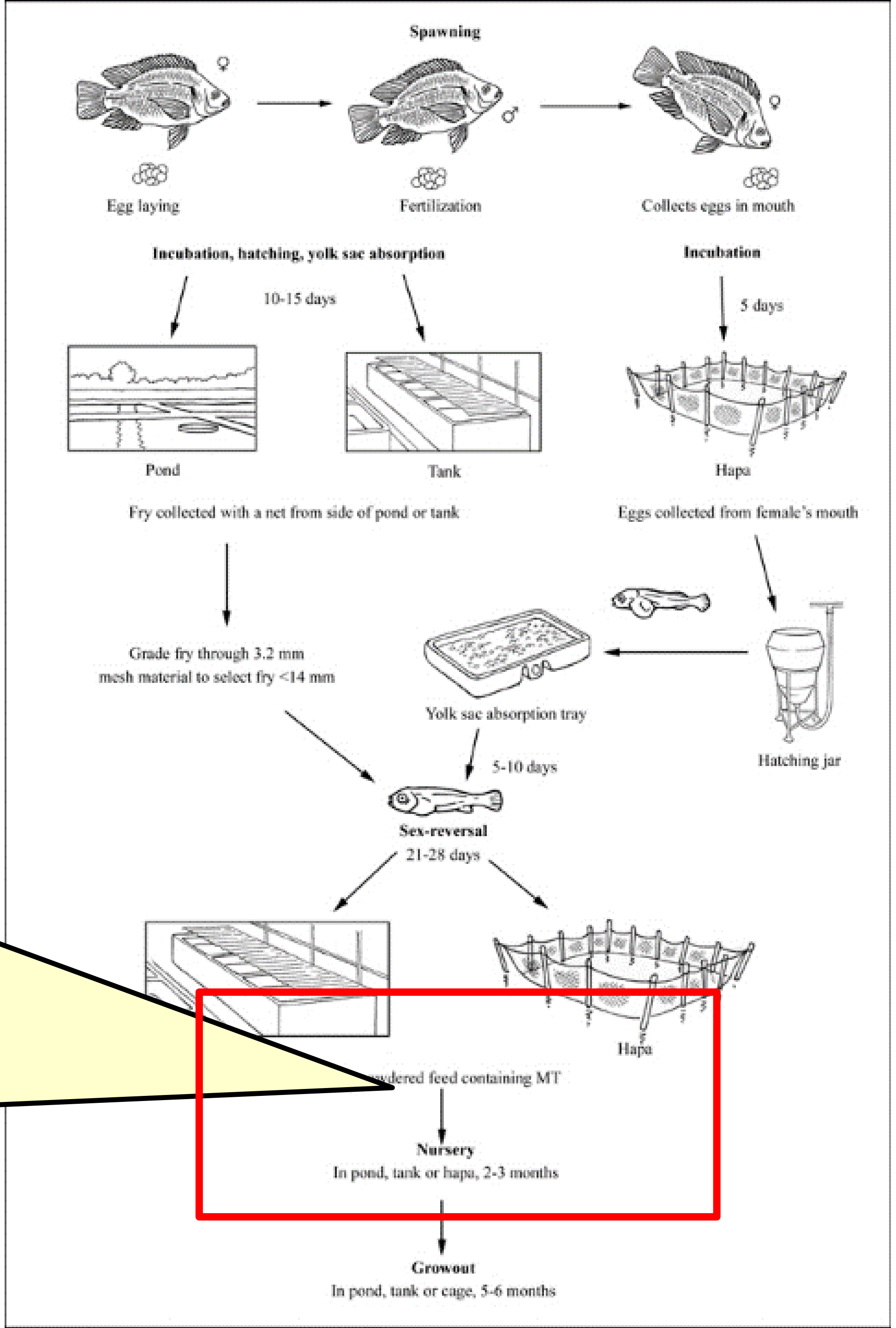
- Outbreaks require all these 3 factors -

Critical control points

Tilapia production cycle
Fish transfer to grow out pond

Nursery
(in ponds, tanks, hapas)
2-3 months

Growout
(in ponds, tanks, cages)
5-6 months



ORIGINAL ARTICLE

WILEY | Journal of Fish Diseases

Production-level risk factors for syncytial hepatitis in farmed tilapia (*Oreochromis niloticus* L)

R M Kabuusu[1] | A T Aire[2] | D F Stroup[3] | C N L Macpherson[4] | H W Ferguson[1]

TABLE 3 Linear regression model for severity of excess tilapia mortality associated with syncytial hepatitis viral infection as function of production factors

Excess mortality	Coefficient	*SE*	*F* test	*p*-Value
Stocking density	365.651	59.599	37.6400	<.000001
Initial weight	−258.106	84.566	9.3154	.002405
Temperature	−1,025.331	122.099	70.5191	<.000001
Dissolved oxygen	5,768.980	749.898	59.1825	<.000001
# of pond cycles	340.179	82.853	16.8578	.000048
CONSTANT	−41,152.417	3,456.541	141.7449	<.000001

Correlation coefficient: $r^2 = .24$; no confounding or interaction was established in both models.

- **Chitralada strain had higher risk**
- **Stocking density** ↑
- **Dissolved oxygen** ↑ **risk**
- **Pond production cycles** ↑

ORIGINAL ARTICLE

WILEY Journal of Fish Diseases

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- **Higher initial weight** ↓
- **Higher temperature** ↓

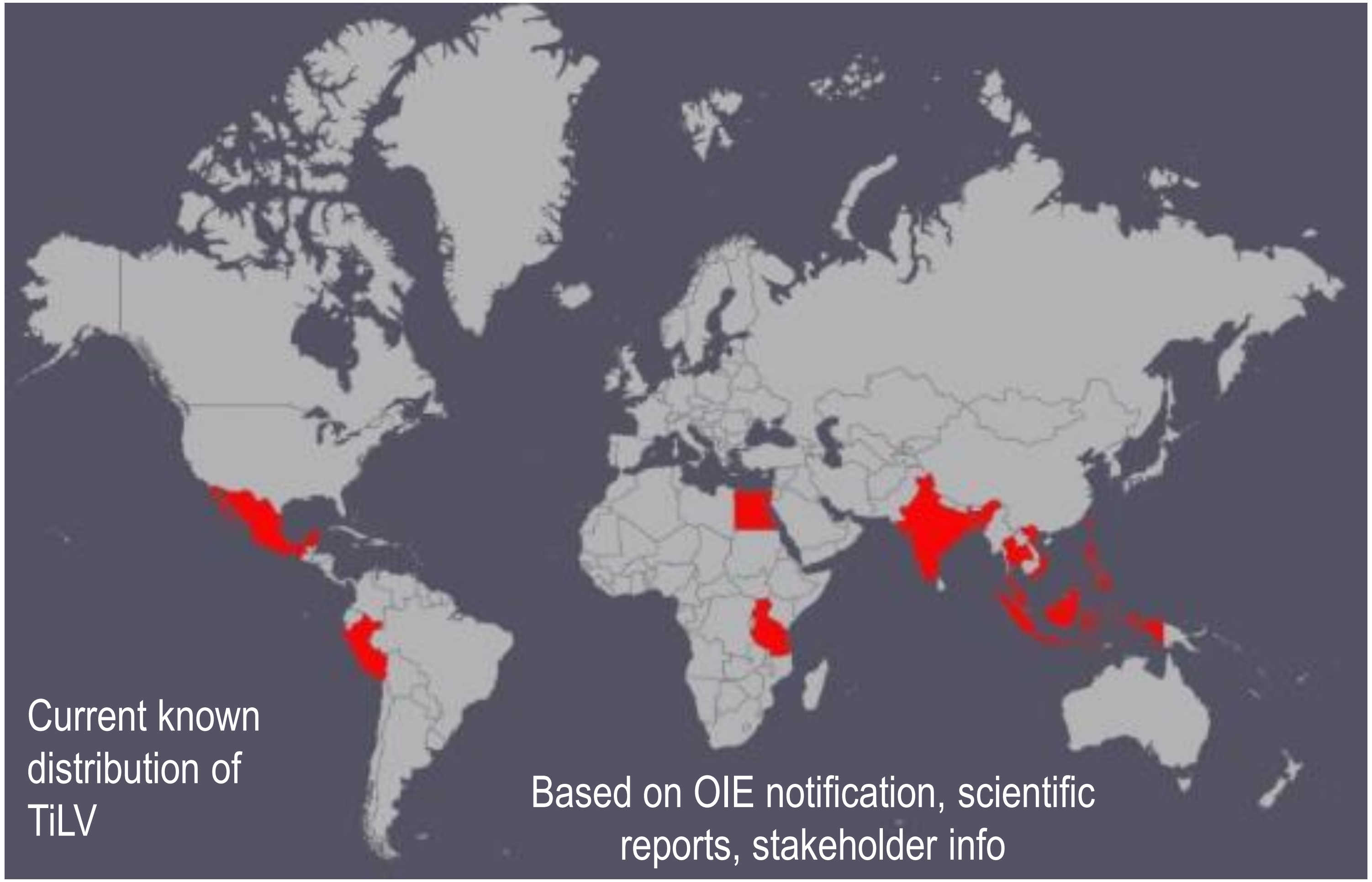
risk

- **Season and stocking year (not associated with severity)**

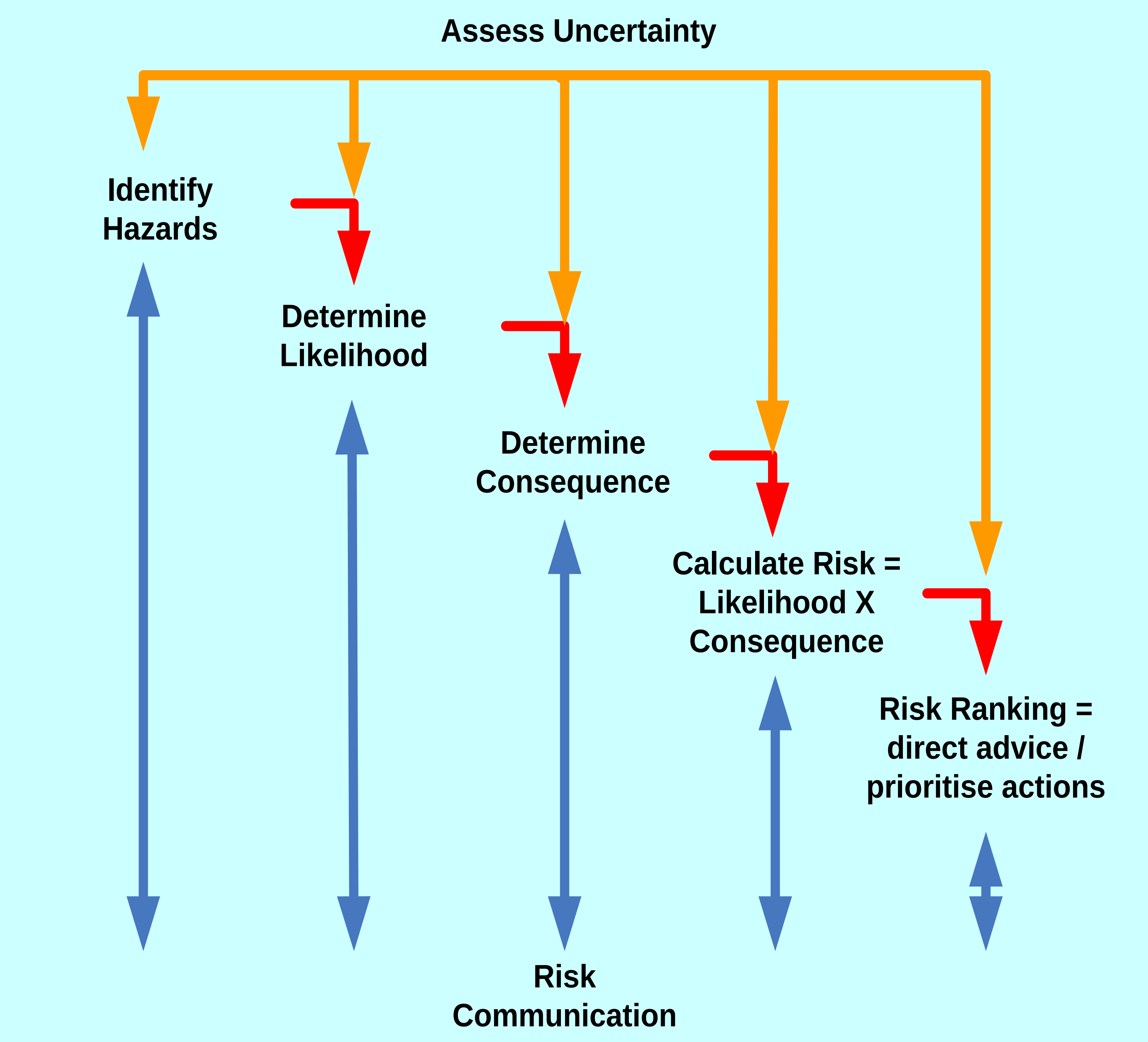
Global:
China
Brasil

Africa:
Ghana, Nigeria, Zambia, Kenya, Zimbabwe

LAC: Brasil, Honduras, Costa Rica, Guatemala



Slide courtesy: Melba Reantaso; FAO



Simplified Risk Analysis Process

They generally incorporate the concepts of:

uncertainty of outcome (of an action or situation)

that leads to a likelihood (probability or chance) of an unwanted event happening, and

a **consequence** or impact (if the unwanted event happens)

risk = likelihood x consequence

Slide courtesy: Melba Reantaso; FAO

Species	Country	Reference
Hybrid tilapia (*Oreochromis niloticus* x *O. aureus* hybrids)	Israel Chinese Taipei	Eyngor *et al*., 2014 OIE, 2017c
Nile tilapia (*O. niloticus*)	Egypt	Fathi *et al*., 2017
	Ecuador	Ferguson *et al*., 2014
	Colombia	Tsofack *et al*., 2017
	Thailand	Dong *et al*., 2017a; Surachetpong *et al*., 2017
	Peru	OIE notification 2018
	Philippines	OIE notification 2017
	Indonesia	Isti *et al*. 2018
	India	Behera *et al* 2018
Black tilapia (*Oreochromis* spp) (wild)	Malaysia	OIE notification 2017
Red tilapia *Oreochromis* sp	Thailand	Dong *et al*., 2017a; Surachetpong *et al*., 2017
Wild tilapinies (Sarotherodon galileus, *Tilapia zilli*, *O. aureus*, and *Tristamellasimonis intermedia*	Sea of Galilee, Israel	Eyngor *et al*., 2014

TiLV Risk Profile:

Host range farmed and wild populations

Slide courtesy: Melba Reantaso; FAO

Risk Profile: TiLV distribution:

TiLV OIE notification, scientific publication, stakeholder information

The presence of a disease in any particular country can be a very sensitive issue and easily subject to misinterpretation; caution is recommended; it is always good to have a reference/source

Country	Reference		
	OIE Notification (as emerging disease) (date of start of event/ date of confirmation of event/ date of report)	Scientific report	Stakeholder information
Bangladesh			√ suspicion (2018)
Colombia		√ (2014)	
Ecuador		√ (2014)	
Ghana			√ unexplained mortalities (2017)
India		√ (2018)	
Indonesia		√ (l2018; local report)	
Israel	√ (2011/2014/2017)	√ (2014, 2016, 2017) First observation in 2009	
Peru	√ (2017/2017/2018)		
Malaysia	√ (2017/2017/2017)	√ (2017)	
Mexico	√ (2018/2018/201)		
Philippines	√ (2017/2017/2017)		
Tanzania		√ (2018)	
Taiwan Province of China	√ (2017/2017/2017)		
Thailand	√ (2015/2017/2017)	√ (2016.2017, 2018)	
Uganda		√ (2-18)	
Vietnam			√ suspicion (2017)

Risk management measures

- **Movement restriction**
- **Surveillance program**
- **Farm level biosecurity and husbandry**
- **Emergency preparedness and response**

Risk of TiLV spreading via frozen fillet?

TiLV genomic was detected until 28 days post freezing

Clinically-infected fish

The virus still infective

Fish no.	C_t values			CPE formation (days post inoculation)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
1	17.04	17.22	16.22	3	10	9
2	17.73	17.79	16.39	3	5	9
3	17.36	17.31	17.41	3	10	9
4	17.45	18.68	16.83	5	5	7
5	20.36	24.31	24.48	6	–	–
6	17.89	21.38	16.75	4	6	6
7	22.43	24.68	20.36	6	6	6

No infection for frozen fillet at 90 and 120 days

Clinically-infected fish

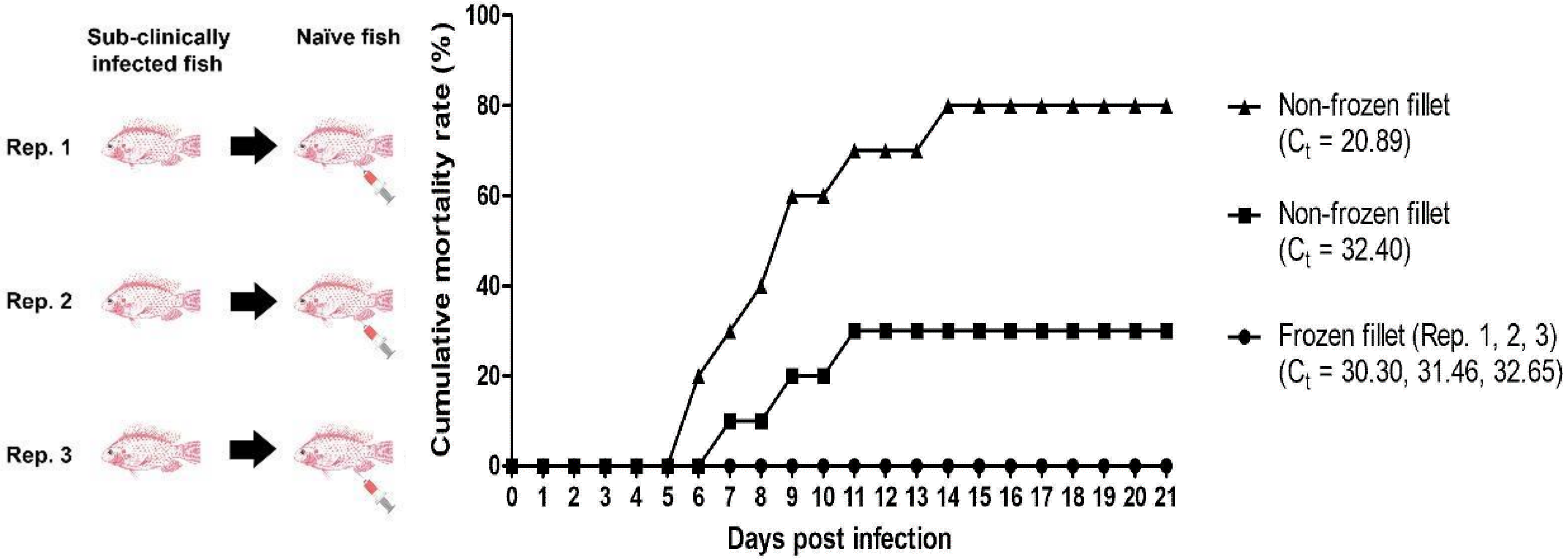
Fish no.	C_t values		CPE formation (days post inoculation)	
	Day 90	Day 120	Day 90	Day 120
1	26.80	29.27	–	–
2	17.99	24.13	–	–
3	24.72	23.51	–	–
4	20.27	N/A	–	N/A
5	18.21	N/A	–	N/A

No infection for frozen fillet at 0 to 28 days

Subclinically-infected fish

Fish no.	C_t values			CPE formation (days post inoculation)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
1	31.09	29.64	26.49	–	–	–
2	27.31	35.40	26.50	–	–	–
3	34.49	35.08	31.98	–	–	–
4	28.85	31.99	28.36	–	–	–
5	32.03	36.25	26.72	–	–	–
6	27.15	30.80	27.37	–	–	–
Mean ± SD	$30.15 \pm 2.90^{*,***}$	$33.19 \pm 2.74^{*}$	$27.90 \pm 2.12^{**,***}$			

Low risk of TiLV transmission via frozen fillet



Thammatorn et al., Journal of Fish Diseases *in press.*





Strategies for prevention and control in the presence of TiLV

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What is biosecurity?

A set of management and physical measures designed to reduce the *risk* of introduction, establishment and spread of *pathogenic agents* to, from and within an *aquatic animal* population

OIE aquatic animal health code, 2015

Identified transmission routes

Infected live fish and eggs

Potential risk

Exposure via water

Fish processing on site

Mechanical transmission

Potential pathways for introduction and spread into the compartment of the agents

Open cage culture: risk of diseases

Important of Biosecurity

Use pre-caution prior to move aquatic animals

Pathogens introduction via a transport truck?

Important of farms location and disease spreading

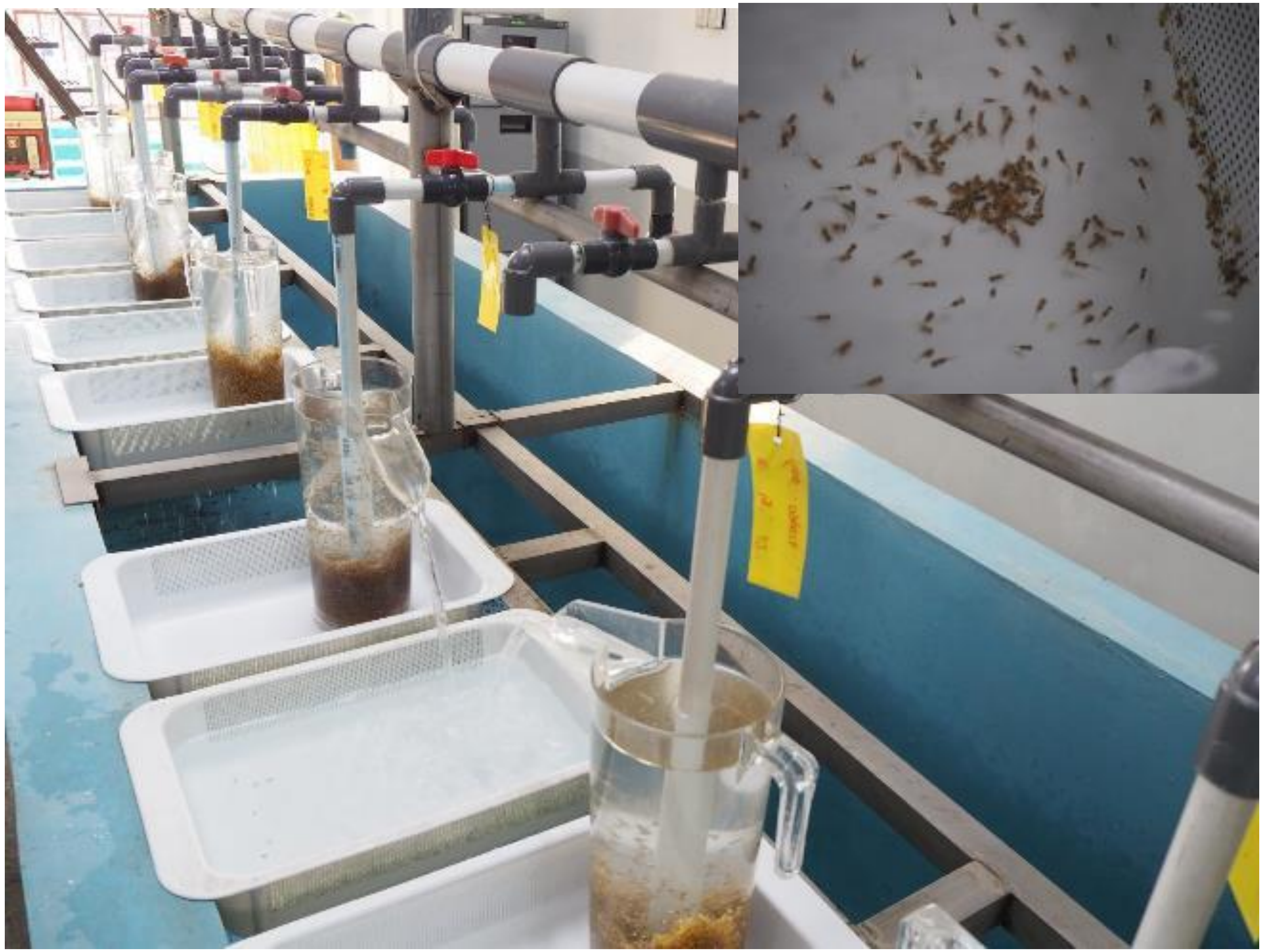
Biosecurity to prevent pathogen introduction

Farm with clear boundary

A farm with surrounding wall

Disinfecting water supply

Stock only certified disease free eggs/animals



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

ORIGINAL ARTICLE

WILEY 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]

[1]Center for Advanced Studies for Agriculture and Food (CASAF), Kasetsart University Institute for Advanced Studies, Kasetsart University (NRU-KU), Bangkok, Thailand
[2]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand
[3]Research Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok, Thailand
[4]Center of Excellence on Environmental Health and Toxicology (EHT), Ministry of Education, Bangkok, Thailand

Correspondence
Win Surachetpong, Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.
Email: fvetwsp@ku.ac.th

Abstract

Tilapia lake virus (TiLV) is an emerging pathogen associated with high mortalities of wild and farm-raised tilapia in different countries. In this study, a SYBR green-based reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay targeting segment three of the virus was developed to detect and quantify TiLV in clinical samples and experimentally challenged fish. All 30 field samples with clinical signs and history consistent with TiLV infection were positive for TiLV as detected by the developed RT-qPCR method. The RT-qPCR technique provided 100 and 10,000 times more sensitive for virus detection than those offered by the RT-PCR and virus isolation in cell culture methods, respectively. The detection limit of the RT-qPCR method was as low as two viral copies/μl. Moreover, the RT-qPCR technique could be applied for TiLV detection in various fish tissues including gills, liver, brain, heart, anterior kidney and spleen. Significantly, this study delivered an accurate and reliable method for rapid detection of TiLV viruses that facilitates active surveillance programme and disease containment.

Highly sensitive and specific method for disease screening

Routine disease monitoring & biosecurity practice



Sampling

&

Disease

screening




Good biosecurity practices

Remove moribund and dead animals properly!

Aquaculture 486 (2018) 75–80

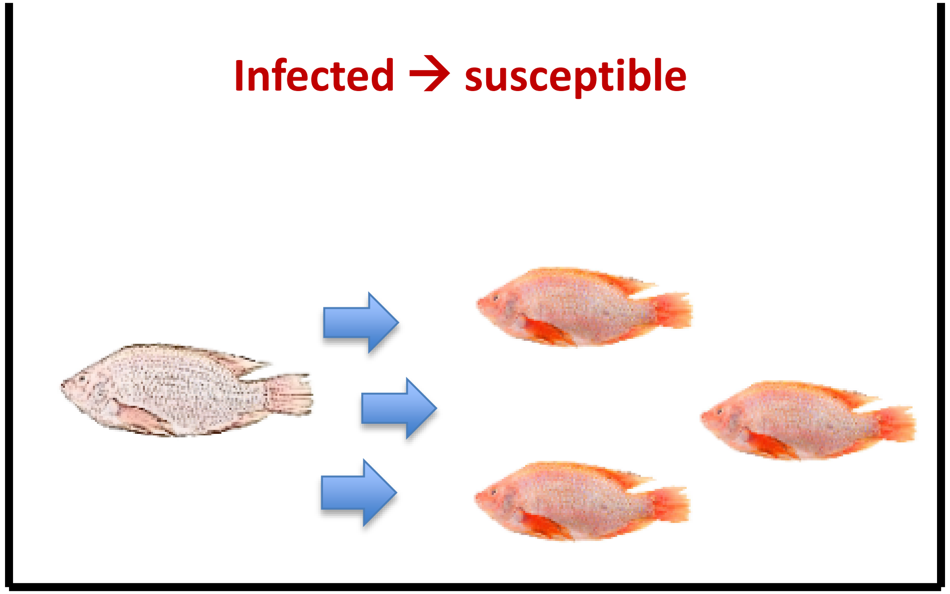
Contents lists available at ScienceDirect

Aquaculture

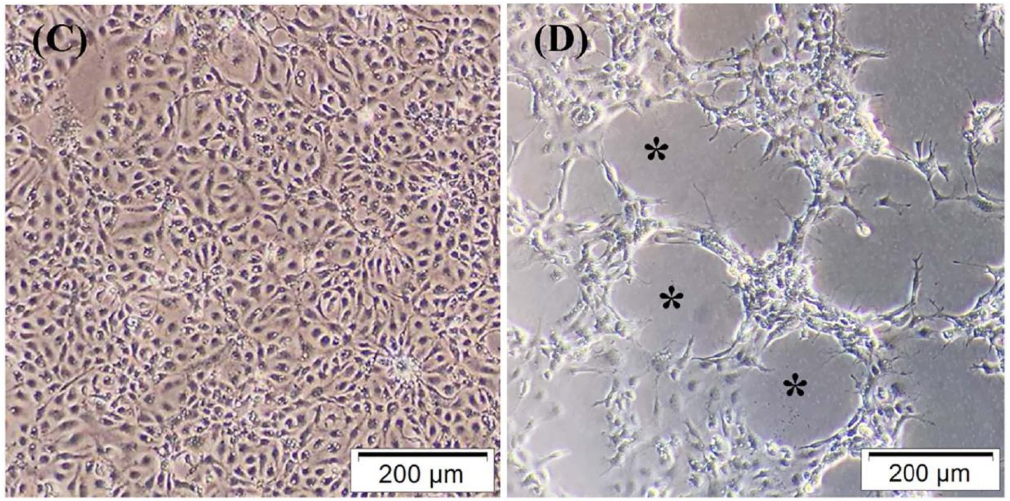
journal homepage: www.elsevier.com/locate/aquaculture

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

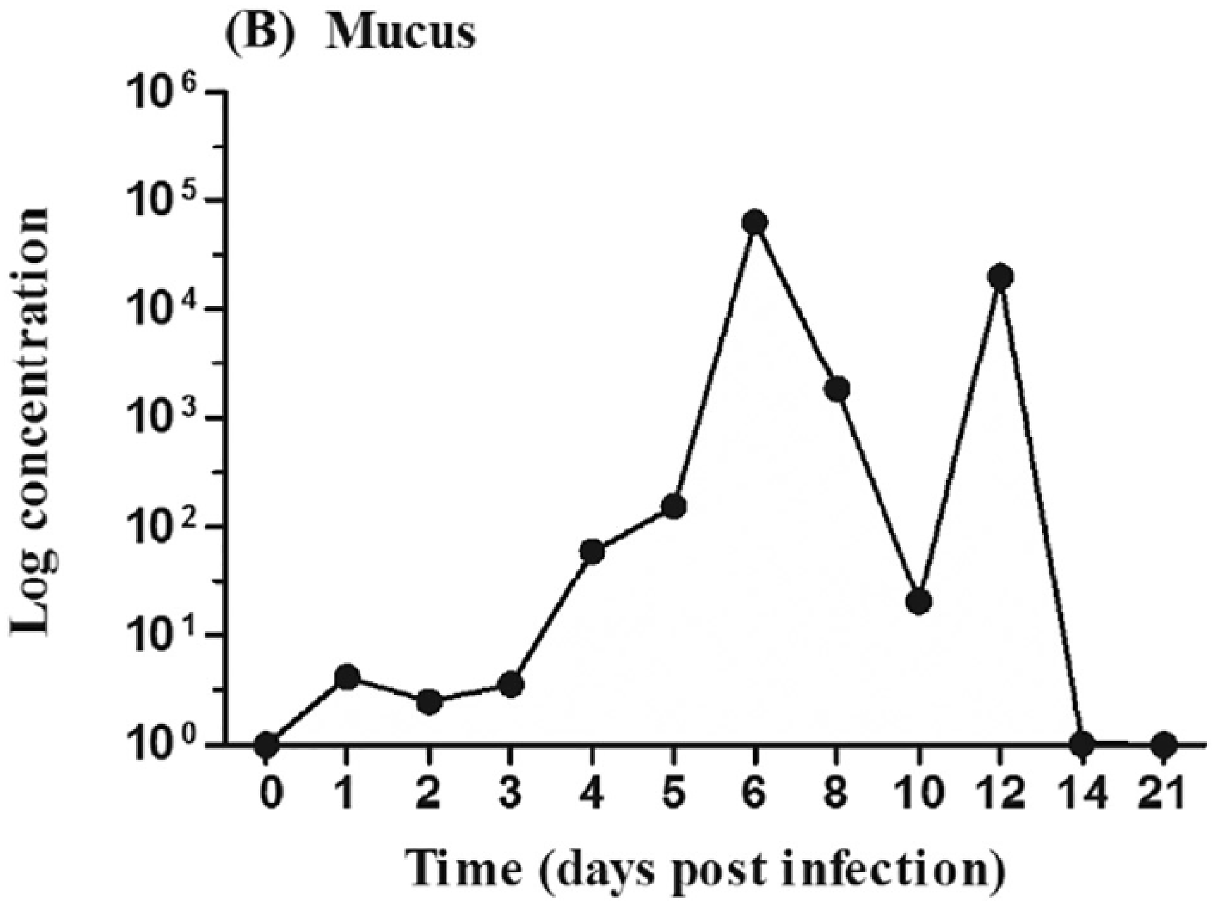
Pavarit Liamnimitr[a], Worrayanee Thammatorn[a], Sonicha U-thoomporn[a], Puntanat Tattiyapong[b], Win Surachetpong[a,b,*]



- **Virus in mucus is still infective!**



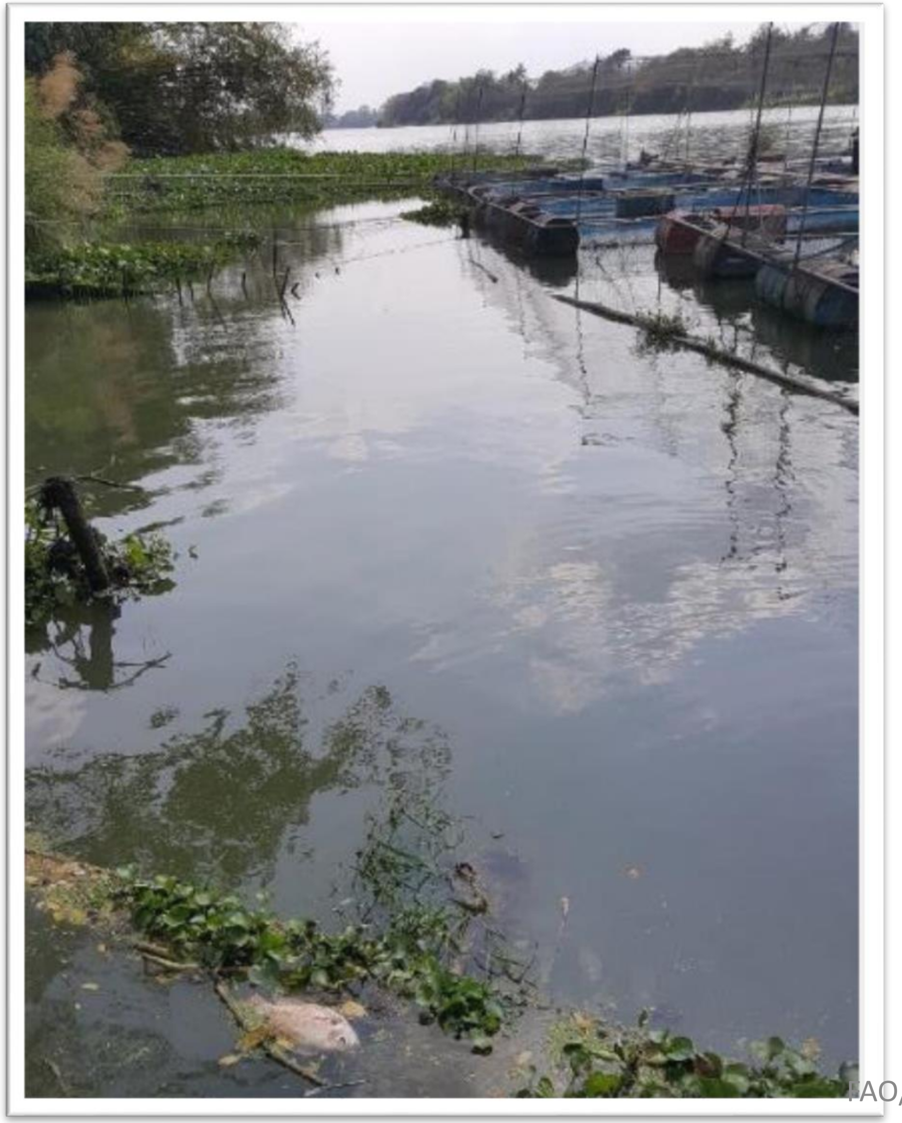
TiLV presents in mucus upto 12 dpi → shedding



Management of dead fish is critical

Liamnimitr et al., 2018 Aquaculture, 486: 75-80

Don't dump dead fish in public water

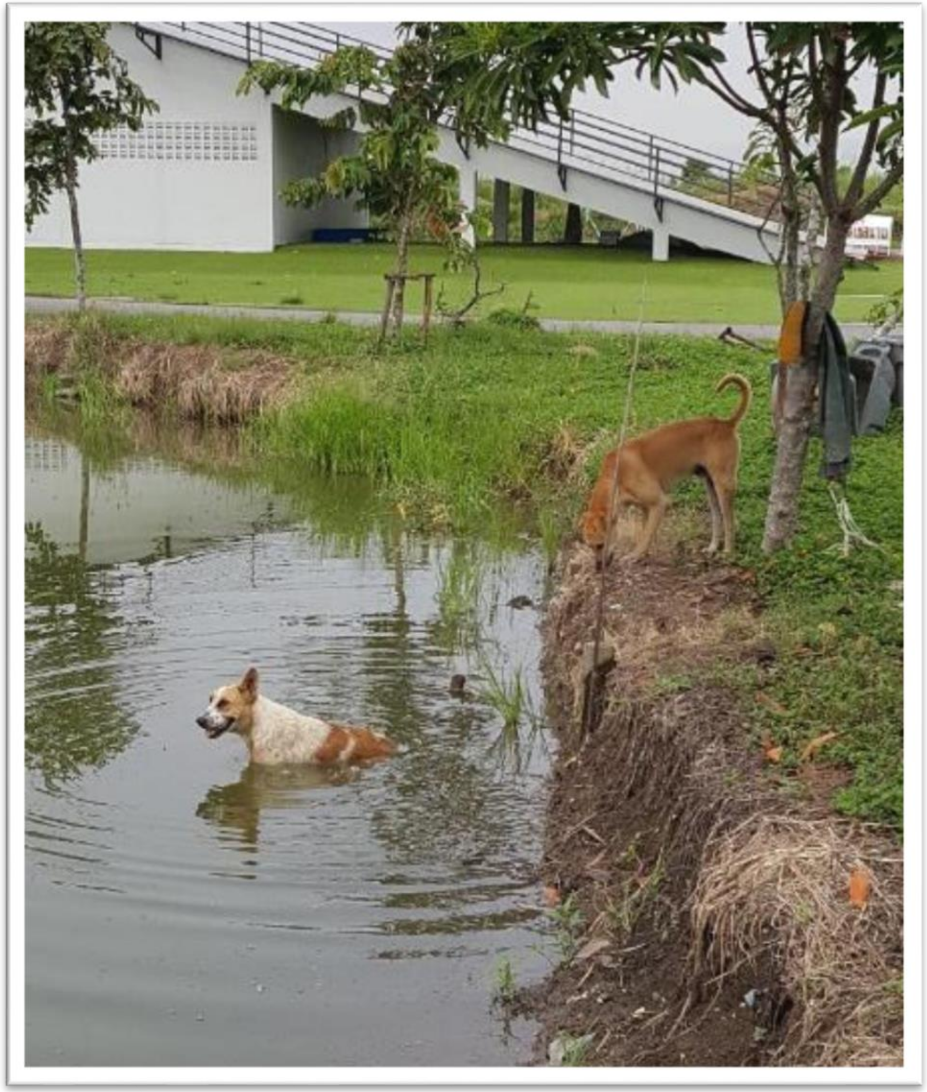


Avoid stocking fish at different ages/size

Minimize handling to reduce stress that predispose to disease

Some farmer said if you want fish to get TiLV → Grading or stress them!!

Transmission by carriers?



Mechanical vectors causing disease to spread

Vectors?

- **No detection of TiLV in fish *Argulus* and mollusk** (prelim study)
- **How long the virus survive in water?**

Aquaculture 497 (2018) 462–468

Contents lists available at ScienceDirect

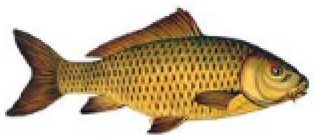
Aquaculture

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

Susceptibility of important warm water fish species to tilapia lake virus (TiLV) infection

Phitchaya Jaemwimol[a], Pattarasuda Rawiwan[a,b], Puntanat Tattiyapong[a,b], Pattrawut Saengnual[c], Attapon Kamlangdee[d], Win Surachetpong[a,b,*]

Most important warm water fish species are resistant to tilapia lake virus (TiLV) infection



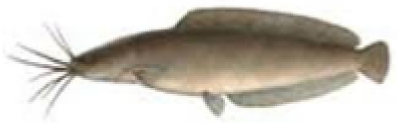
Cyprinus carpio

Trichogaster pectoralis

Barbodes gonionotus

Lates calcarifer

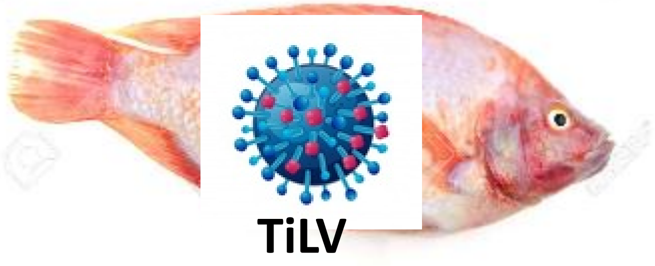
Anabas testudineus



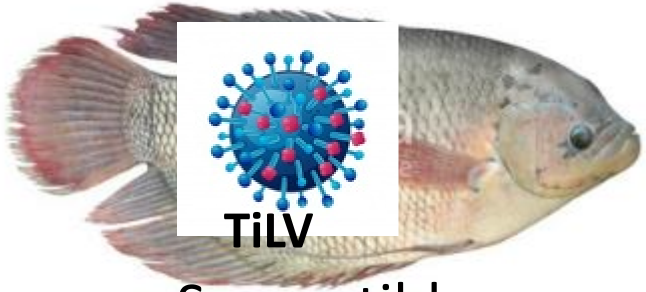
Clarias macrocephalus

Pangasianodon hypophtthalmus

Chana striata



Susceptible
***Oreochromis* sp.**



Susceptible
Osphronemus goramy

Aquaculture 497 (2018) 462–468

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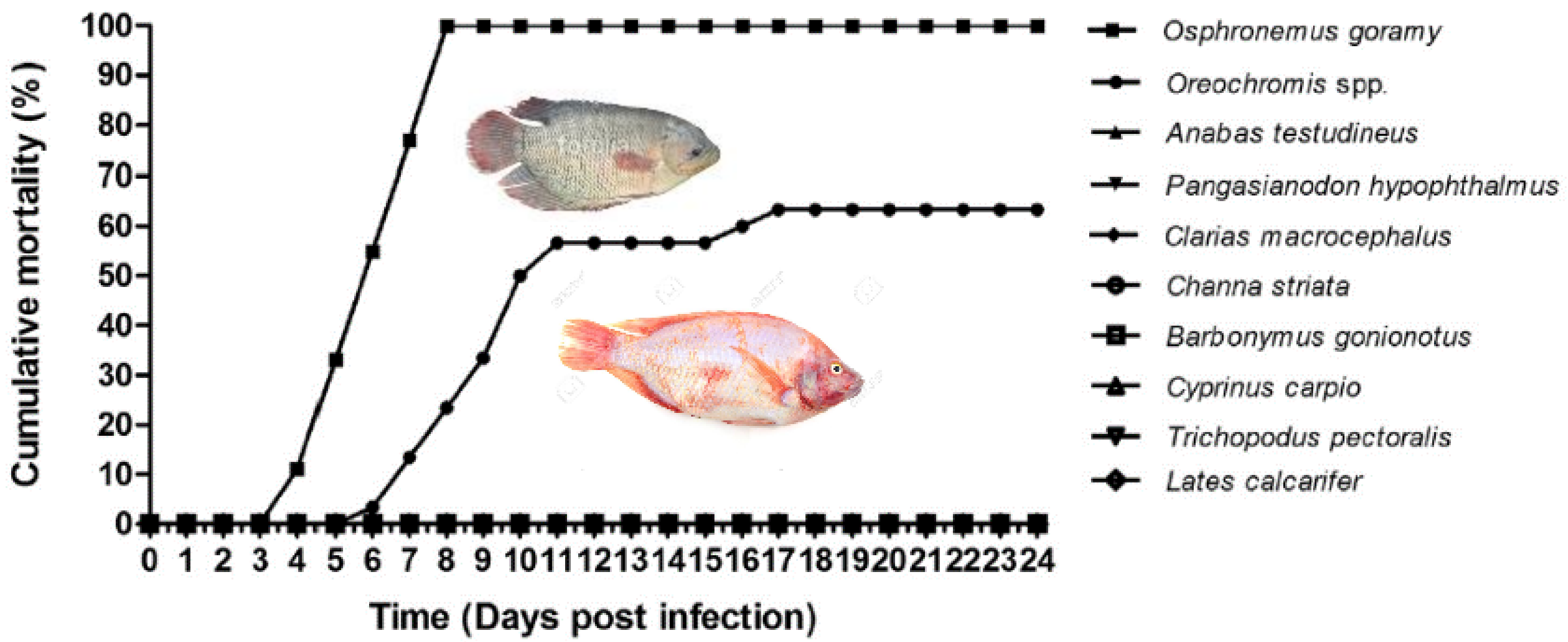
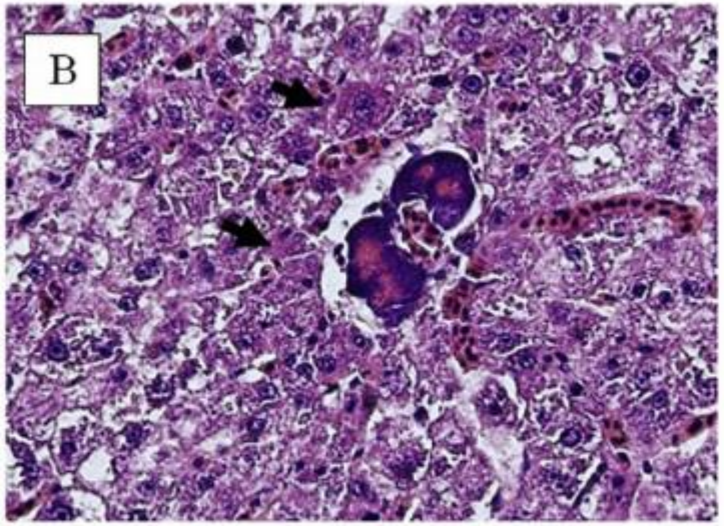
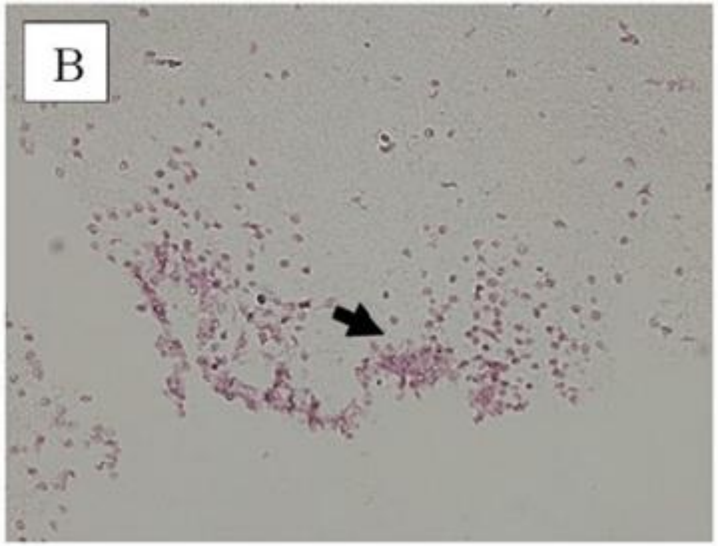
Mortality of ten species after TiLV challenge





Syncytial cell in liver of giant gourami



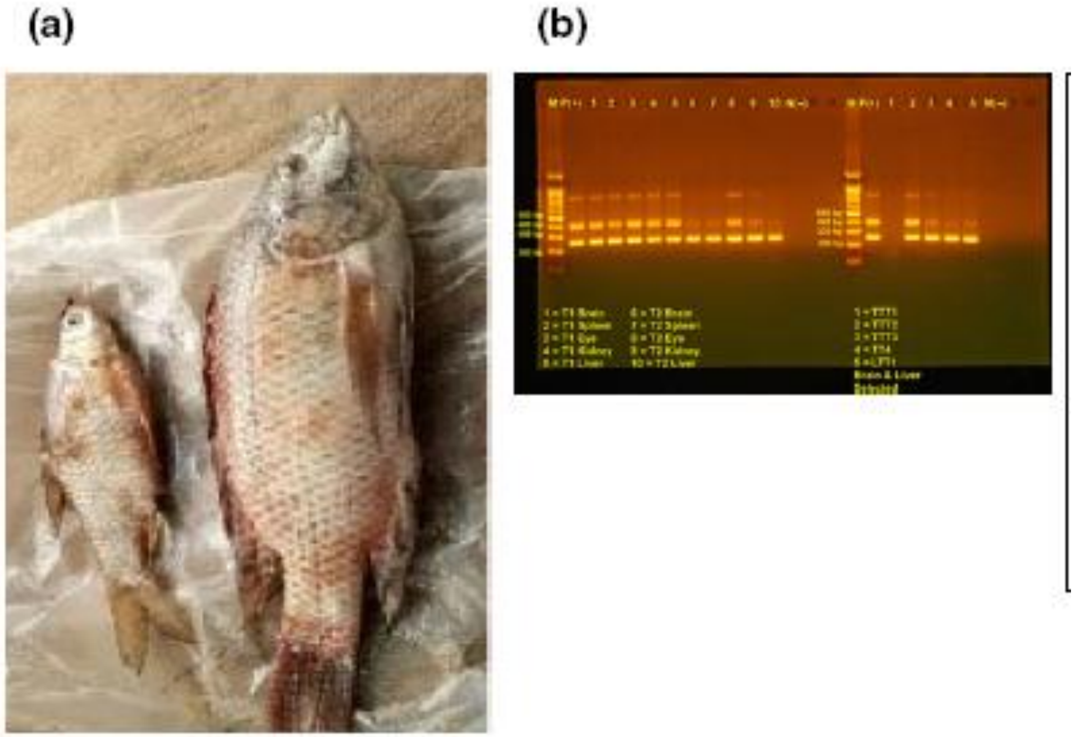
In situ hybridization signal in the brain of infected giant gourami

SHORT COMMUNICATION

WILEY Journal of Fish Diseases

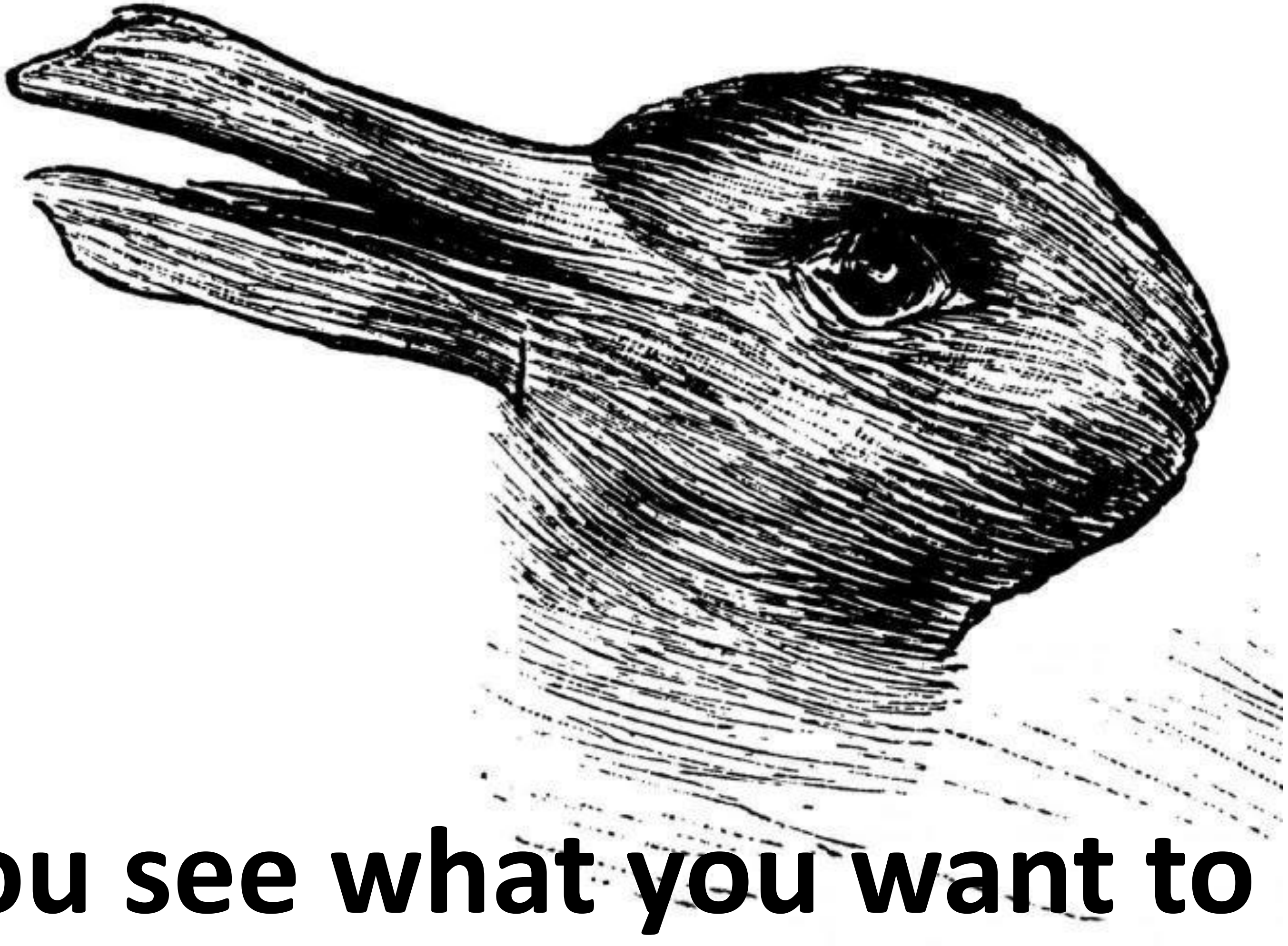
First detection of tilapia lake virus (TiLV) in wild river carp (*Barbonymus schwanenfeldii*) at Timah Tasoh Lake, Malaysia

Azila Abdullah[1] | Rimatulhana Ramly[1] | Mohammad Syafiq Mohammad Ridzwan[1] | Fahmi Sudirwan[1] | Adnan Abas[2] | Kamisa Ahmad[1] | Munira Murni[1] | Beng Chu Kua[1]



- **Healthy and normal river barb**
- **Positive TiLV (2/2)**
- **Pale liver and congested kidney**

Rabbit or Duck?



You see what you want to see!

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*

TiLV + Bacteria + Parasites

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

				Laboratory diagnosis		
Outbreak	Date	Location	Species	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.					

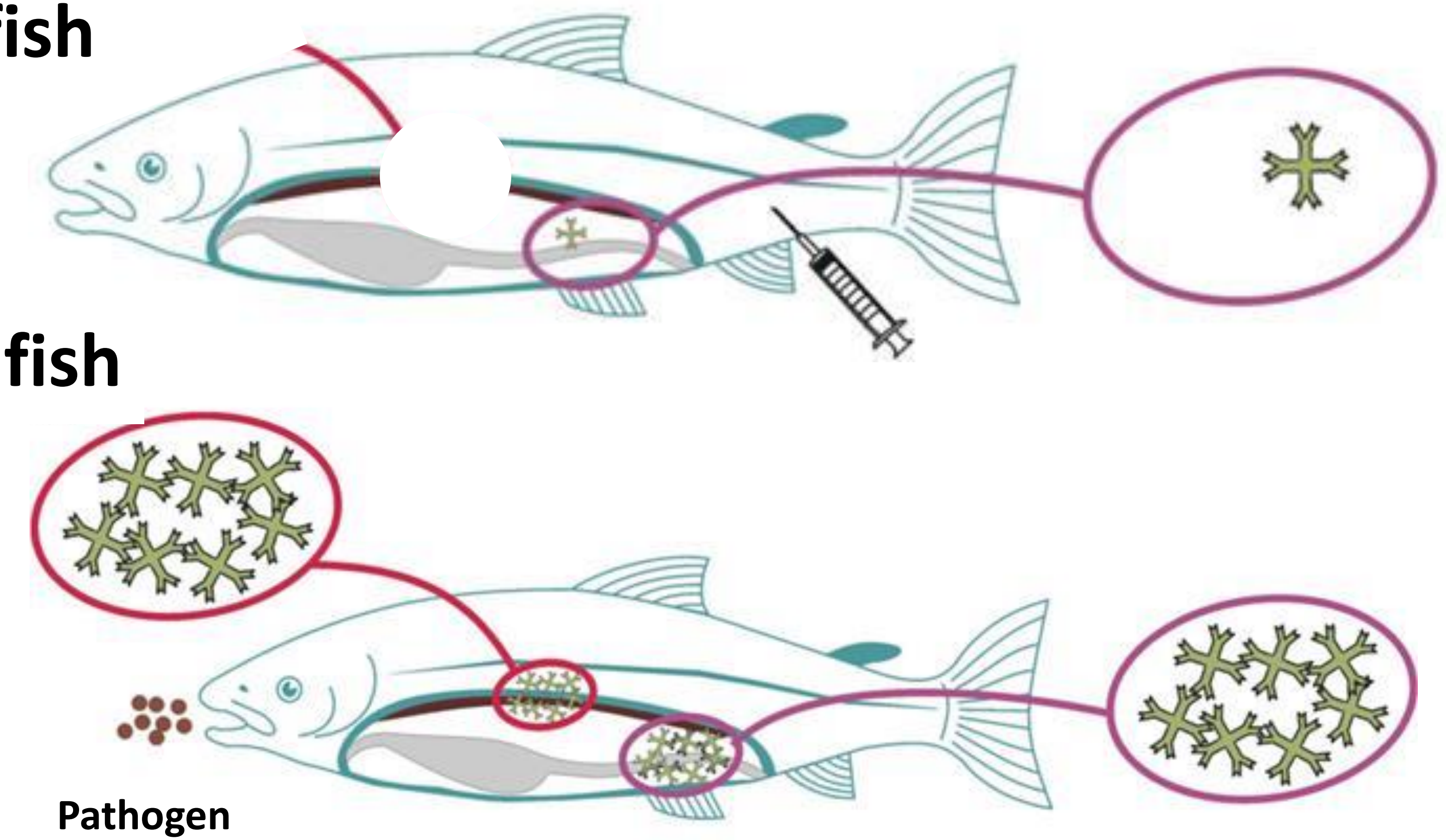
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 6, June 2017

Complex bacteria were isolated from TiLV-infected fish

Flavobacterium, Aeromonas, Streptococcus, Francisella

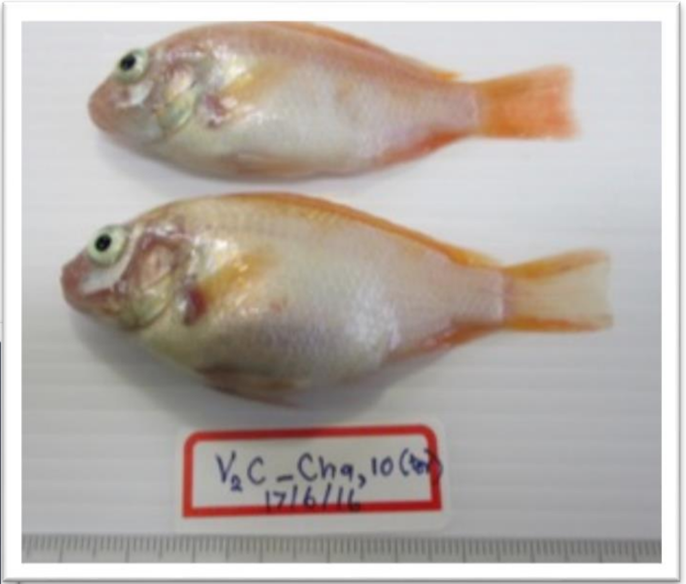
TiLV vaccine

Fish that survive TiLV do not have re-infection –> develop solid immunity?



TILAVAC: Vaccine for the prevention of an emerging viral disease in tilapia

- Live and killed vaccines
- Immersion and injection
- Currently testing in the field, under natural infection conditions

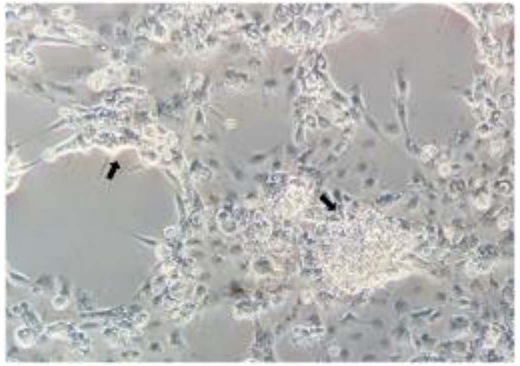


Biosecurity – key considerations for TiLV control

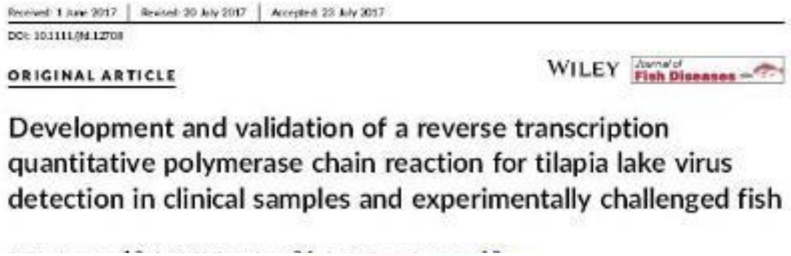
- Fish movement (between sites)
- Sick and dead fish management (quickly remove them)
- Trucks, equipment, boats (disinfectants)
- Personnel (control facility access)
- Potential vectors & other species (???)

How could we detect the disease?

- History, clinical signs, mortality pattern
- Histopathology; liver, brain, spleen
- *In situ* hybridization
- Virus isolation
- RT-PCR, Nested RT-PCR,
  SYBR & TaqMan real-time PCR



TiLV-infected E-11 cells



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

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Short communication

A TaqMan RT-qPCR assay for tilapia lake virus (TiLV) detection in tilapia

Pitchaporn Waiyamitra[a,b], Puntanat Tattiyapong[a,b], Kwanrawee Sirikanchana[c,d], Skorn Mongkolsuk[c,d], Pamela Nicholson[e], Win Surachetpong[a,b,*]

Prof. Kevin Fitzsimmons

Expert: New tilapia virus 'is going to be a mess,' but not the next EMS

Researcher not worried about the long-term consequences.

June 9th, 2017 13:10 GMT   Updated June 12th, 2017 13:05 GMT

Is Tilapia Lake Virus (TiLV) the new ISA or EMS?

Not quite, University of Arizona tilapia guru Kevin Fitzsimmons told **IntraFish**.

"Long term, I'm not that worried, because people will breed in resistance fairly quickly," he said. "It's not going to end the tilapia industry by any means."

Egypt is free from Tilapia lake virus: FAO

Al-Masry Al-Youm

August 4, 2017
5:24 pm

On Thursday, Saudi Arabia's Ministry of Environment, Water and Agriculture imposed a temporary ban on imports of fish from Egypt, based on a warning issued by the FAO on May 26 regarding the virus.

Take home messages….

- The problem is more complex than just only the virus (bacteria, parasites or other virus) may impact on the mortality rate
- Don’t panic…the problem is manageable
- Biosecurity and Farm management
- Vaccine is one of the important priority for this emerging virus

Thank you....Q & A

Grand palace, Bangkok, THAILAND