

Ranaviruses in Frogs and Fish in Southeast Asia

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Emerging disease

Iridovirus/ranavirus diseases have been reportedly isolated from fishes and amphibians in the region;

- **Ranavirus** disease in guppy fish and dwarf gourami in Singapore;
- **Ranavirus** disease in goldfish, marble goby and frog in Thailand (1998-2003);
- **Ranavirus** disease in frog in Guangdong and Hainan (Weng *et al.*, 2002) and soft-shelled turtle in Shenzhen, China (Chen *et al.*, 1999).

Emerging disease > Notifiable disease

- *Ranavirus* can infect both fishes and frogs and parts of their major capsid protein gene sequence analysis are similar (Kanchanakhan *et al.*, 2002; Prasankok *et al.*, 2002; Weng *et al.*, 2002).
- Ranavirus is listed as notifiable disease under the OIE.

Isolation of a FV3-like iridovirus from a cutaneous ulceration and systemic inflammation with exuberant hematopoiesis or CSE disease of cultured frog, *Rana tigrina* Cantor, in Thailand

Somkiat Kanchanakhan, U-malai Saduakdee,
Aranya Kreethachat & Supranee Chinabut

Aquatic Animal Health Research Institute (AAHRI),
DOF

- 'ulcerative disease of cultured tiger frog' (Kanchanakhan *et al.*, 1999)

Cutaneous ulceration and Systemic inflammation with Exuberant hematopoiesis



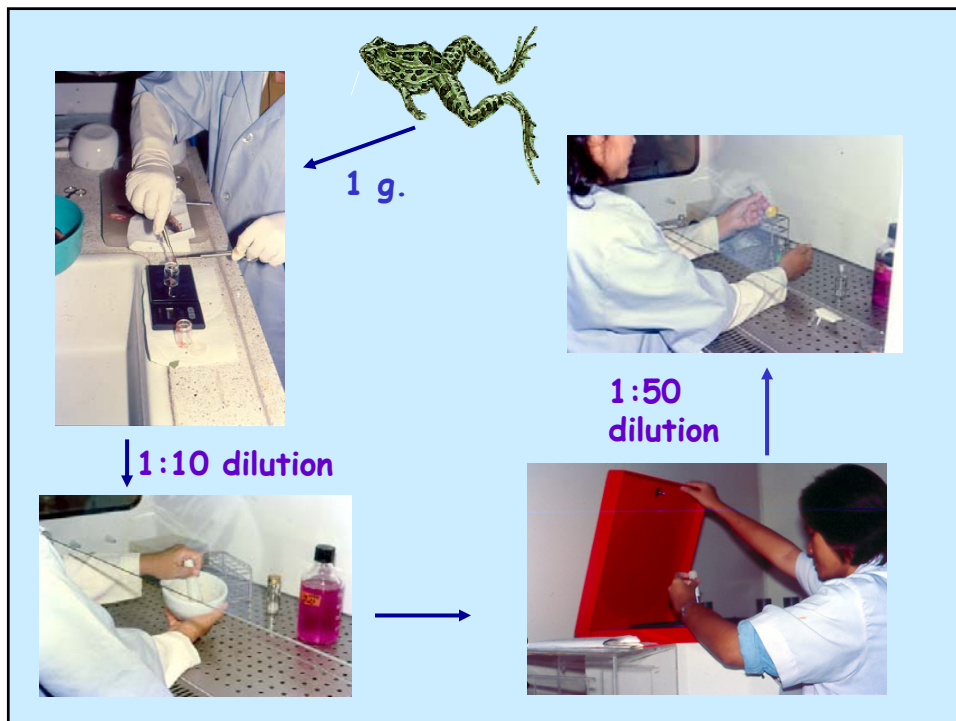
Results

- Bacterial isolation; **negative** from 7/7 frogs
- Histopathological changes
 - **Skin**; chronic excavating processes producing ulcers
 - **Heart**; congestion, small to moderate mix population of mostly mature white blood cells including monomorphic and polymorphic nuclear leukocytes, macrophages and eosinophilic granulocytes in the circulation
 - **Spleen**; focal necrosis and chronic response from macrophages and lymphocytes

- **Livers**; congestion with small infiltration of mixed leukocytes. Many large blast-like leukocytes have mitotic figures, some with abnormal extrusion of chromatin

RTL 6873 – 6883; Cutaneous ulceration and Systemic inflammation with Exuberant hematopoiesis.

The Registration of Tumors in Lower Vertebrate Animals,
Dept of Pathology, The George Washington University.



Extracts inoculation on to EPC cells at 25C



Results (cont.)

- Viral isolation; **positive** 70 / 107 tissue extracts (65% prevalence) from diseased frog/tadpole.
- **Round CPE on EPC cells within 2-4 days**
- Viruses isolated from 8/9 Provinces in Central Thailand.

Viral isolation results

Code	Province	Date	Size frog	Virus/extracts
AV9803	Bkk	1/98	LF	2/2
AV9806	Bkk	2/98	LF	1/9
AV9829	Nonthaburi	7/98	LF	11/11
AV9830	Nonthaburi	7/98	LF	0/3
AV9835	Ayuthaya	8/98	LF	2/3
AV9837	Nonthaburi	9/98	LF	2/7
AV9838	Nonthaburi	9/98	LF	5/5
AV9842	Pathumthani	9/98	LF	0/3
AV9843	Nonthaburi	10/98	LF	1/5

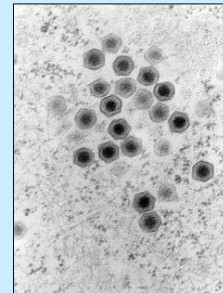
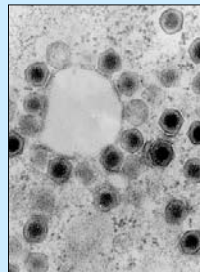
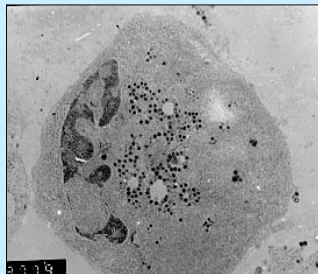
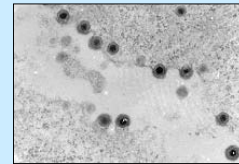
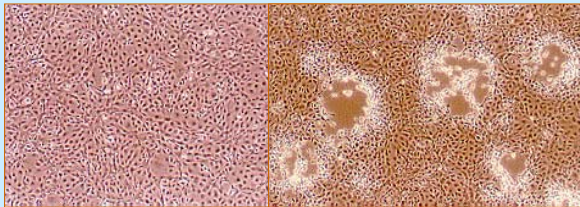
Viral isolation results (cont.)

Code	Province	Date	Size frog	Virus/extracts
AV9844	Nakonayok	10/98	LF	5/5
AV9845	Aungthong	11/98	LF	8/10
AV9905	Nonthaburi	2/99	SF	3/6
AV9909	Nonthaburi	3/99	T	5/5
AV9910	Nonthaburi	3/99	T	3/11
AV9914	Suphanburi	4/99	LF	3/3
AV9917	Suphanburi	6/99	LF	11/11
AV9922	Pichit	6/99	LF	3/3
AV9926	Rachaburi	7/99	LF	5/5
Total	8/9 Provinces			70/107

Diseased frogs and Tadpoles



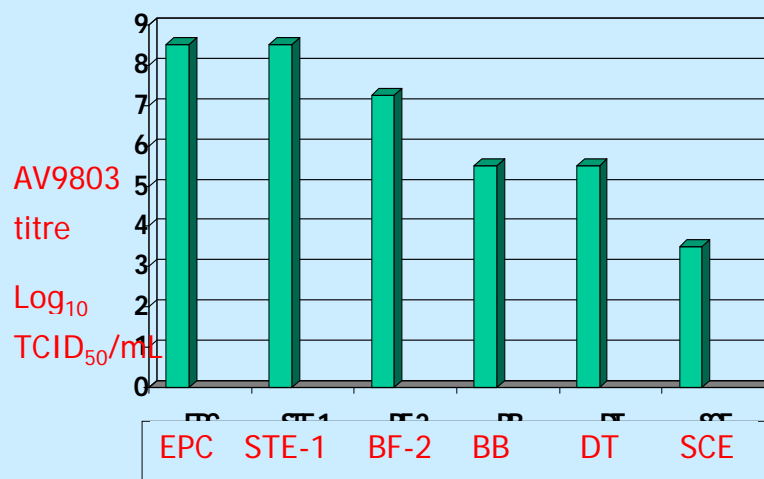
CPE and viral particle



Physico-chemical properties

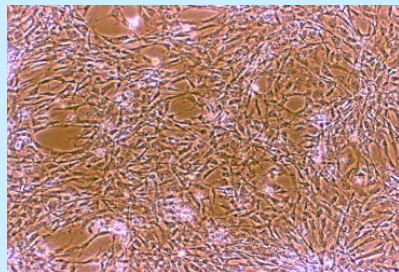
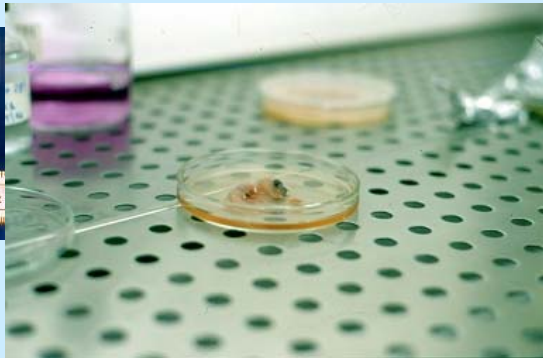
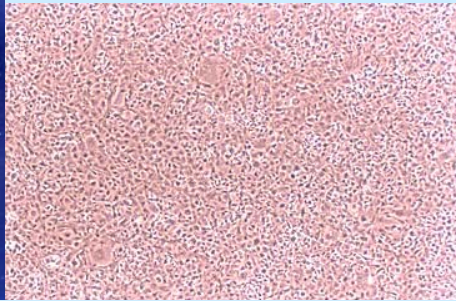
- First frog virus isolated in Thailand.
- **Virus shape**; icosahedral symmetry with lipid-containing envelop at ~128 nm diameter
- **Type of genome**; DNA (>4 log₁₀ TCID₅₀/m titre reduction)
- **CPE**; spherical plaque forming,
- **Susceptible cells**; EPC, BF-2, BB, DT, STE-1, SCE

Susceptible cells;



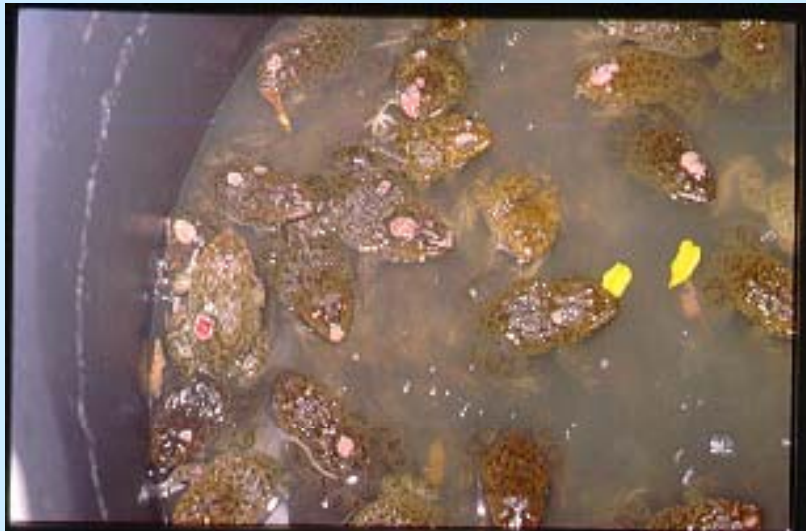
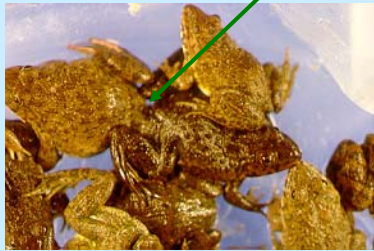


**Soft-shelled
turtle embryo,
STE**



SCE

- **RTRV** is found to be **associated** with CSE disease of frog that caused moderate to high mortality.
- **RTRV** caused death in fish and reptile cell lines.
- **RTRV** were also isolated from other frog diseases.
 - Cloudy eyes and lenses
 - Paralysis syndrome



Ranavirus-affected frog brooders, Nonthaburi province

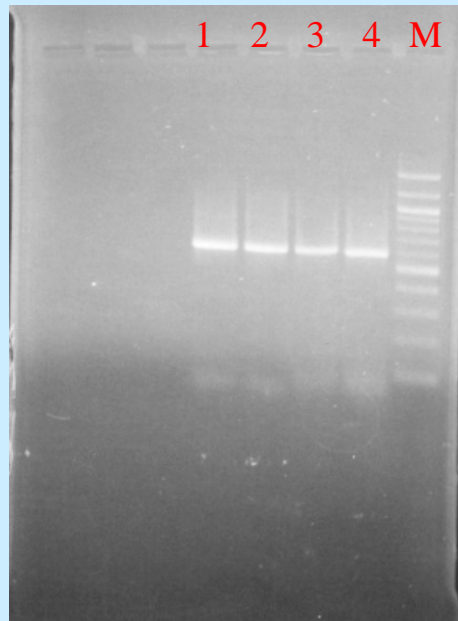
A Case Report: An Iridovirus found in a diseased gold fish

U-malai Saduakdee
Virology Unit, AAHRI

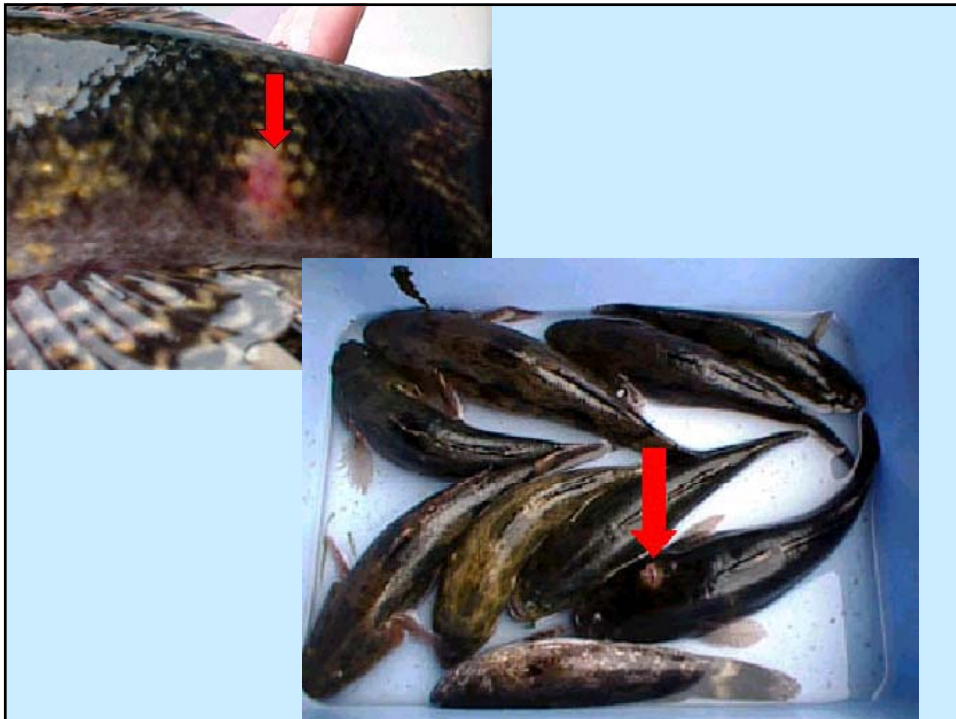
Gel Photo

Lane 1&2 = Frog
iridovirus DNA

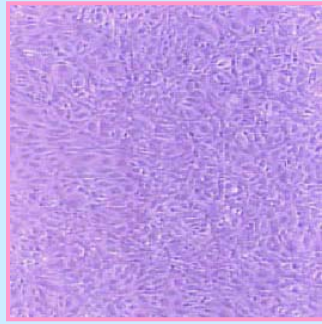
Lane 3&4 = Gold fish
iridovirus DNA



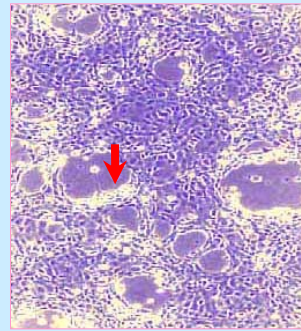
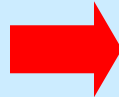
- Prasankok, P., M. Chutmongkonkul and S. Kanchanakhan. 2005.
Characterization of an iridovirus isolated from diseased marble goby *Oxyeleotris marmoratus* (Bleeker, 1852),
Disease of Asia Aquaculture (DAA –V)



Cytopathic effect (CPE)

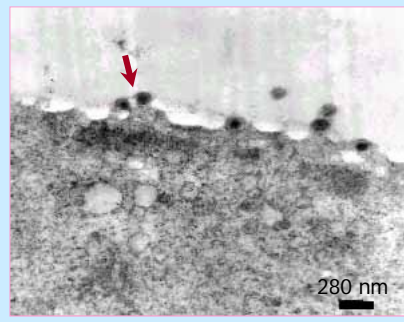
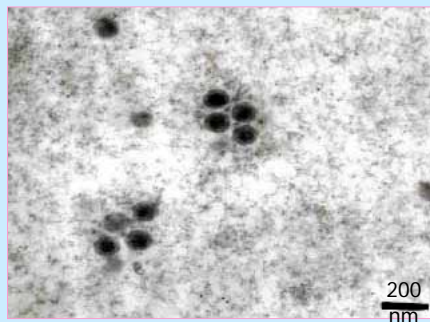


Normal EPC



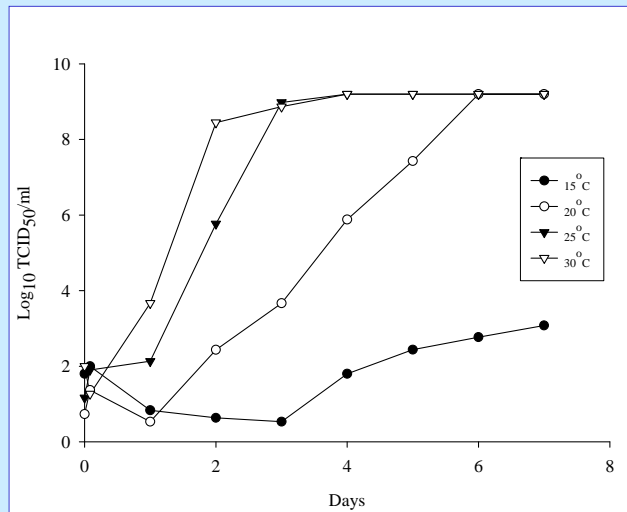
EPC+ virus

Viral Morphology

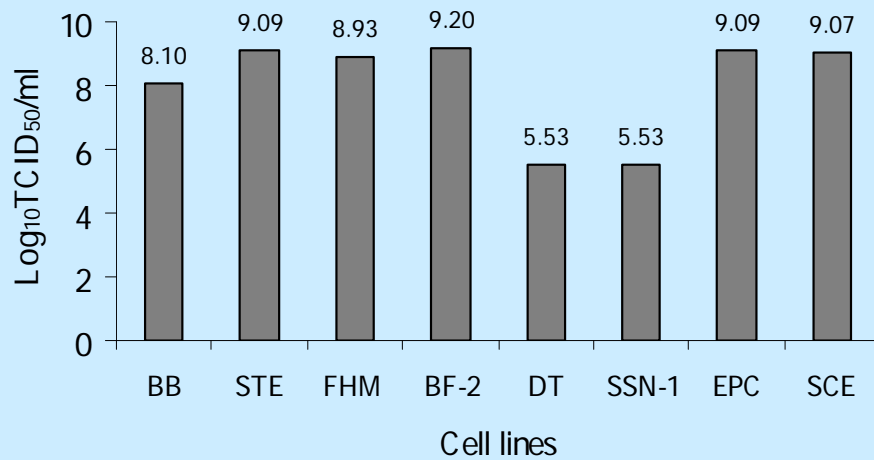


- icosahedral symmetry
- nucleocapsid size :130 nm

Optimum growth temperature of marble goby virus at 15, 20, 25 and 30C

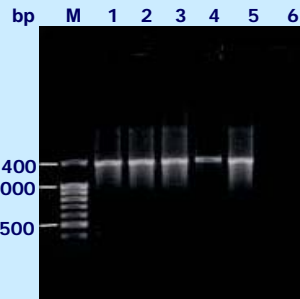
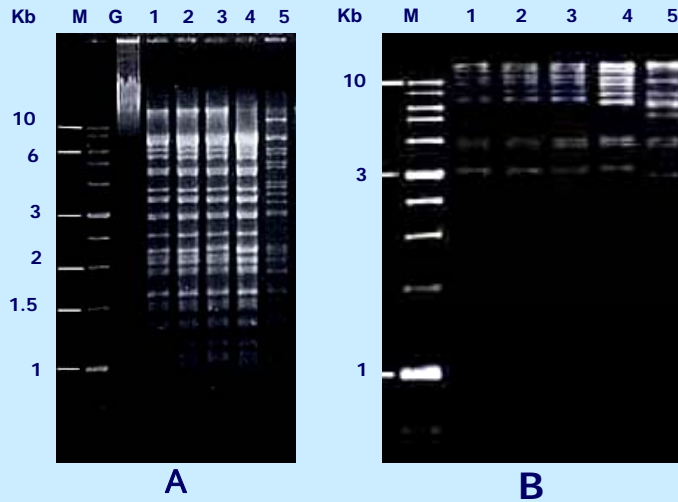


Cell lines susceptibility of marble goby virus were grown in fish and reptile cell lines



Restriction endonuclease analysis

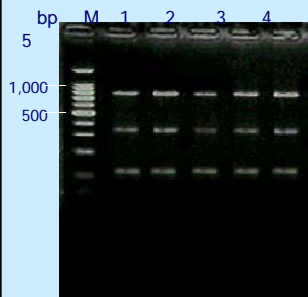
Agarose gel electrophoresis analysis of viral DNA of iridovirus cleaved with *Bam* HI(A) and *Xba* I(B). Marker(line M), Genomic DNA (G) OMRV (line 1), TFIV (line 2), RTV-S (line 3), RTV-P (line 4), RTV-R (line 5)



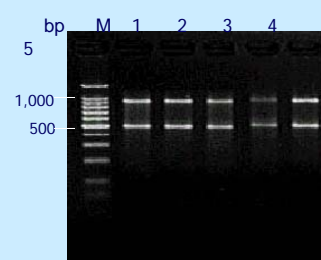
Agarose gel electrophoresis analysis of PCR products from amplification of 1,400 bp fragment of MCP gene cleaved with *Mbo* II (A), *Alu* I(B) and *Sau3A* I(C). Marker(line M), OMRV (line 1), TFIV (line 2), RTV-S (line 3), RTV-P (line 4), RTV-R (line 5)

DAA V

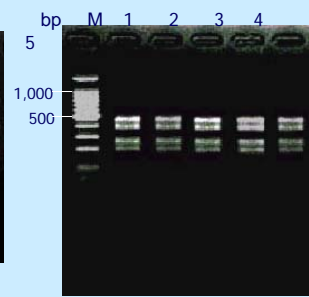
PCR-RFLP



Mob II



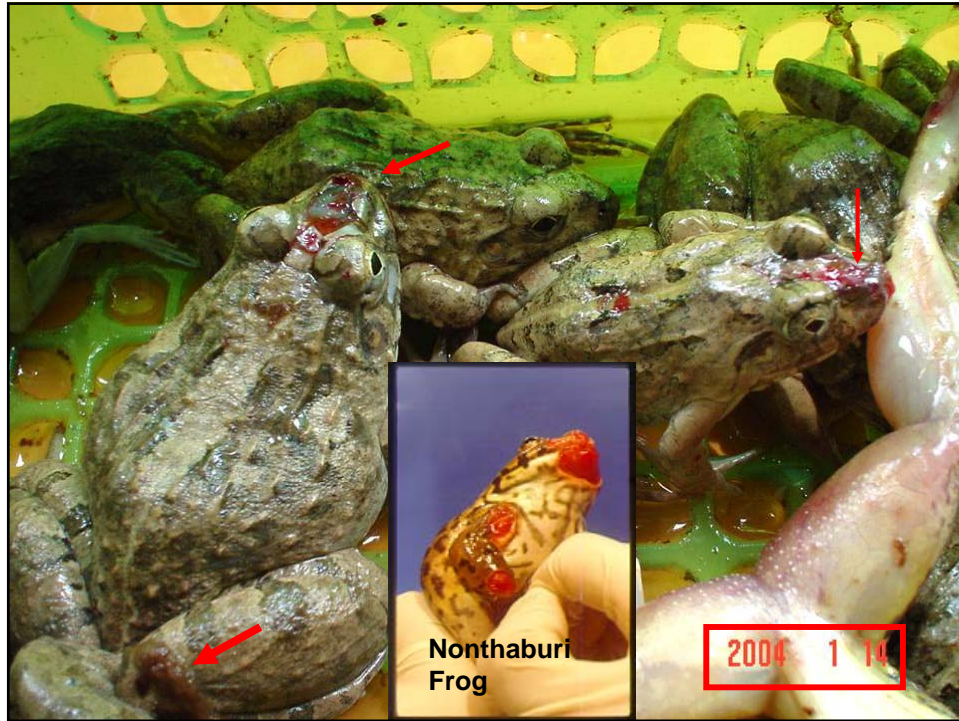
Alu I



Sau3 AI

- Kanchanakhan, S., J. Polchana, T. Nuanchan and S. Tandavanitj. 2004. Isolation and Characterisation of Ranavirus from Diseased Frog, *Rana* spp., Imported from Cambodia. Seminar on Fisheries year 2004, Department of Fisheries, Bangkok. (Abstract).





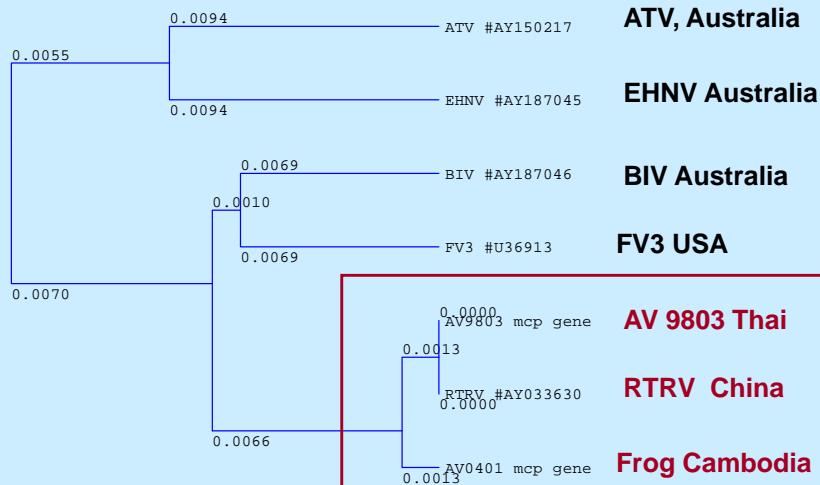
Primers designed from *Rana tigrina* ranavirus GenBank
 #AF389451.1 (He *et al.*, 2002)
 (<http://www-genome.wi.mit.edu/cgi-bin/primer3>)

Code	Primer SQ	PRC product
RA-MCF	5'tgcaatattttattccacagtca	1521 bp
RA-MCR	5'ttgcgacaaacggacactt	

mcp gene: Blast results

Code	(mcp) gene	Identities
AF389451.1	Rana tigrina ranavirus (TFV)	1517/1521 (99%)
FVU36913	Frog virus 3	1489/1515 (98%)
AY187046.1	Bohle iridovirus	1429/1456 (98%)
AY187045.1	Epizootic haematopietic necrosis virus	1437/1472 (97%),
AY150217.1	Ambystoma tigrinum stebbensi virus	1461/1515 (96%),

[GENETYX : Evolutionary tree]
Date : 2004.7.9
Method: UPGMA



Summaries



1998

- Ranavirus diseases in culture frog, marble goby and goldfish have the same etiological agent.

- Minor variation of MCP gene between strains or isolates .



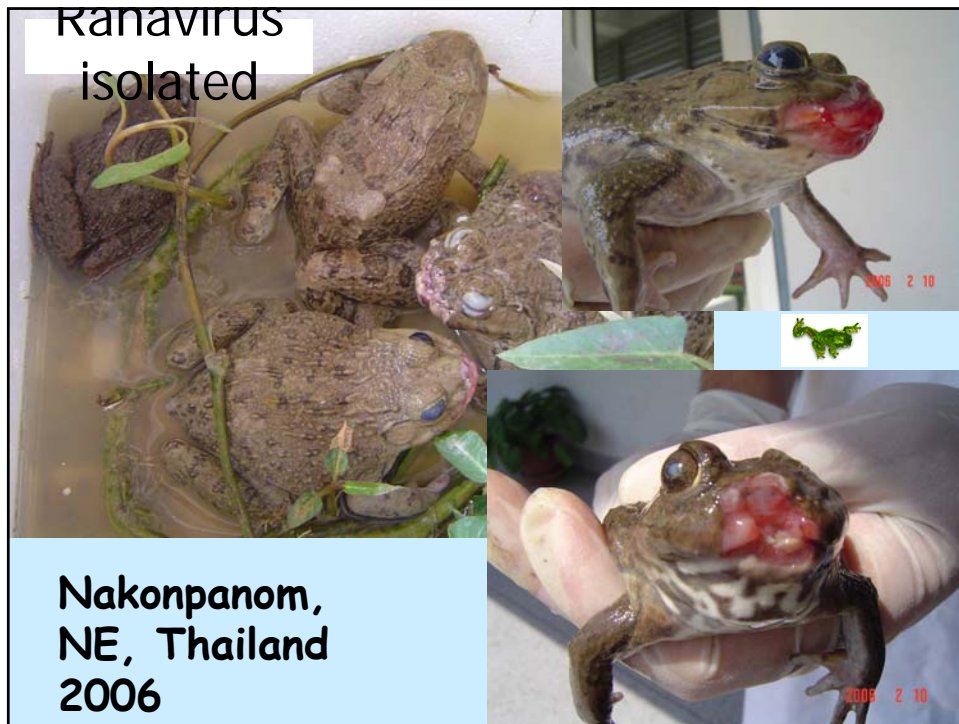
2000

Gold fish

2002

- Places where ranavirus isolated 1998-2004





Discussions (1/2)

- Wild amphibian population decline was noted in 2000-2003 in Thailand. It might be a link to a new emerging disease, ranavirus.
- Ranavirus can be isolated from frogs with different clinical diseases.
- Ranavirus can infect both fishes and frog.
- As ranavirus can propagate on reptile cell lines – can reptiles be healthy carriers?

Discussions (2/2)

- There is a need to know frog immune system after recovered from ranavirus infection / vaccination for understanding cycle of the outbreaks.
- Live frog / fish trades are mostly likely to spread the virus.
- As a notifiable disease, please contact your OIE Aquatic Focal Point for national reporting system.

Thank You for

- the organizer for partially support.
- Prof, John C. Harshbarger for histopathology examination



Thanks for your attention