Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

VIRTUAL COURSE

26 March to 15 April 2021

Design of an Active Surveillance for Tilapia Lake Virus (TILV) Disease and Its Implementation

TCP/INT/3707: Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus

CHECKLIST #6

02 April 2021

Checklist 6: TiLV Risk Profile

Win Surachetpong
fvetwsp@ku.ac.th

TCP/INT/3707: Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus

Learning objectives:

- To understand the requirements and criteria for Checklist 6
- To gain knowledge on the different levels of diagnostics in general
- To get to know the TiLV risk profile

Outline : TiLV risk factors → Clustering of the cases

- Host : Susceptible species, Life stages, Stress
- Environment : Season, Climate, Contacts, Locations
- Agent : (Virulence, Survivability)

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Host : Susceptible species

Life stages

Stress factors

Susceptible fish species for TiLV

- Wild tilapia *Tristamellasimonis intermedia*
- Hybrid tilapia (*O. niloticus* × *O. aureus hybrids*)
- Nile tilapia (*O. niloticus*)
- Red tilapia (*Oreochromis* spp.)
- Grey tilapia (*O. niloticus x O. aureus*)

Sarotherodon galilaeus

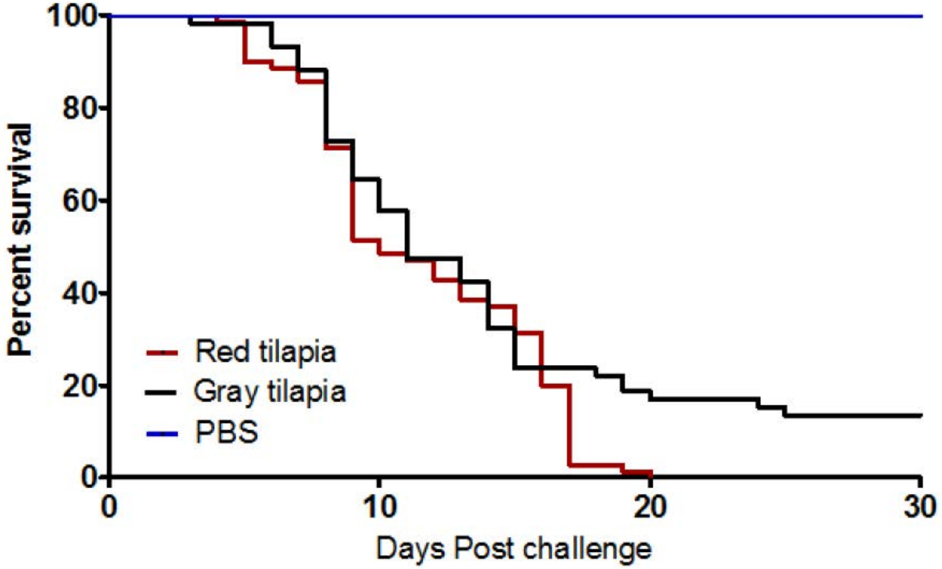
Tilapia zilli

Oreochromis aureus

https://commons.wikimedia.org/wiki/File:Cichlidae_(10.3897-zse.96.55837)_Figure_9.jpg
https://upload.wikimedia.org/wikipedia/commons/0/07/St._Peter%27s_Fish.jpg
https://commons.wikimedia.org/wiki/File:Blue_Tilapia.jpg

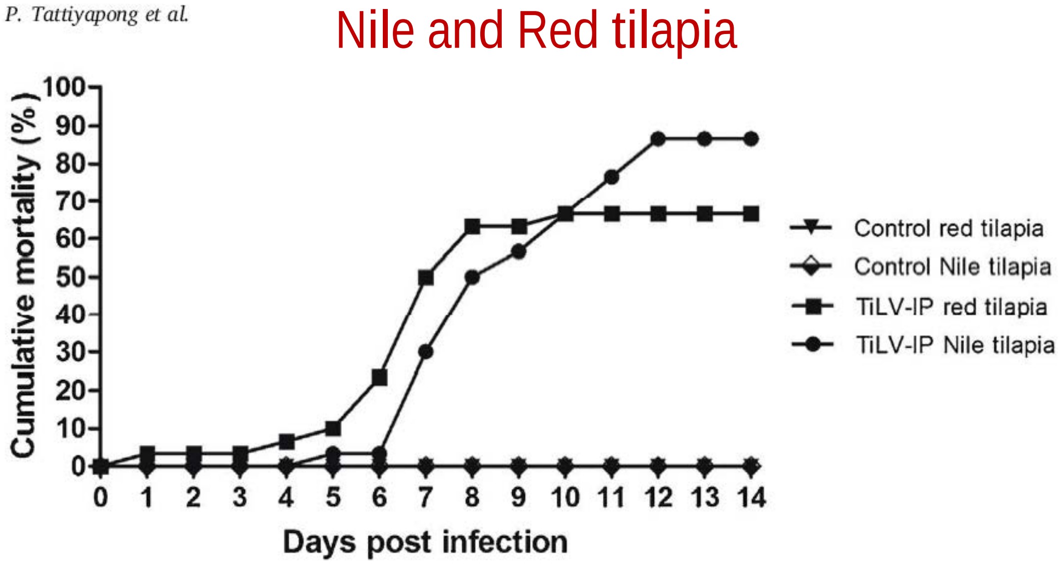
High mortalities after TiLV infection in tilapia

Red and Gray tilapia

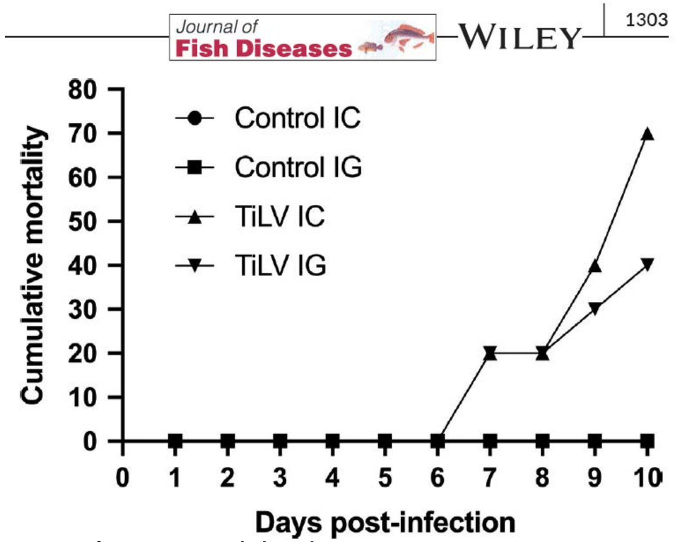


Mugimba et al 2020, Viruses

Nile and Red tilapia



Nile tilapia with intragastric challenge



Pierezan et al 2019, J Fish Dis

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

Aquaculture 497 (2018) 462–468

Contents lists available at ScienceDirect

Aquaculture

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

ELSEVIER

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,*]

Cyprinus carpio

Trichogaster pectoralis

Barbodes gonionotus

Lates calcarifer

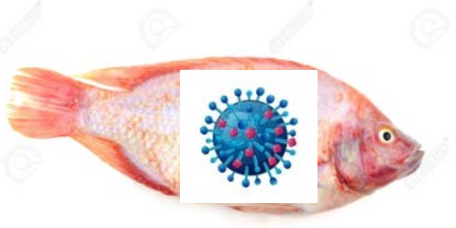
Anabas testudineus

Clarias macrocephalus

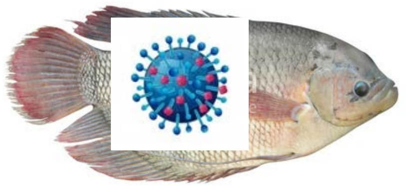
Pangasianodon hypophthalmus

Chana striata

TiLV susceptible



Oreochromis spp.



Osphronemus goramy

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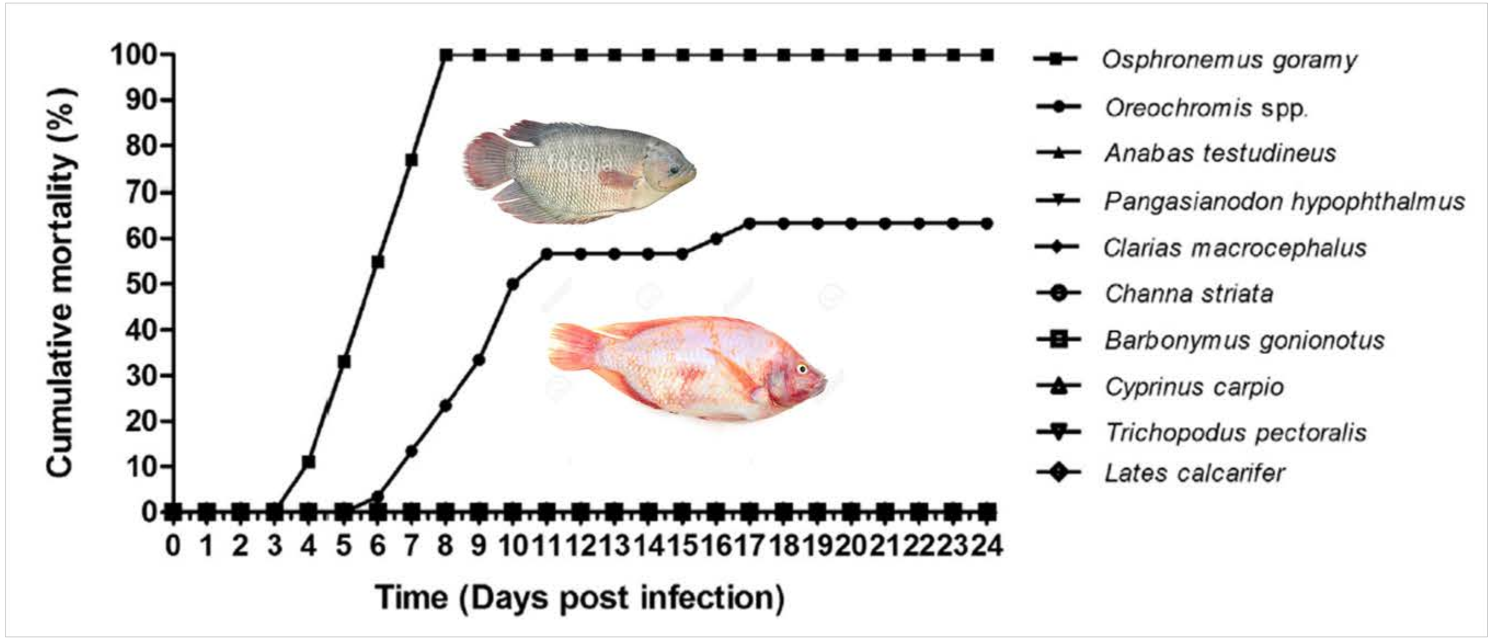
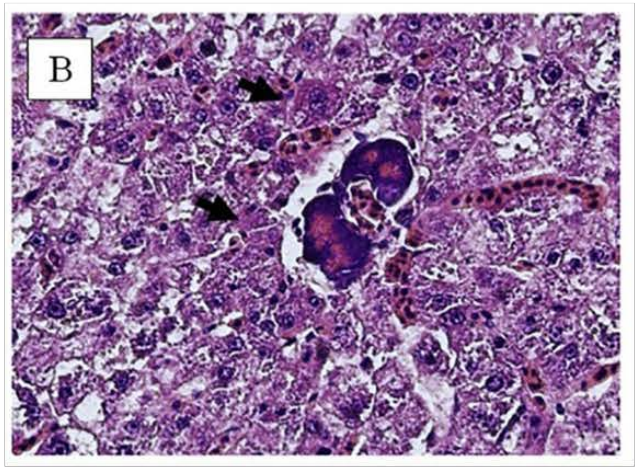
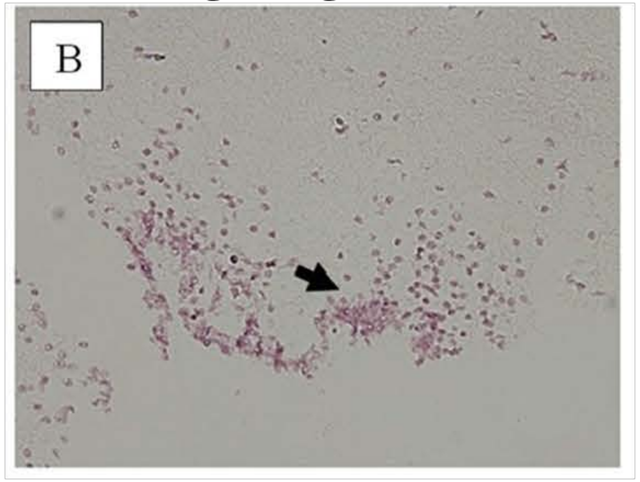
Mortality of ten species after TiLV challenge



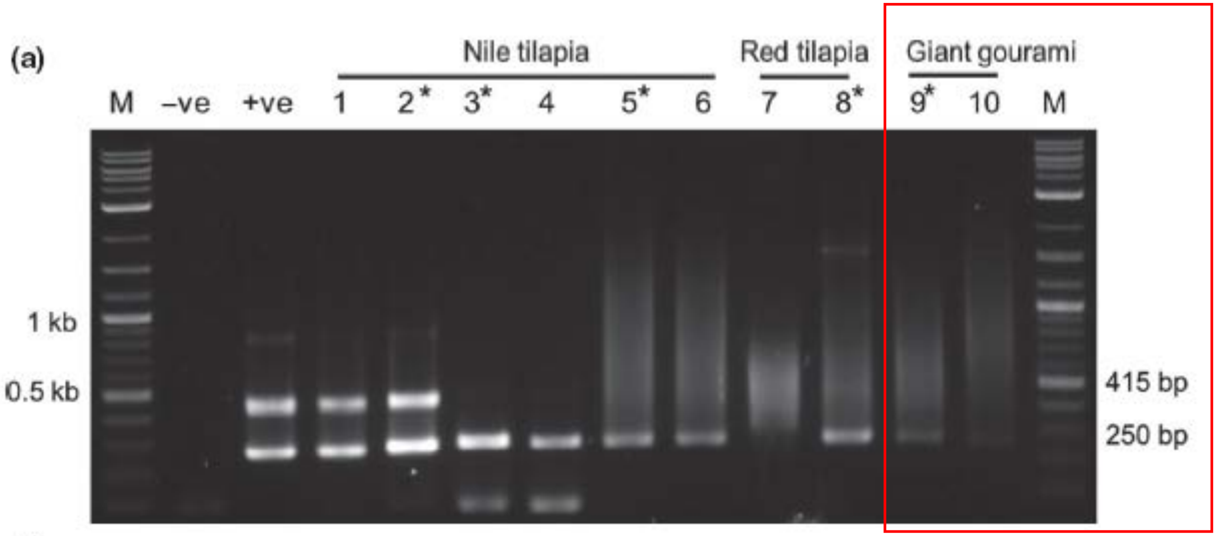


Syncytial cells in liver of giant gourami



In situ hybridization signal in the brain of infected giant gourami

TiLV was detected in blood samples of two cultured giant gourami



(b)

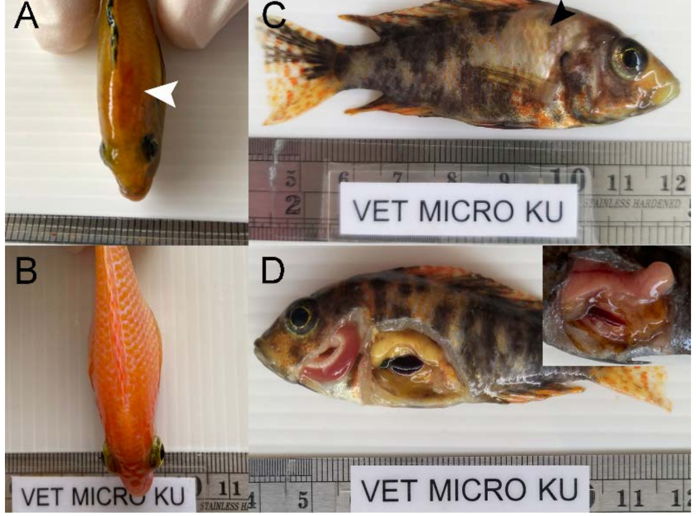
Samples	Origin	Amplicon selected for sequencing	% identity to the type strain Til-4-2001 (KU751816)
2*	Farm 1- batch 1	250 bp	97.2
3*	Farm 1- batch 2	250 bp	96.8
5*	Farm 1-batch 2	250 bp	98.0
8*	Farm 1- batch 3	250 bp	94.0
9*	Farm 1-batch 3	250 bp	97.6

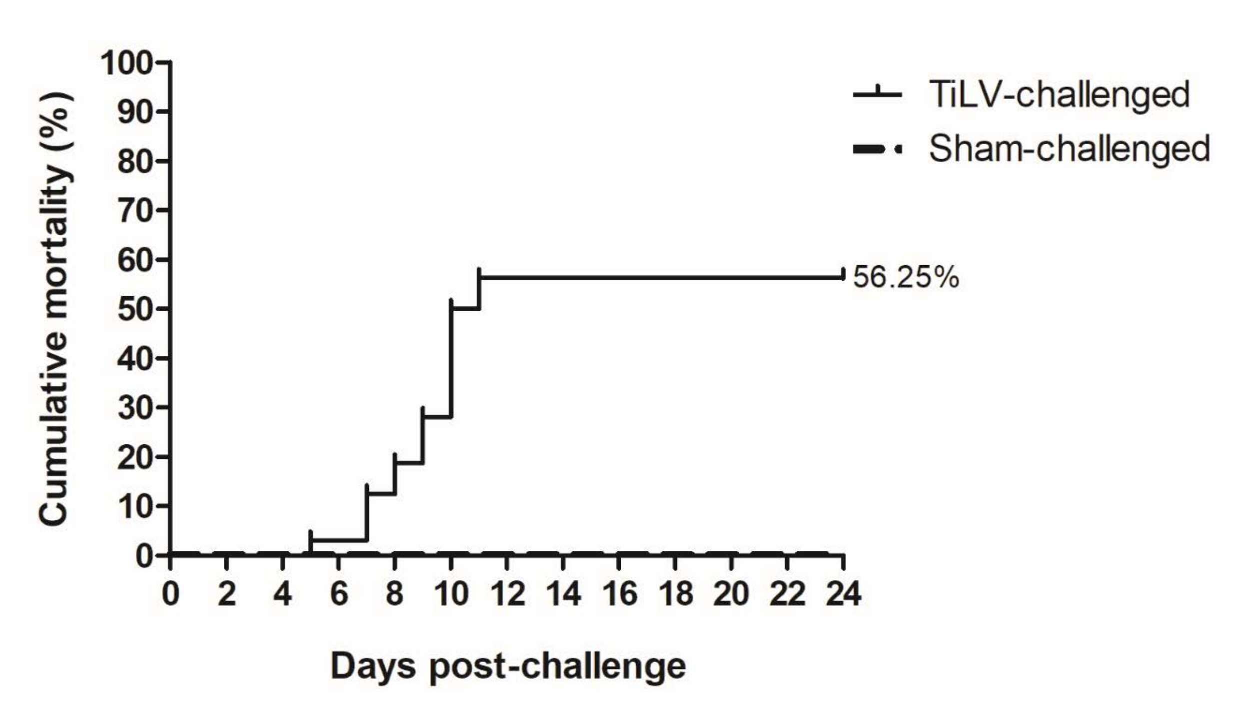
FIGURE 3 A. Representative PCR detection results of liver samples collected from Nile tilapia and blood samples collected from red tilapia and giant gourami. M, marker (NEB); -ve, no template control; +ve, positive control using RNA extracted from TiLV-infected fish as template; 1–10, tested fish samples; * represents the samples that were sent for sequencing. B. Selected 250-bp amplicons (asterisks) were sequenced, and per cent identity to the type strain Til-4-2001 (KU751816) was indicated

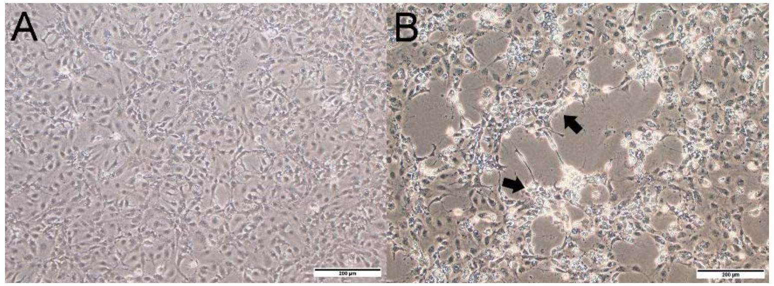
Chiamkunakorn et al 2019, J Fish Dis

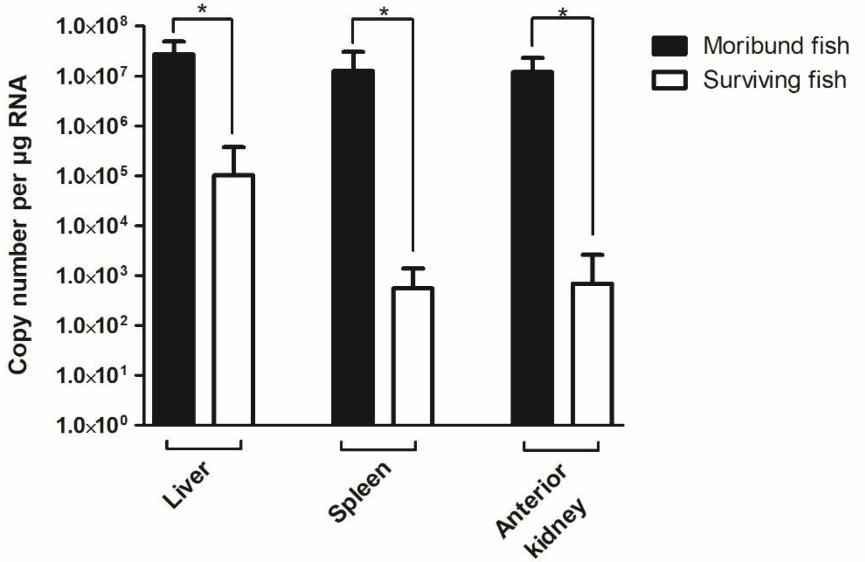
TiLV can infect ornamental African cichlids

- High mortality, virus detected in tissues









Yamkasem et al., 2021 (under review)

Additional fish species susceptible to TiLV?

Turk. J. Fish.& Aquat. Sci. 21(4), 205-209
http://doi.org/10.4194/1303-2712-v21_4_05

PROOF
SHORT PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Detection of Tilapia Lake Virus (TiLV) in Healthy Fish from the Pre-Existing Disease Environment Using Different RT-PCR Methods

Patharapol Piamsomboon[1], Janenuj Wongtavatchai[1,*]

Table 2. TiLV detection in wild Nile tilapia (*Oreochromis niloticus*, n=29), Climbing perch (*Anabas testesdineus*, n=12), snakeskin gourami (*Trichogaster pectoralis*, n=9) and farmed barramundi (*Lates calcalifer*, n=20)

Samples	TiLV RT-PCR result*
Collection Site 1	
Nile tilapia	2/5
Climbing perch	0/5
Collection Site 2	
Nile tilapia	5/5
Climbing perch	0/3
Snakeskin gourami	0/2
Collection Site 3	
Nile tilapia	0/6
Snakeskin gourami	0/4
Collection Site 4	
Nile tilapia	0/10
Collection Site 5	
Nile tilapia	0/3
Climbing perch	0/4
Snakeskin gourami	0/3
Farmed barramundi	2/20

- No virus isolation and histopathology

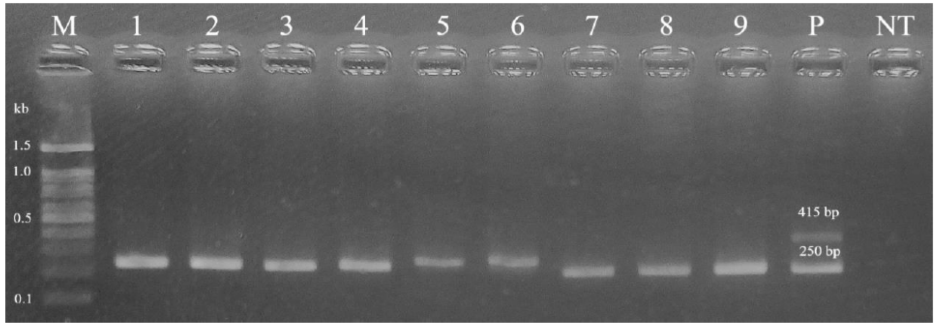


Figure 1. TiLV detection of samples obtained from Nile tilapia in the natural reservoir (Lane 1-7) and farmed barramundi (Lane 8 - 9) using semi-nested RT-PCR. M, 100 bp DNA ladder; NT, negative control; P, positive control.

SUSTAINABLE DEVELOPMENT GOALS

Received: 28 March 2018 | Revised: 29 May 2018 | Accepted: 31 May 2018
DOI: 10.1111/jfd.12843

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]

- No virus isolation and histopathology

(a) (b) (c)

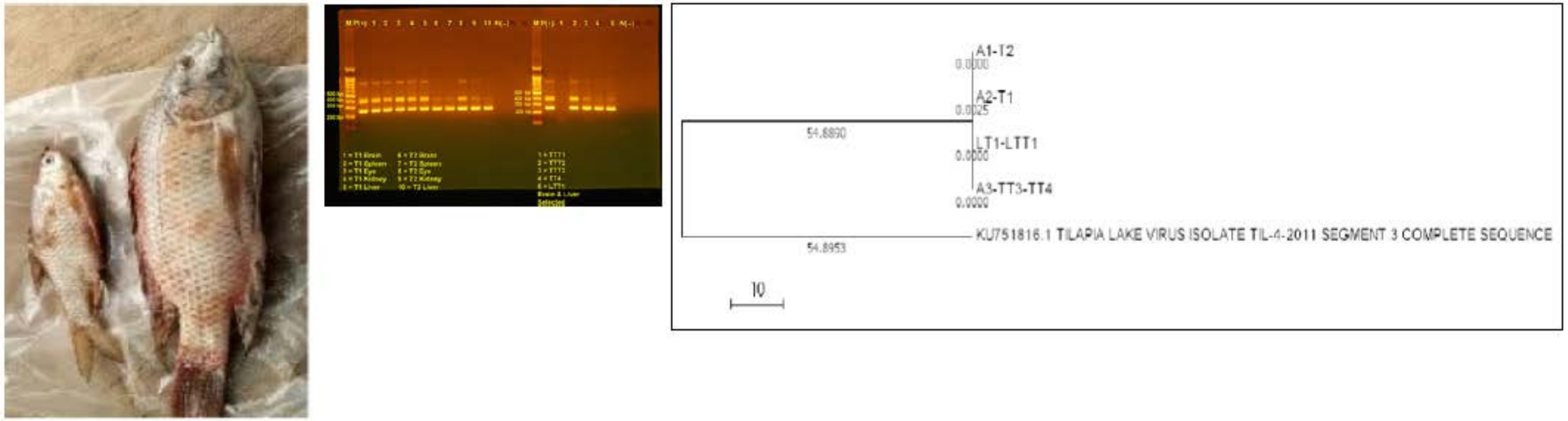


FIGURE 1 (a) Clinical signs observed in river carp (left) and wild tilapia (right) showing reddish discoloration of fins, body and scales. Photo courtesy: Perlis Biosecurity Division, Department of Fisheries Malaysia. (b) Gel electrophoresis–polymerase chain reaction (PCR) technique showed infected tilapia (TT) and river carps (LTT). N = negative control; M = DNA marker; P = positive control; 1 = TT1; 2 = TT2; 3 = TT3; 4 = TT4; 5 = LTT1. Photo courtesy (b): Lab-Ind. Resources Sdn. Bhd. (c) Phylogenetic tree showing similarity of the sequence from this study with Israel strain

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Aquaculture 515 (2020) 734567

Contents lists available at ScienceDirect

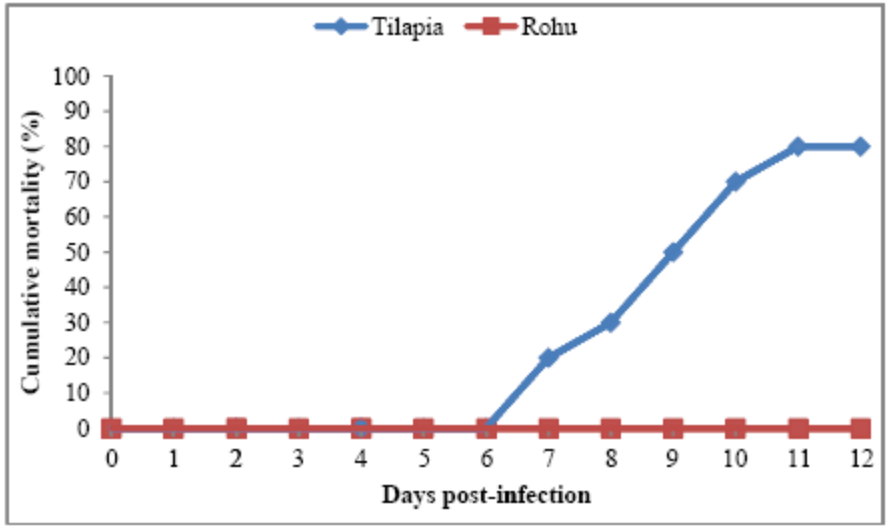
Aquaculture

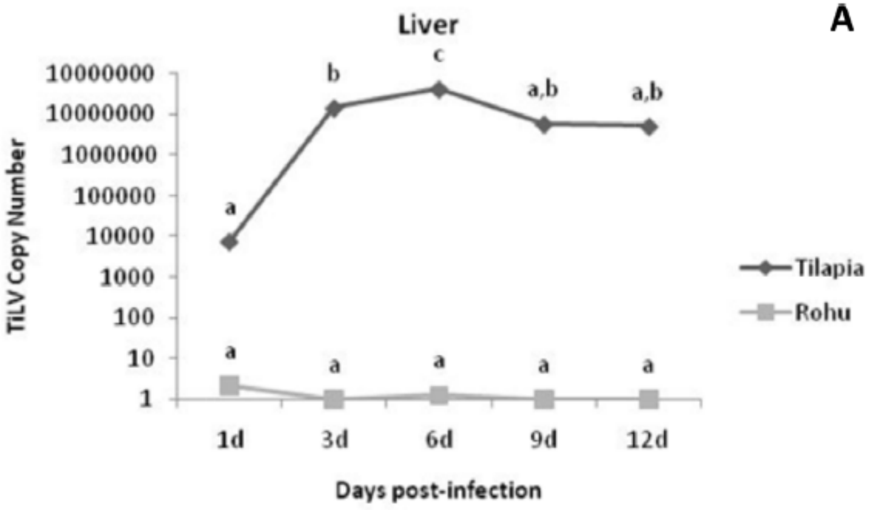
journal homepage: www.elsevier.com/locate/aquaculture

Susceptibility of Indian major carp *Labeo rohita* to tilapia lake virus

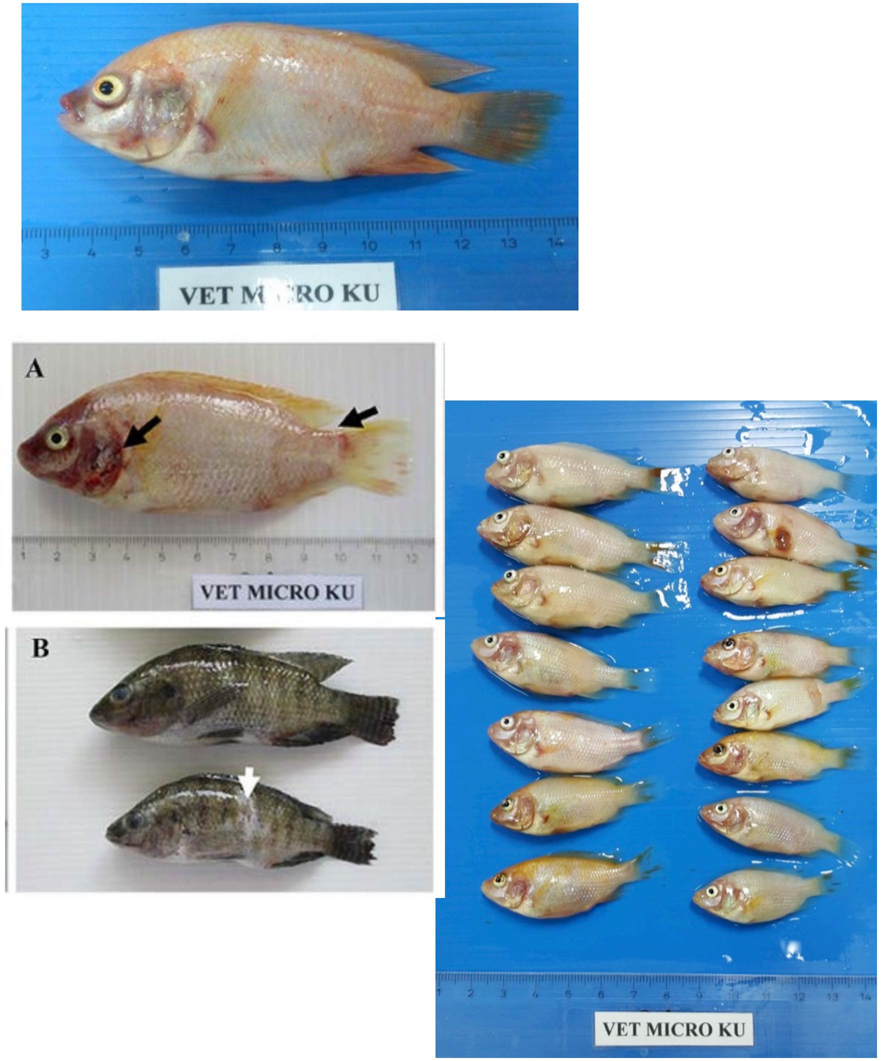
Pravata K. Pradhan[a,*,1], Anutosh Paria[a,1], Manoj K. Yadav[a], Dev K. Verma[a], Shubham Gupta[a], T.R. Swaminathan[b], Gaurav Rathore[a], Neeraj Sood[a,**], Kuldeep K. Lal[a]

- No infection in Indian major carp

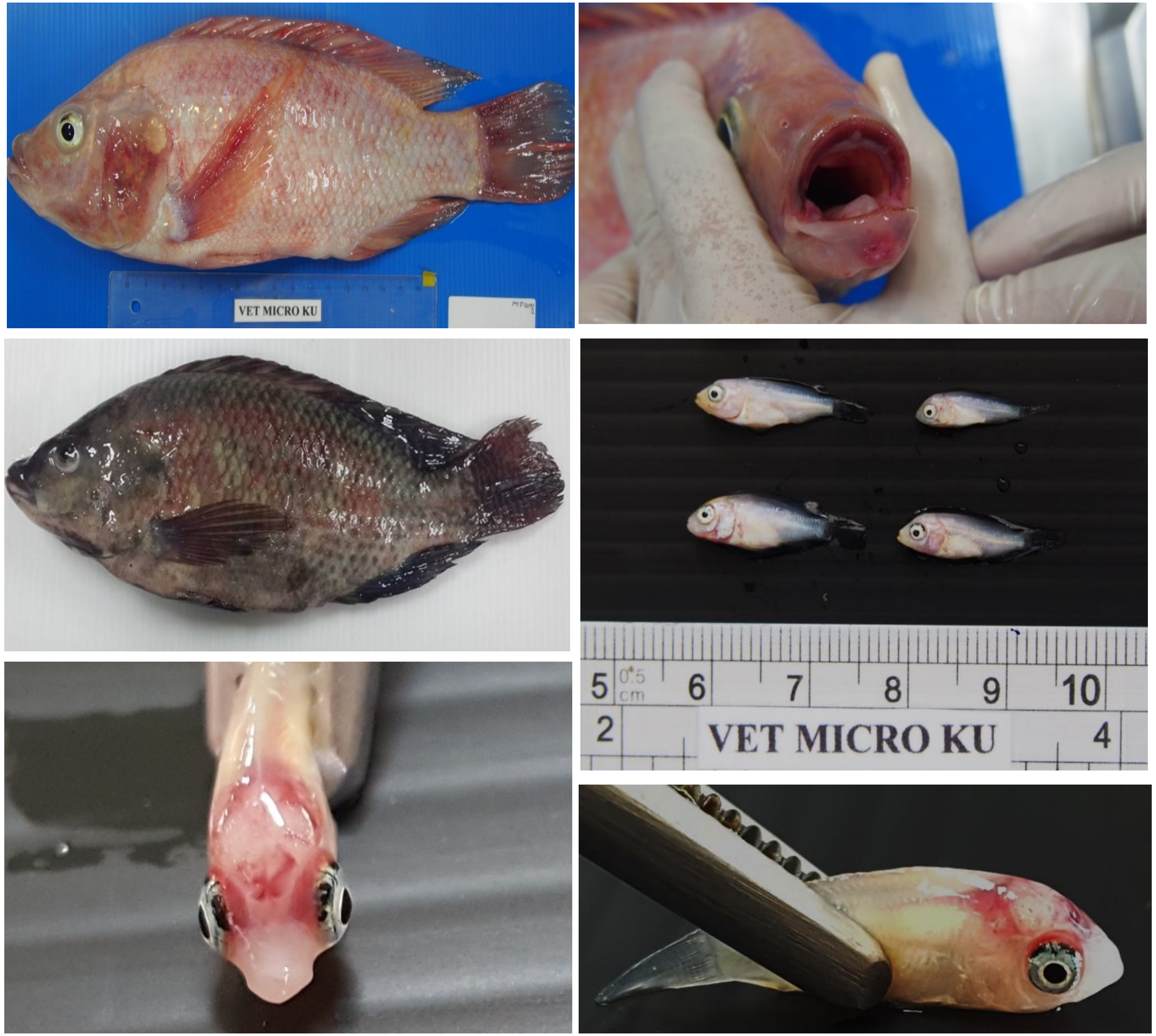




All life stages are susceptible to TiLV



Tattiyapong et al., 2017



Yamkasem et al., 2019

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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.

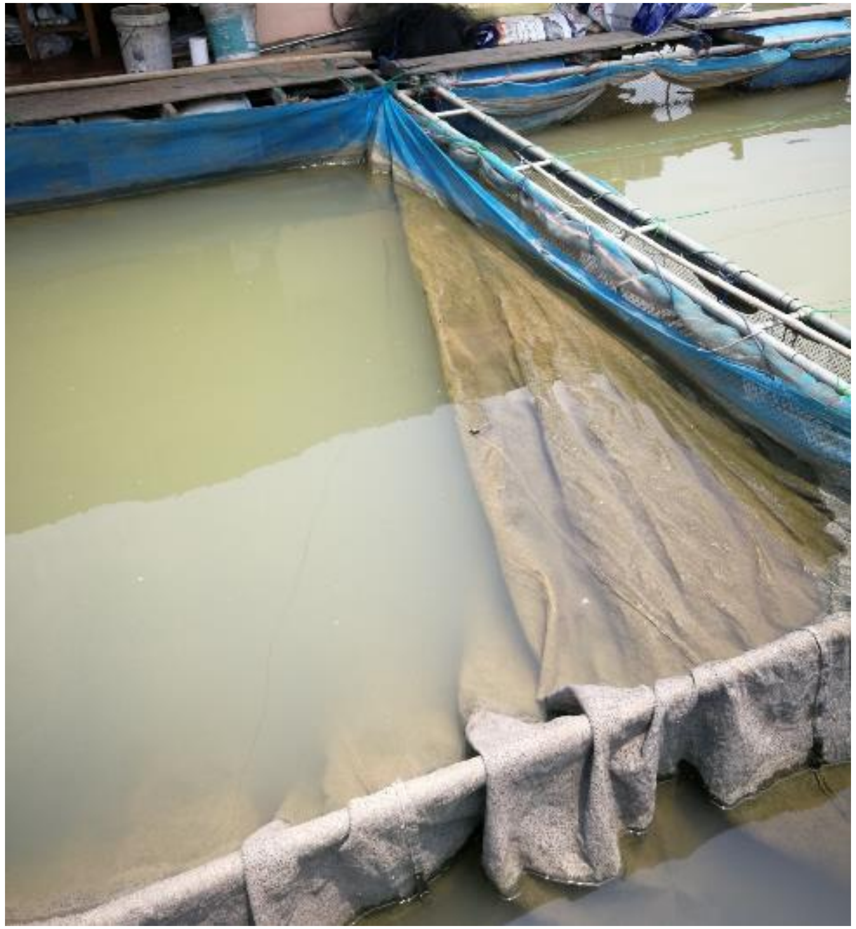
- Chitralada strain had higher risk ↑
- Stocking density ↑
- Pond production cycles ↑
- Higher initial weight ↓

Minimize handling to reduce stress that predisposes to disease

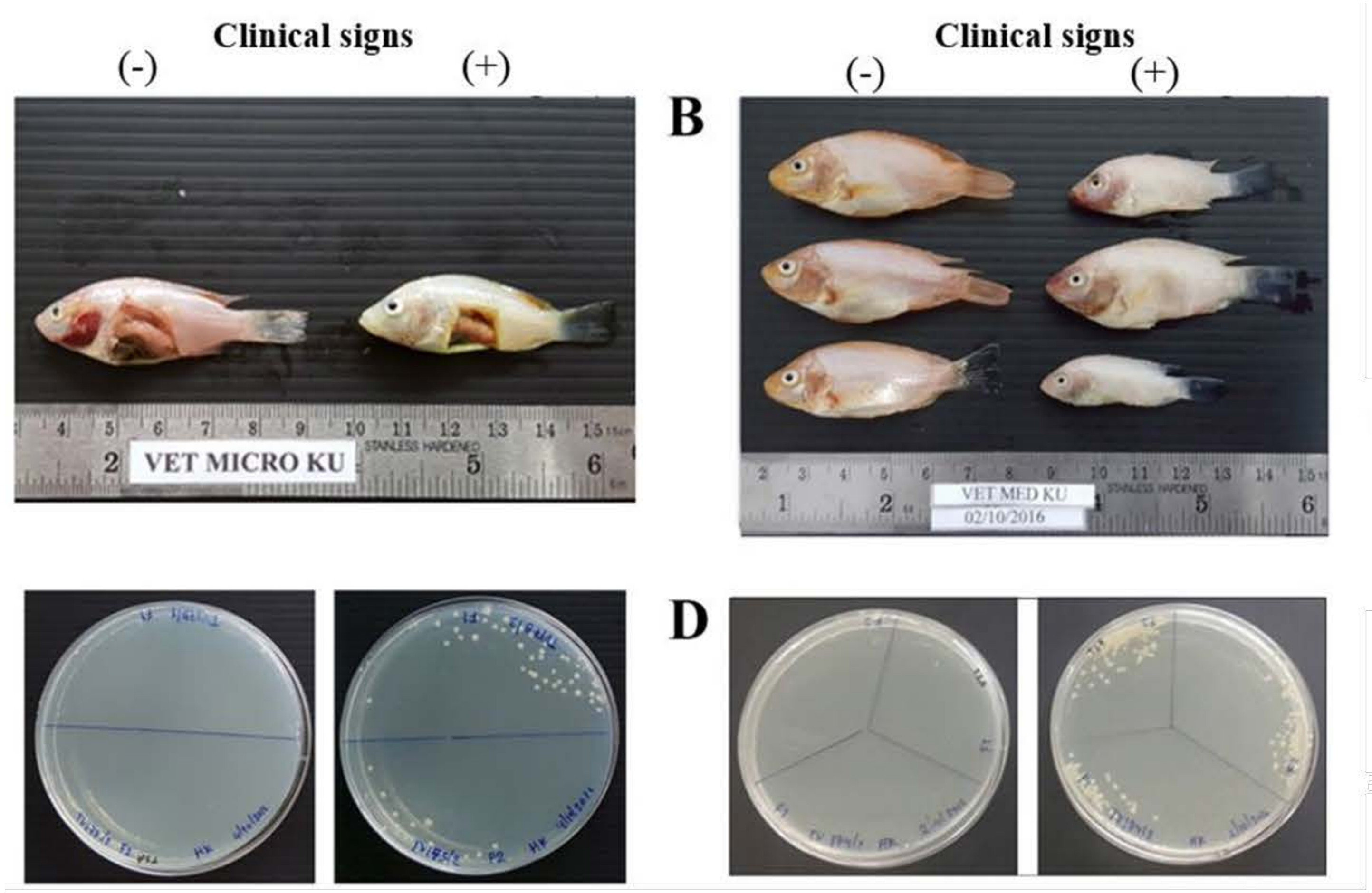
Grading or stress factors

e.g. poor water quality, overcrowding

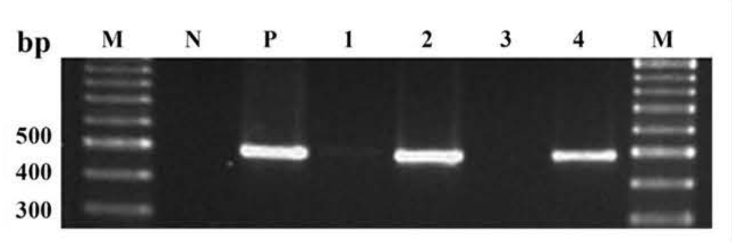
→ predispose fish to TiLV infection



Co-infections of TiLV and bacteria worsen the clinical outcome



Bacteria and TiLV are frequently found in the moribund fish.



Nicholson et al., 2020 Aquaculture. 734746

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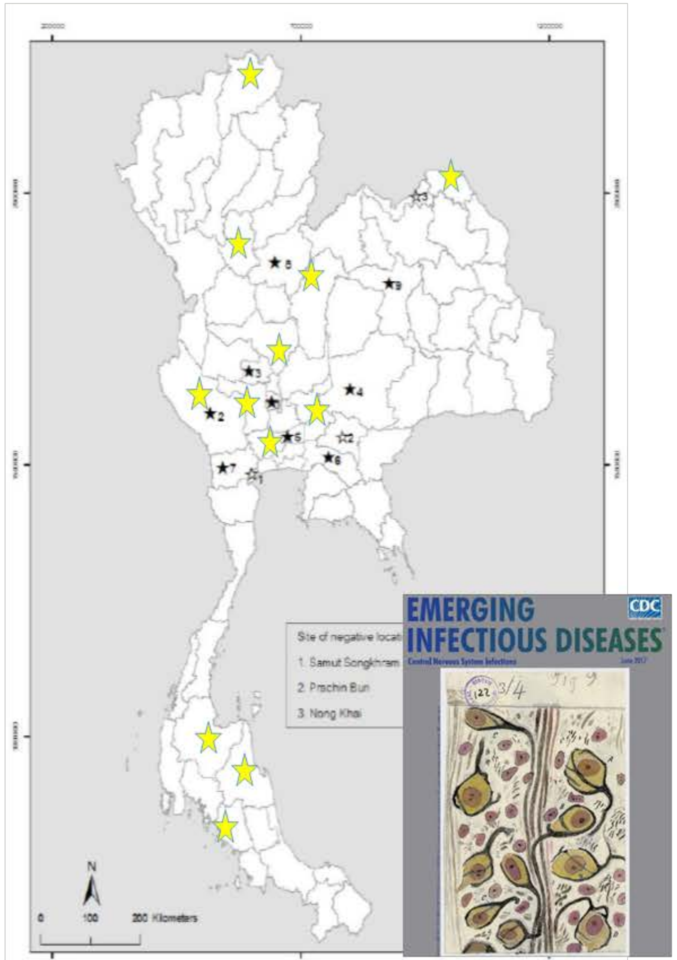
Multiple infections of TiLV and other pathogens



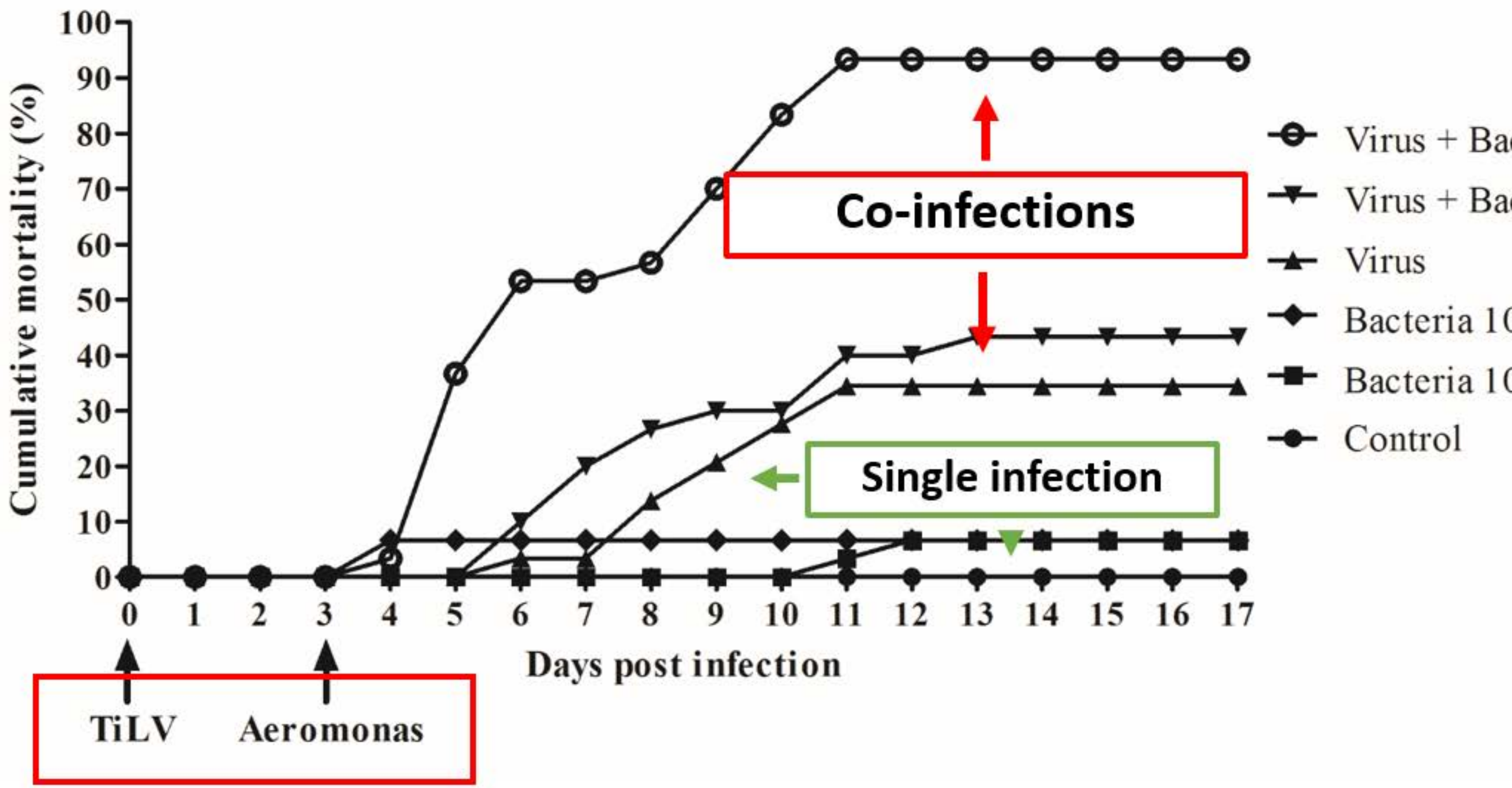
Technical Appendix Table 1. Description of TiLV outbreaks in Thailand*

Outbreak	Date	Location	Species	Laboratory diagnosis: Ectoparasite†	Laboratory diagnosis: Bacteria identification‡	Laboratory diagnosis: 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	24/05/2016	Khon Kaen	T	2+	*Aeromonas*	+ (TV7)

***Outbreaks of massive tilapia death were investigated in 9 provinces during Oct 2015 to May 2016. Epidemiologic information and laboratory findings were shown.**

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

Co-infections of TiLV and bacteria worsen the clinical outcome



Nicholson et al., 2020 Aquaculture. 734746

Environment :

- Season and climate
- Contacts, locations

Permissive temperature for TiLV

- Normal temperature for tilapia aquaculture 24-28°C
- In Israel, outbreak occurs during hot season (May to October) Eyngor et al., 2014
- TiLV associated with "Summer mortality" in Egypt
- In Thailand, the disease could be found throughout the year

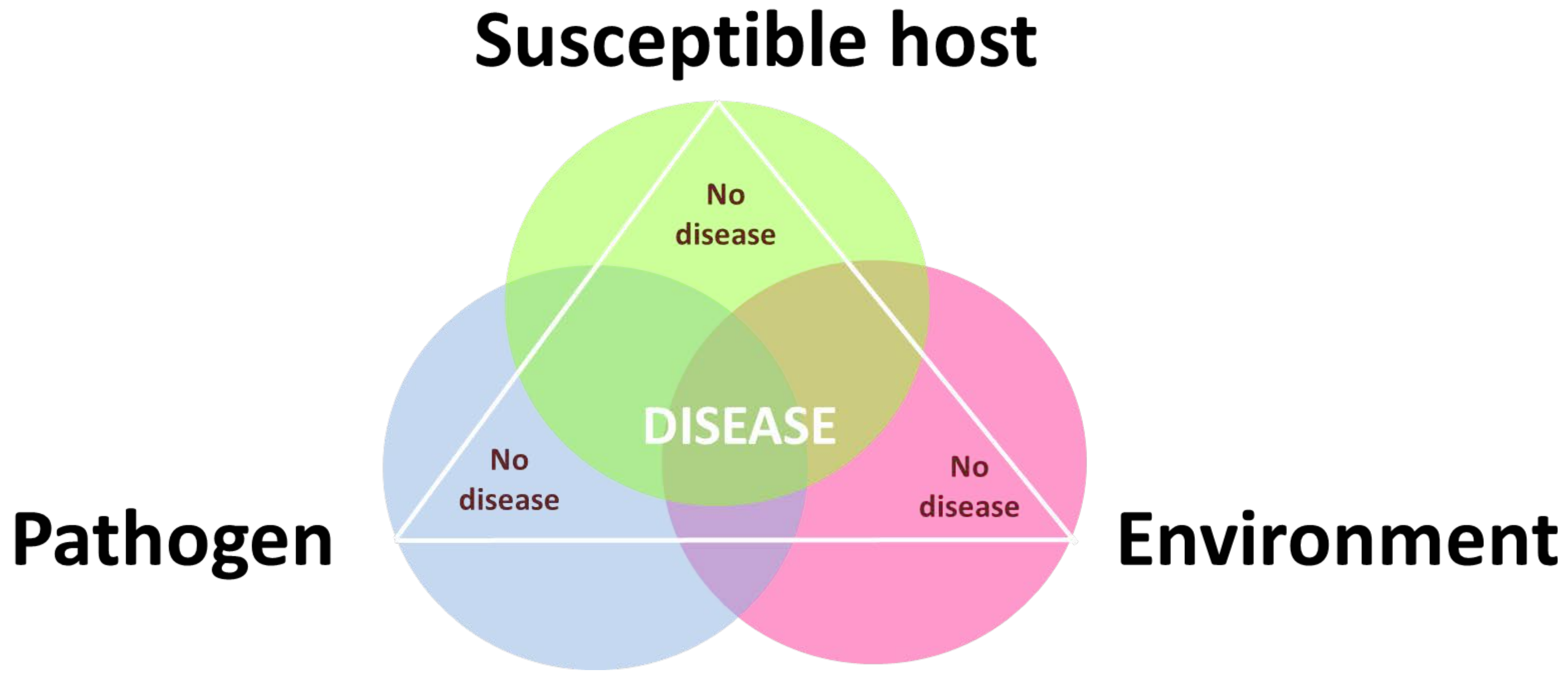
Permissive temperature for TiLV

TiLV-affected countries	Mortality impact	Onset of mortality	Susceptible life stages (weight in grams)	Susceptible temperature (Celsius scale)	References
Israel	Mass mortality	N/A	N/A	22–32°C	Eyngor et al. (2014)
Ecuador	>80%	4–7 days post-transfer from hatchery to a farm facility	3 g	25–27°C	Ferguson et al. (2014)
	90%	N/A	3 g	N/A	Del-Pozo et al. (2017)
Egypt	5%–15%	N/A	>100 g	>25°C	Fathi et al., (2017)
Thailand	20%–90%	Peak in 14 days	1–50g	N/A	Surachetpong et al., (2017)
	20%–90%	N/A	Fertilized egg, yolk sac larvae, fries, and fingerlings	N/A	Dong, Ataguba, et al. (2017))
Philippines	33.79%	N/A	Fingerlings	N/A	OIE (2017a)
Chinese Taipei	6.40%	N/A	N/A	N/A	OIE (2017b)
Malaysia	0.7%–15%	N/A	N/A	N/A	OIE (2017c)
	25%	Peak in 5–9 days after the first death	7–20 g	N/A	Amal et al. (2018)
India	80%–90%	N/A	20–80 g	N/A	Behera et al. (2018)
Mexico	0%–2.71%	N/A	N/A	N/A	OIE (2018)
Peru	100%	N/A	N/A	N/A	OIE (2018c)
	Low mortality	N/A	Fingerlings < 2g, 80 g	N/A	Pulido et al. (2019)

Surachetpong et al., 2020 J Fish Dis

Impact of environment and farm locations

The disease triangle

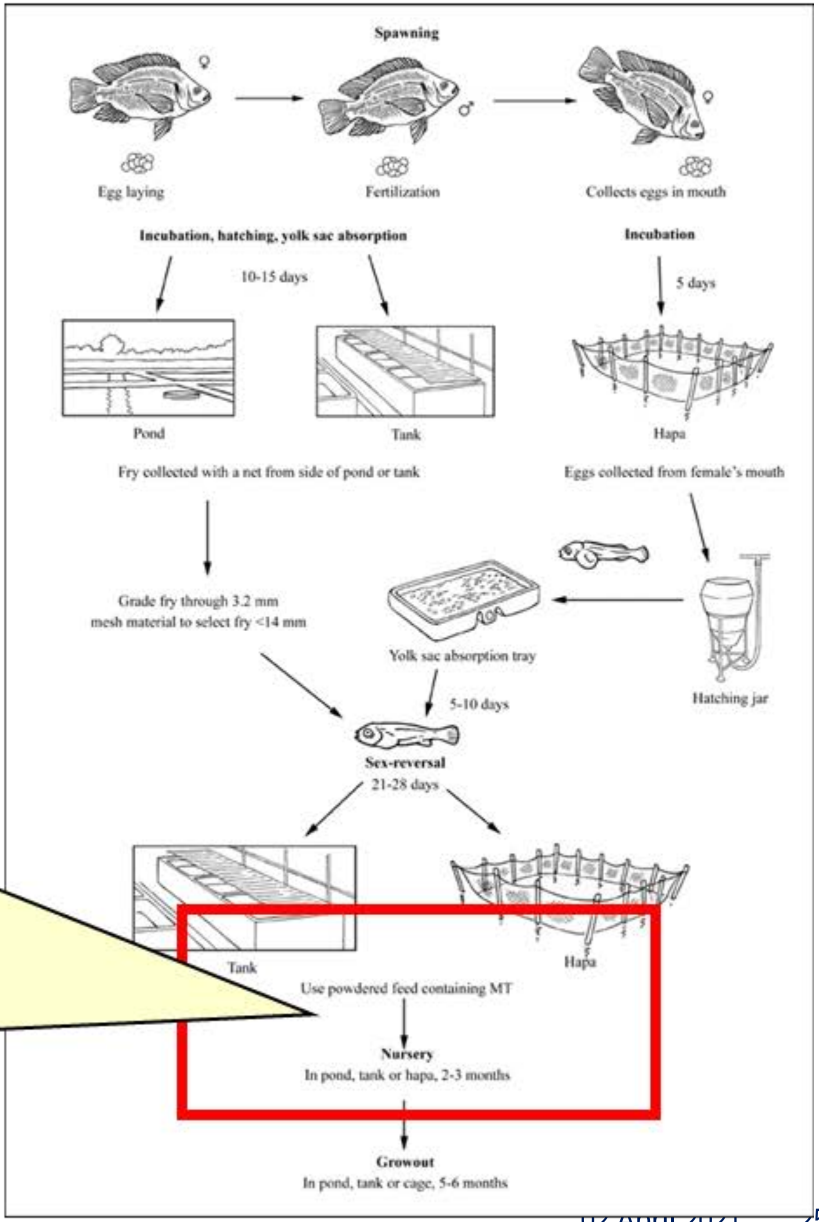


Critical control points

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



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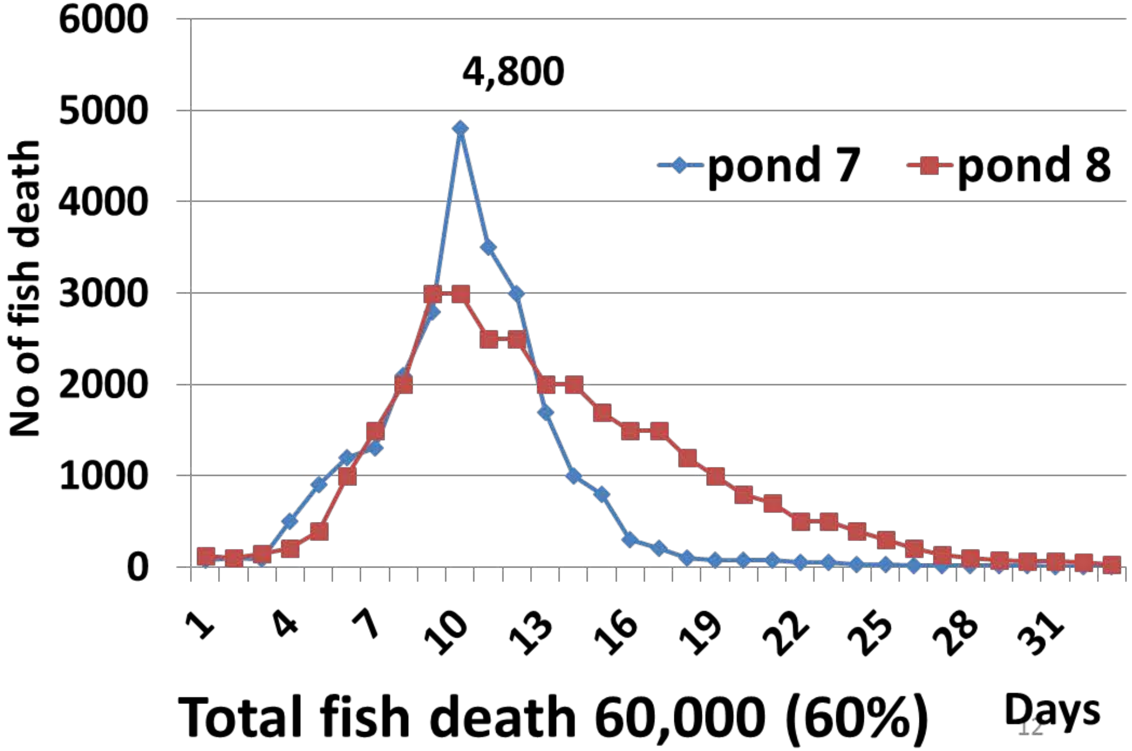
Tilapia One Month Mortality Syndrome (TOMMS)

Months in cage/pond culture

0 1 2 3 4 5-8

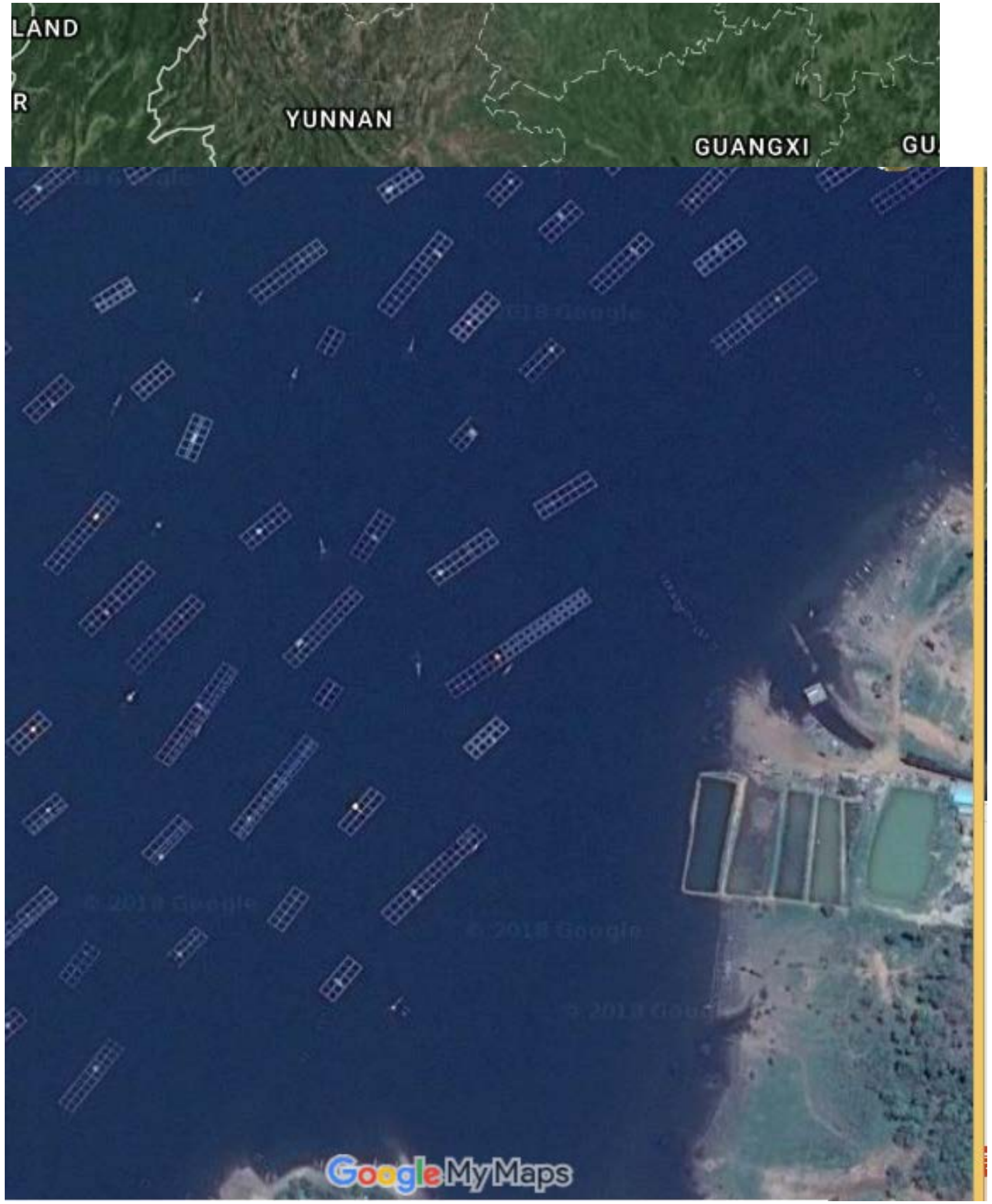
2-30 g 800-1,000 g

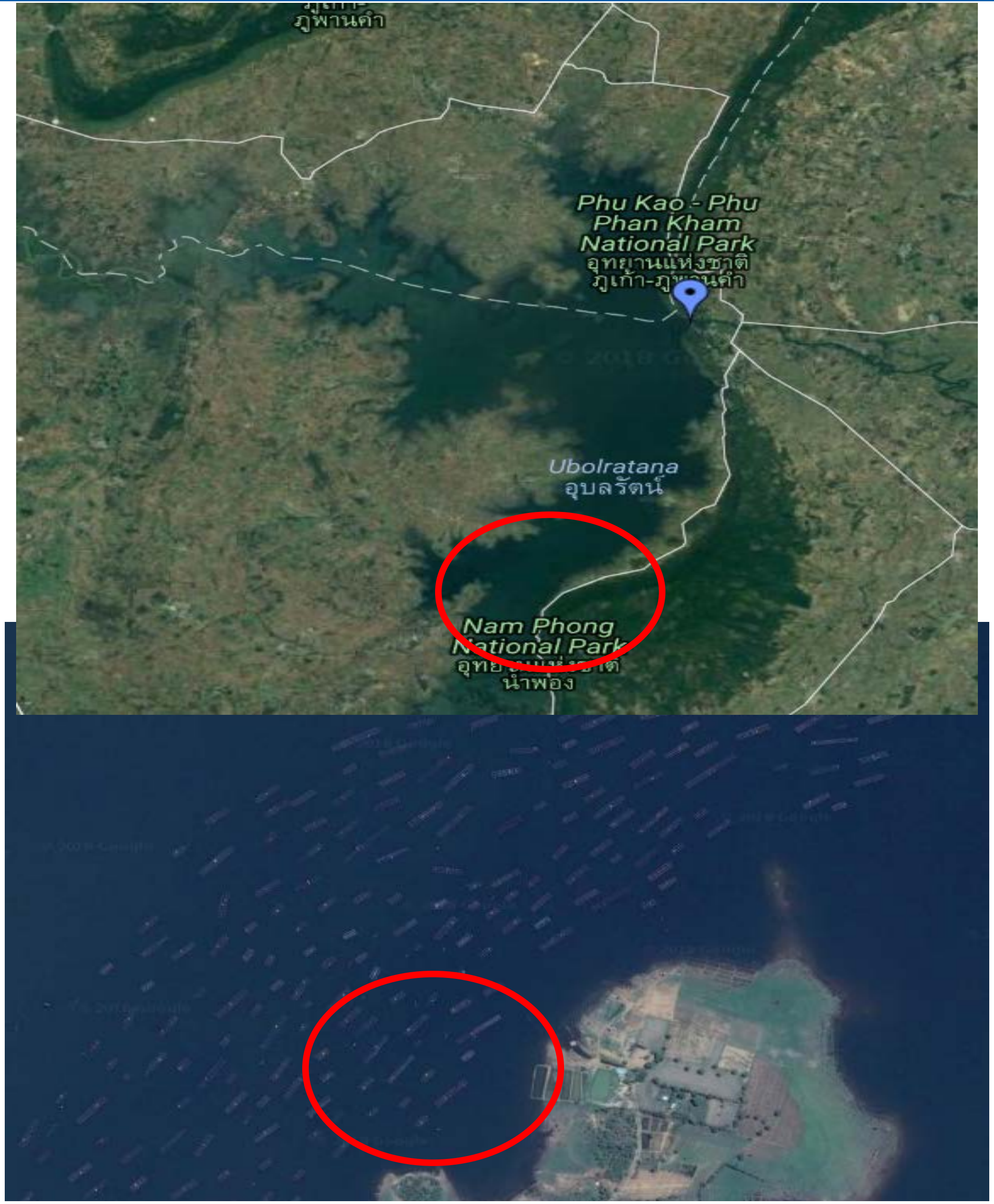
Pattern of fish death



Total fish death 60,000 (60%)

Important of farms location and disease spreading





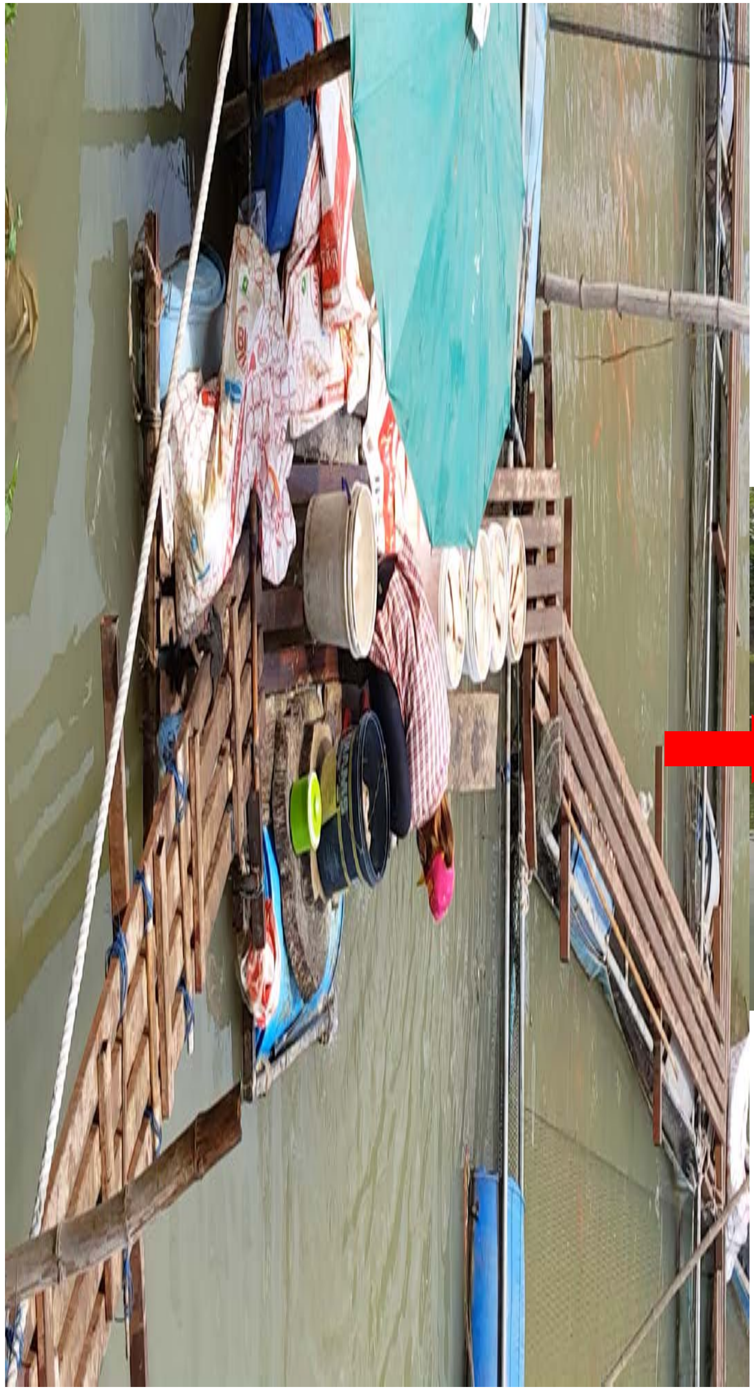
Stocking fish at different ages/size

Disease circulation in the farm/environment

Farm with good biosecurity is less likely to have TiLV

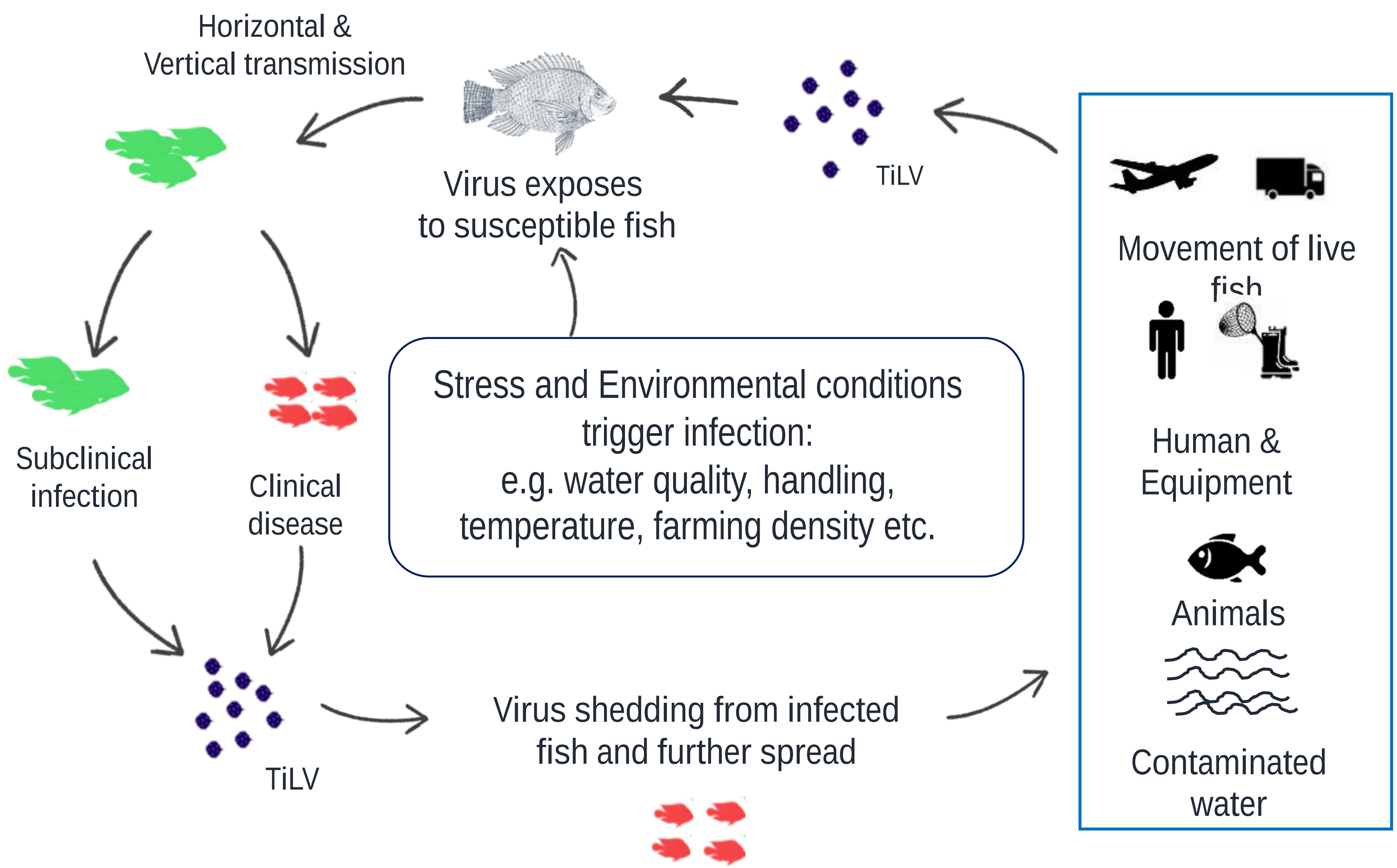
Farm with clear boundary

Surrounding wall



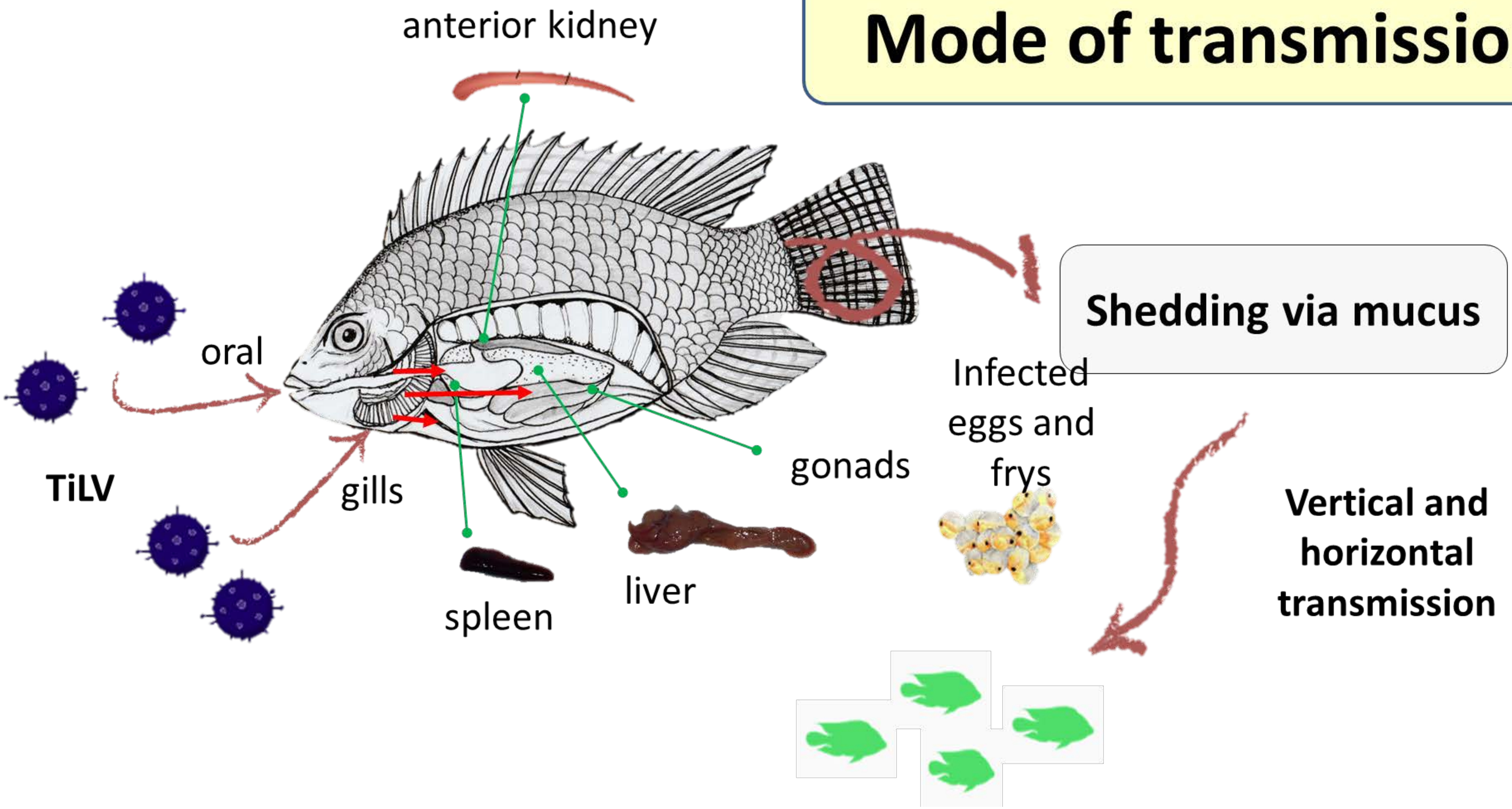
Important of Biosecurity

TiLV distribution and risk of disease introduction in fish farms



Surachetpong et al., 2020 J Fish Dis

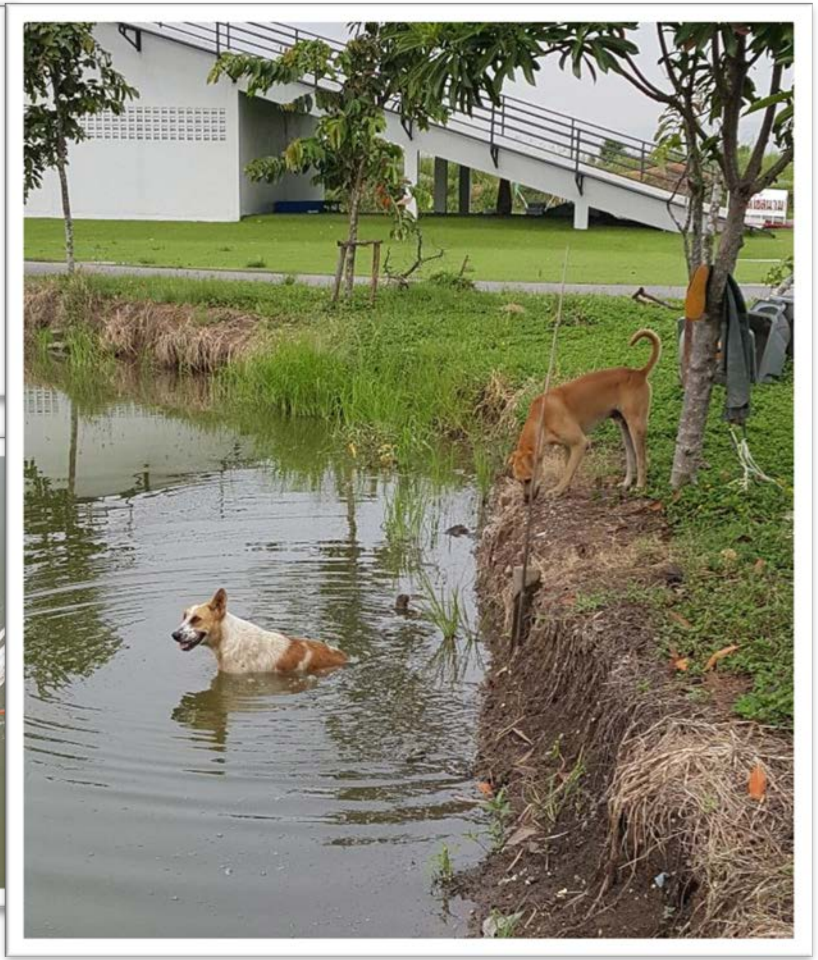
Mode of transmission



Surachetpong et al., 2020 J Fish Dis

How quickly the farmers manage moribund/dead fish

Transmission by vectors or carriers?



Spread the virus?

No detection of TiLV in fish parasite and mollusk

(manuscript in preparation)

Agent (TiLV):

- Virulence
- Survivability

Sequence comparison between Thai and Israel TiLV

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Isael       TTGCTCTGAGCAAGAGTACCAGCAGATTTGTAAGGTACAATTCAAGGATTATTTGGAGAT  60
Thailand    TTGCTCTGAGCAAGAGTACCAGCAGATTTGTAAGGTACAATTCAAGGATTATTTAGAGAT
            ****************************************************** *****

Isael       CGACGGGGTTGTTAAAGTTGGGCACAAGGCATCCTACGATGCTGAGCTAAGGGAACGGCT  120
Thailand    CGACGGGGTTGTTAAAGTTGGGCACAAGGCATCCTACGATGCTGAGCTAAGGGAACGGCT
            ************************************************************

Isael       ATTGGAACTACCACATCCAAAGAGTGGCCCGAAGCCTCGTATTGAGTGGGTGGCACCACC  180
Thailand    ATTGGAACTACCACATCCAAAGAGTGGCCCGAAGCCTCGTATCGAGTGGGTGGCACCACC
            ****************************************** *****************

Isael       CAGACTTGCGGACATATCCAAGGAAACAGCTGAGCTAAAGAGGCAATATGGATTCTTCGA  240
Thailand    CAGACTTGCGGACATATCCAAGGAGACAGCTGAGCTAAAGAGGCAATATGGATTCTTCGA
            ************************ ***********************************

Isael       GTGCTCAAAGTTCCTCGCCTGCGGTGAGGAGTGTGGTCTTGACCAAGAGGCAAGAGAACT  300
Thailand    GTGCTCAAAGTTCCTCGCCTGCGGTGAGGAGTGTGGTCTTGACCAAGAGGCAAGAGAGCT
            ********************************************************* **

Isael       TATACTGAACGAGTACGCACGTGATAGAGAATTTGAGTTCCGCAATGGAGGGTGGATACA  360
Thailand    TATACTAAACGAGTACGCACGTGATAGAGAATTTGAGTTCCGCAACGGAGGGTGGATACA
            ****** ************************************** **************

Isael       AAGGTATACAGTTGCTTCTCACAAGCCTGCTACACAGAAGATATTACCTCTACCGGCTAG  420
Thailand    GAGGTATACAGTTGCTTCCCATAAGCCTGCTACACAGAAGATATTACCTCTACCGGCTAG
            ****************** ** **************************************

Isael       TGCTCCACTTGCTCGTGAGCTTTTGATGTTGATTGCTAGAAGCACAACTCAGGCAGGGAA  480
Thailand    TGCCCCACTTGCTCGTGAGCTTTTGATGTTGATTGCTAGAAGCACAACTCAGGCAGGGAA
            *** ********************************************************

Isael       AGTACTGCATA
Thailand    AGTACGGCATA
            ***** *****
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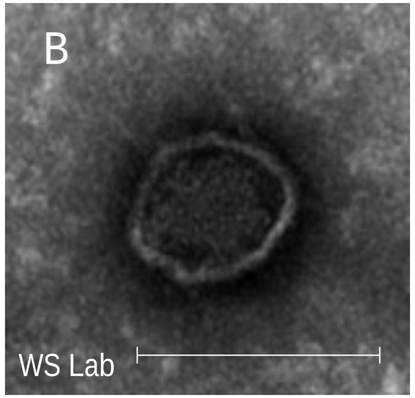
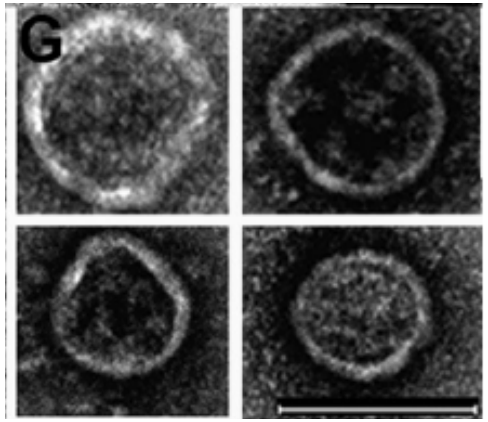
98% identity



Thai isolate

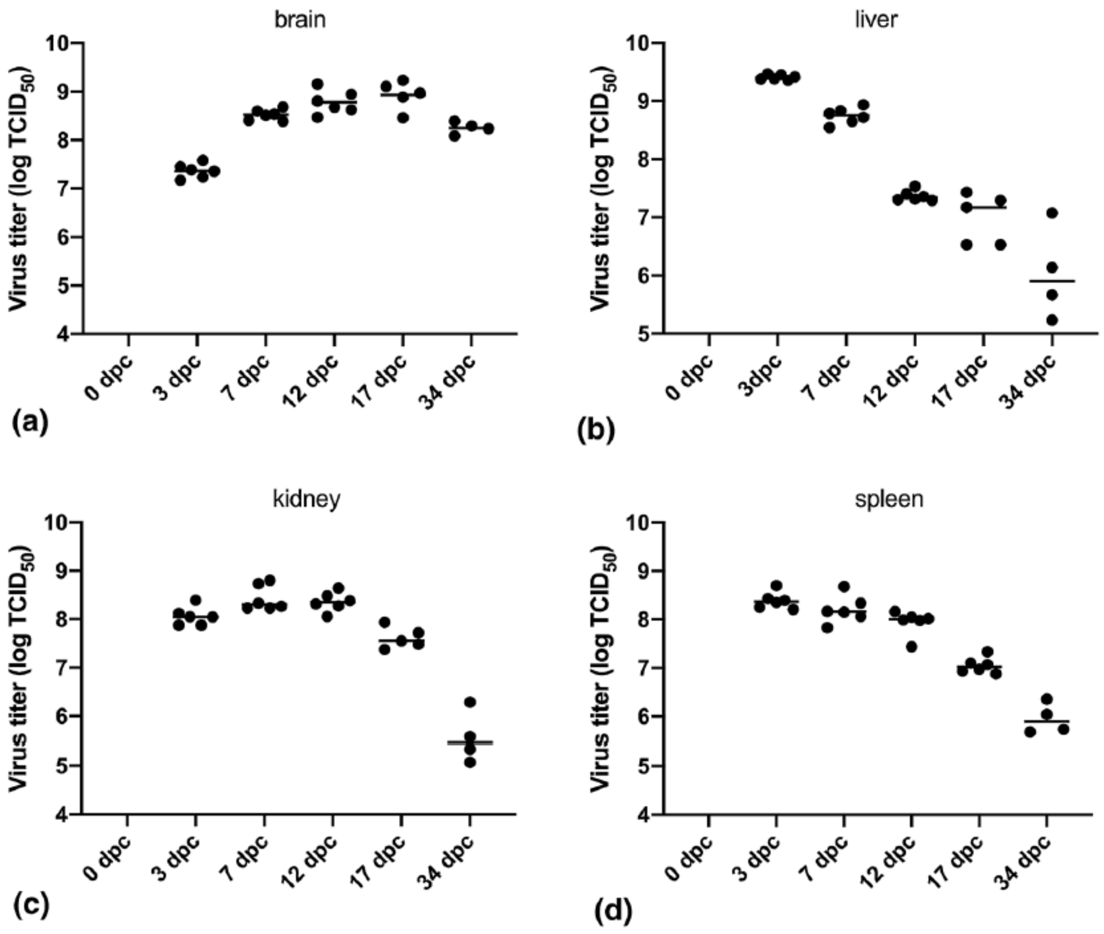


Israel isolate

Surachetpong et al., 2017 Emerg Infect Dis

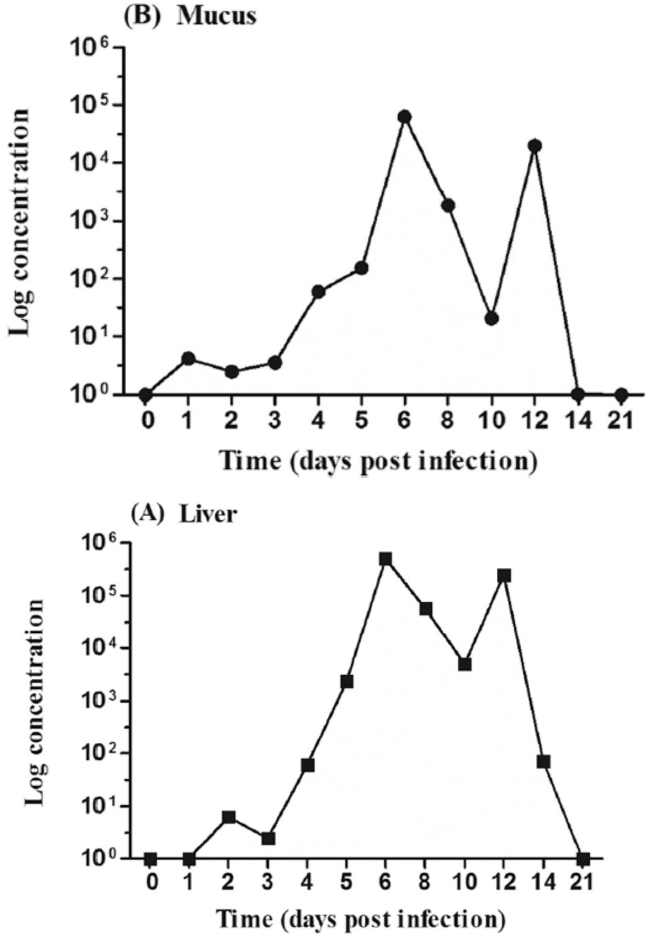
Persistence of TiLV in fish and survival in environment

TiLV persists in multiple organs of challenged fish until 34 days



Mugimba et al., 2020 Sci Reports

TiLV could be detected in mucus of cohabitation fish until 12 days



Liamnimitr et al., 2018 Aquaculture

SUSTAINABLE DEVELOPMENT GOALS

TiLV is detected in faeces and water of TiLV challenge fish/tank.

TABLE 2 Quantification of tilapia lake virus in faeces and water of intragastric and intracoelomic injection-exposed fish 10 days post-infection

Sample ID	Status	Cycle threshold	LOG_{GE}/1 ng RNA
Faeces IC_{ch} 7	Moribund	20.18	5.71
Faeces IC_{ch} 8	Survivor	23.76	4.72
Faeces IC_{ch} 9	Survivor	19.96	5.77
Faeces IC_{ch} 10	Survivor	19.90	5.78
Faeces IG_{ch} 4	Dead	25.98	4.11
Faeces IG_{ch} 5	Survivor	22.77	4.99
Faeces IG_{ch} 6	Survivor	ND	ND
Faeces IG_{ch} 7	Survivor	22.39	5.10
Faeces IG_{ch} 8	Survivor	29.84	3.05
Faeces IG_{ch} 9	Survivor	31.40	2.62
Faeces IG_{ch} 10	Survivor	ND	ND
Water IC_{ch}	NA	31.95	2.30
Water IG_{ch}	NA	32.56	2.47
Water IC_{con}	NA	ND	ND
Water IG_{con}	NA	ND	ND

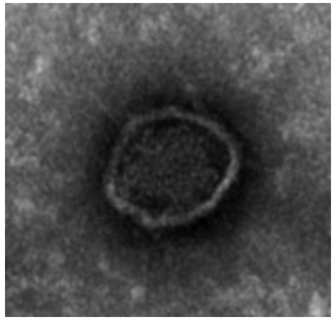
Abbreviations: IC_{ch}, intracoelomic challenge; IC_{con}, intracoelomic control; IG_{ch}, intragastric challenge; IG_{con}, intragastric control; NA, not applicable; ND, no fluorescence detection.

Conclusion

- TiLV risk profile

  - Host: Susceptible species, life stage

  - Environment: Stress, temperature and factors that affect the disease progression



  - Agent: Virulence and Persistence in fish and environment

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

Thank you for your attention!

Win Surachetpong

fvetwsp@ku.ac.th

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Norad

TCP/INT/3707:
Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus