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Chinese Giant Salamander Iridovirus (GSIV)

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Dr. Matthew J. Gray, the course coordinator

Outline of this talk

- Farming Industry
- Epidemiology
- Symptoms & Histopathology
- Diagnosis/Detection
- Basic Features of GSIV
- Control and Prevention
- Protein data base of the host
- Future study

Chinese giant salamander (*Andrias davidianus*)

Cited from http://www.bbc.com/earth/story/20150316-amazing-giant-chinese-salamanders

The largest living amphibians
(Credit: Daniel Heuclin/NPL)

They are a tasty treat in China
(Credit: BBBar/Alamy)

The Chinese salamanders are sometimes called "**wa wa yu**", meaning "baby fish", because their distress call resembles a baby's cry.

Farming Industry in China

Culture of Chinese giant salamander (*Andrias davidianus*)

- Culture in earth and concrete ponds
- Seed production:

  2 million per year
- Farmed:

  4-5 million

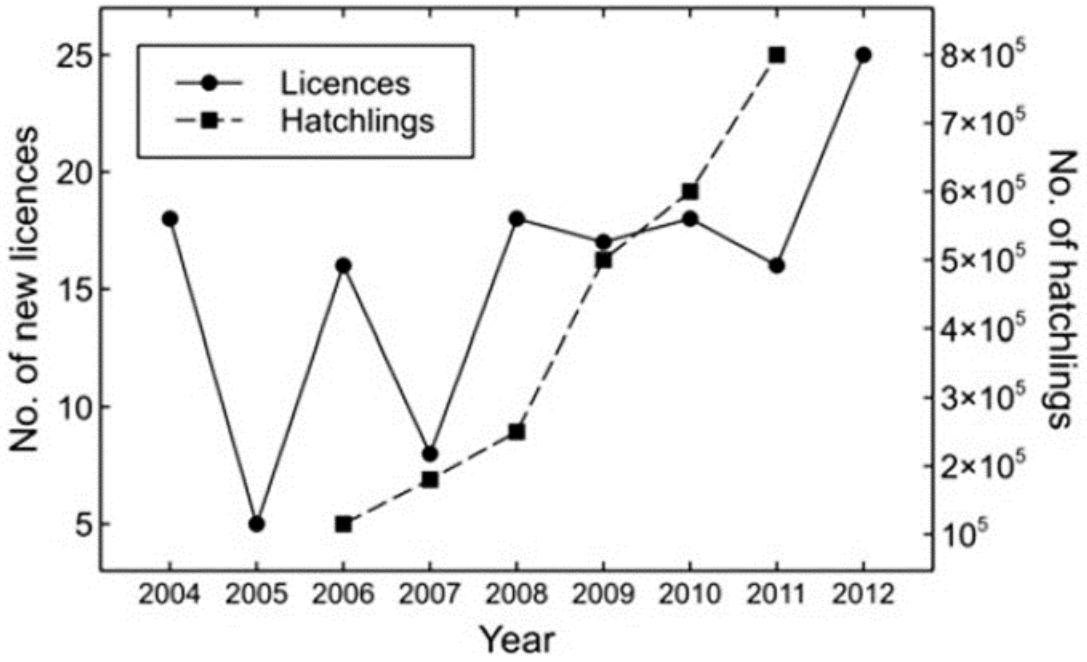
Farming Industry

Conservation threats and opportunities

Cunningham et al. 2015

http://journals.cambridge.org

Fauna & Flora International, Oryx, 1–9



Numbers of newly licensed Chinese giant salamander farms and of salamander offspring produced in Shaanxi Province

Farming Industry

Conservation threats and opportunities

Cunningham et al. 2015

http://journals.cambridge.org

Fauna & Flora International, Oryx, 1–9

Typical Chinese giant salamander farm, with rearing pens for young animals. The inset is a close-up view of a rearing pen, showing the high stocking density.

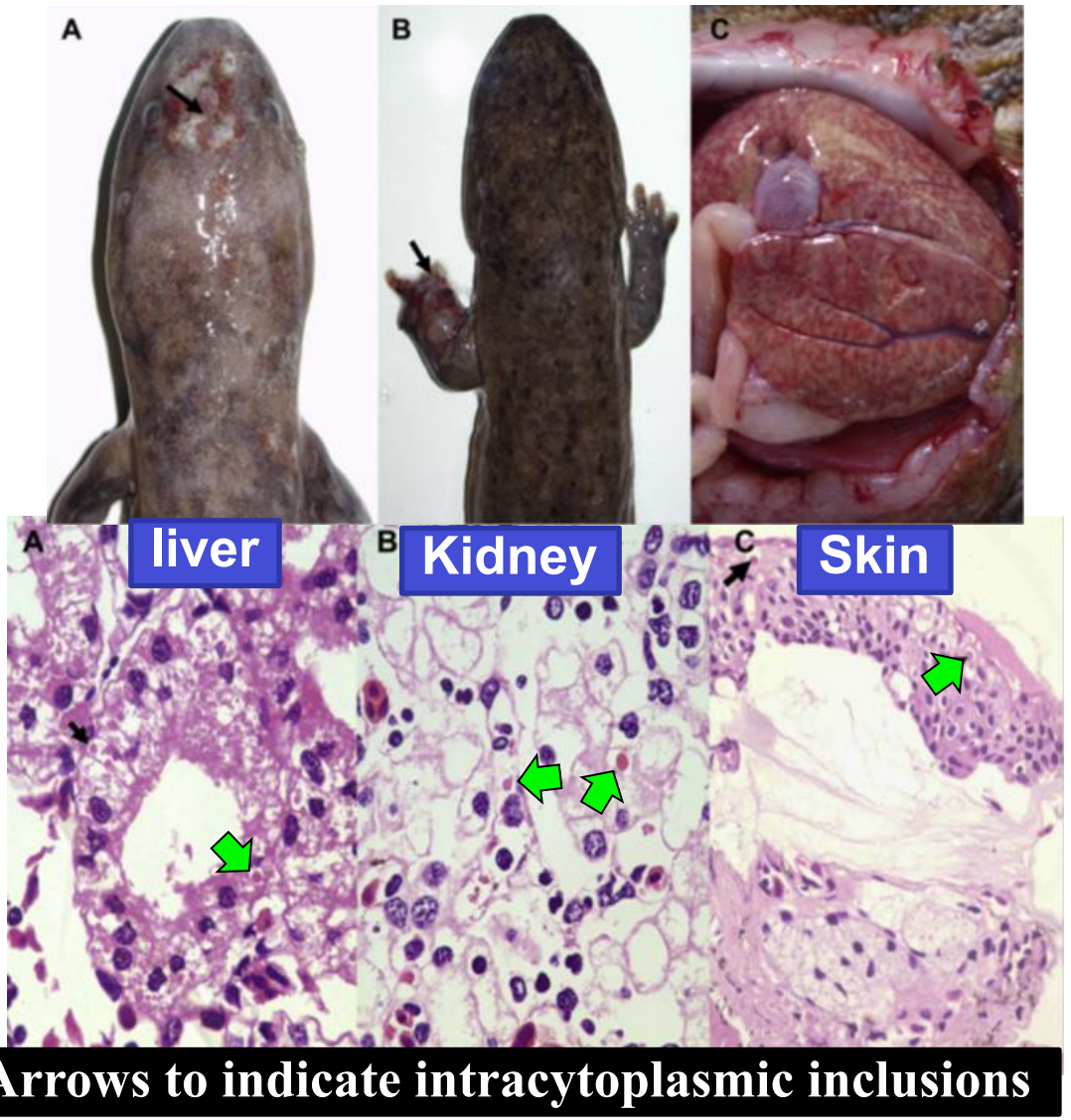
First Case Report of a viral disease

Hanzhong, Shaanxi
陕西省汉中市

Gross lesions in an infected Chinese giant salamander. (A) Cutaneous erosions (arrow) and swollen areas on the head. (B)

Ecchymoses, swelling and necrosis in a forelimb (arrow). (C) The liver was pale and swollen with multifocal haemorrhages

Y. Geng et al. J. Comp. Path. 2011, Vol. 145, 95e102

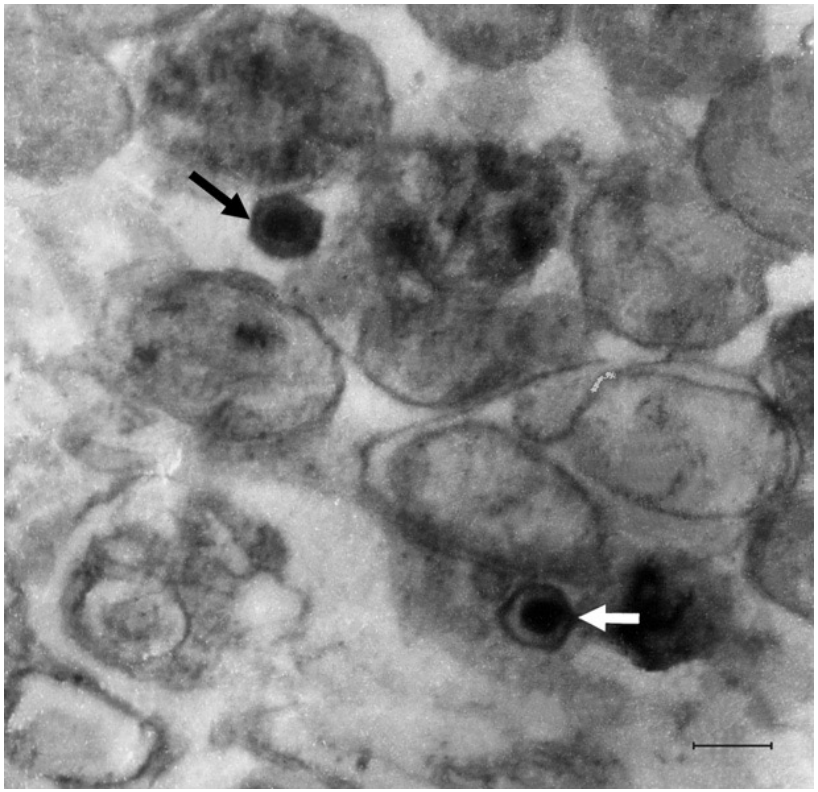


First Case Report

PCR Detection and Sequence analysis

A GenBank BLAST (MCP gene) search on the sequence revealed 95-98% identities to ranaviruses from amphibian:

- **ATV (AY548301, EU512397, EU360297; 95%),**
- **FV3 (DQ897669, GQ144407; 96% and AY548484, FJ459783; 97%),**
- **Rana esculenta virus (FJ358611, FJ515796; 98%),**
- **common midwife toad ranavirus (FM213466; 98%)**
- **Rana catesbeiana virus (FJ207464; 97% and AB474588; 98%).**



Epidemiology

The host:

Chinese giant salamander, *Andrias davidianus*

The viral pathogen: (different isolates, they are most likely being the same virus with different names)

Andrias davidianus ranavirus (ADRV)

Giant salamander iridovirus (GSIV)

Chinese giant salamander iridovirus (CGSV)

Chinese giant salamander iridovirus (CGSIV)

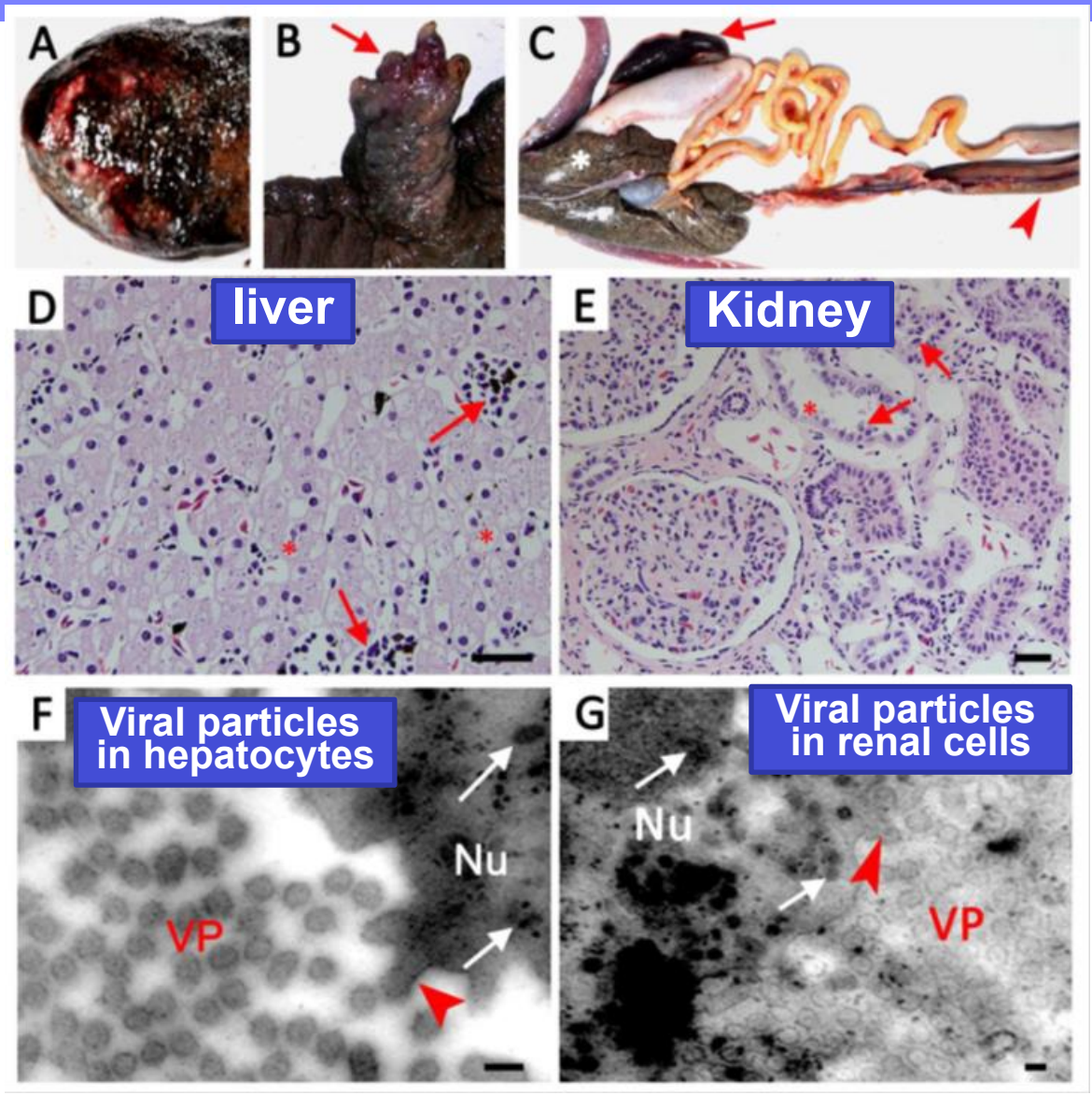
Epidemiology

NUS National University of Singapore

First Case Report to CDC

Iridovirus Infection in Chinese Giant Salamanders, China, 2010

Dong W et al. CDC Letter, Volume 17, Number 12—December 2011



Epidemiology

Y. Geng et al. J. Comp. Path. 2011, Vol. 145, 95e102

First Report of a Ranavirus Associated with Morbidity and Mortality in Farmed Chinese Giant Salamanders *(Andrias davidianus) Received, Aug 3rd, 2010 Accepted, Nov 23rd, 2010*

From February to May 2010, an outbreak of disease occurred amongst farmed Chinese giant salamanders (*Andrias davidianus*) in Hanzhong County, Shanxi Province, China.

Should be Shaanxi

Dong W, et al. Iridovirus infection in Chinese giant salamanders, China, 2010. Emerg Infect Dis. 2011 Dec

Iridovirus Infection in Chinese Giant Salamanders, China, 2010

The mesocosms (ambient temperature <20°C) are maintained primarily in mountainous caves and mountainous ditches. During June–October 2010, a high mortality rate was reported in salamanders in ditch mesocosms in Shaanxi, Sichuan, and Henan, reaching an epidemic peak in July. Mortality rate reached 95% in the affected areas

Epidemiology

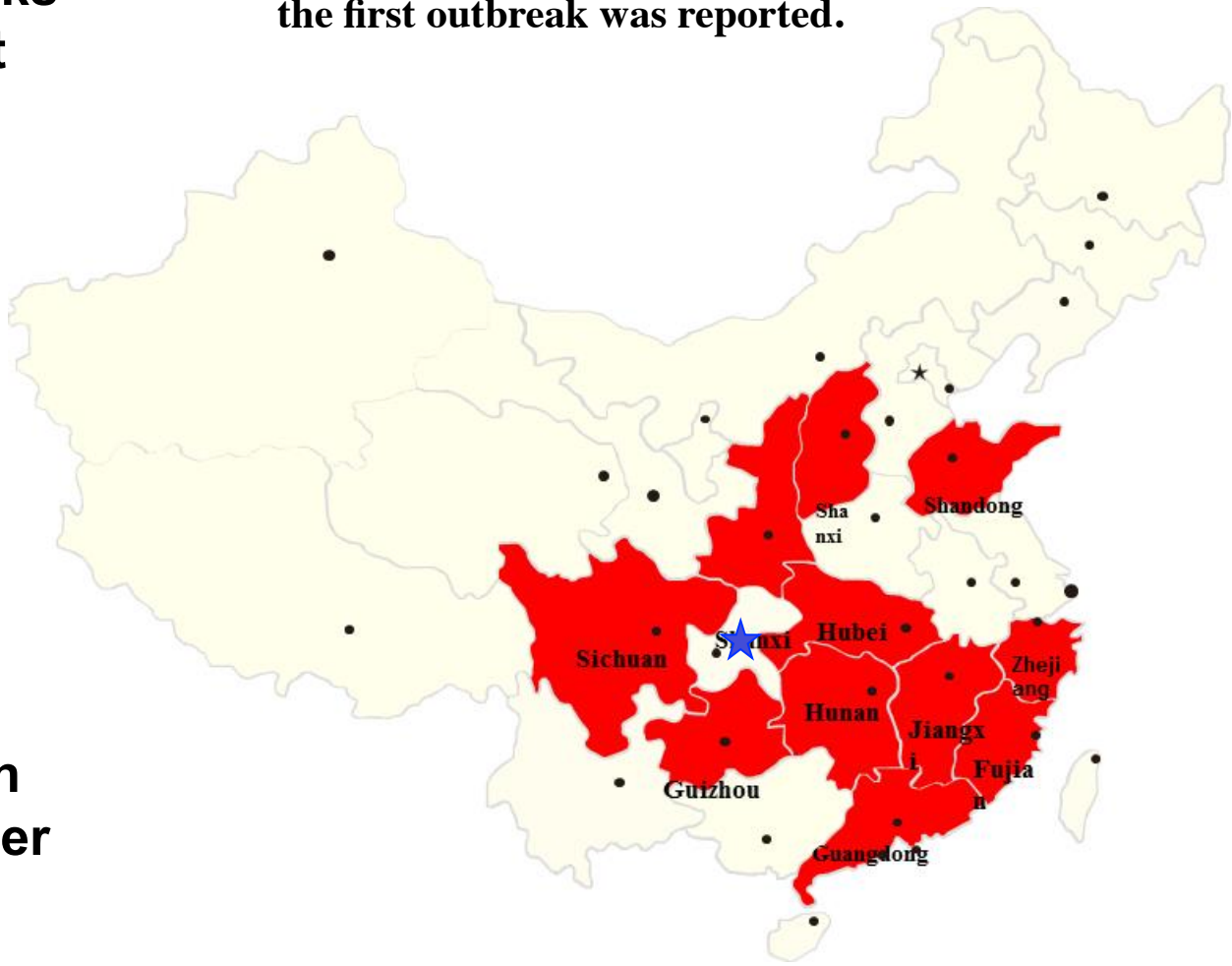
- Disease outbreaks often in May-Oct
- Water Temp:

  15-28 ℃
- Mortality:

  Juvenile 100%

  Adult 70%
- Water born transmission
- Co-infection with bacteria and other viruses

The star indicates the Shaanxi province, where the first outbreak was reported.

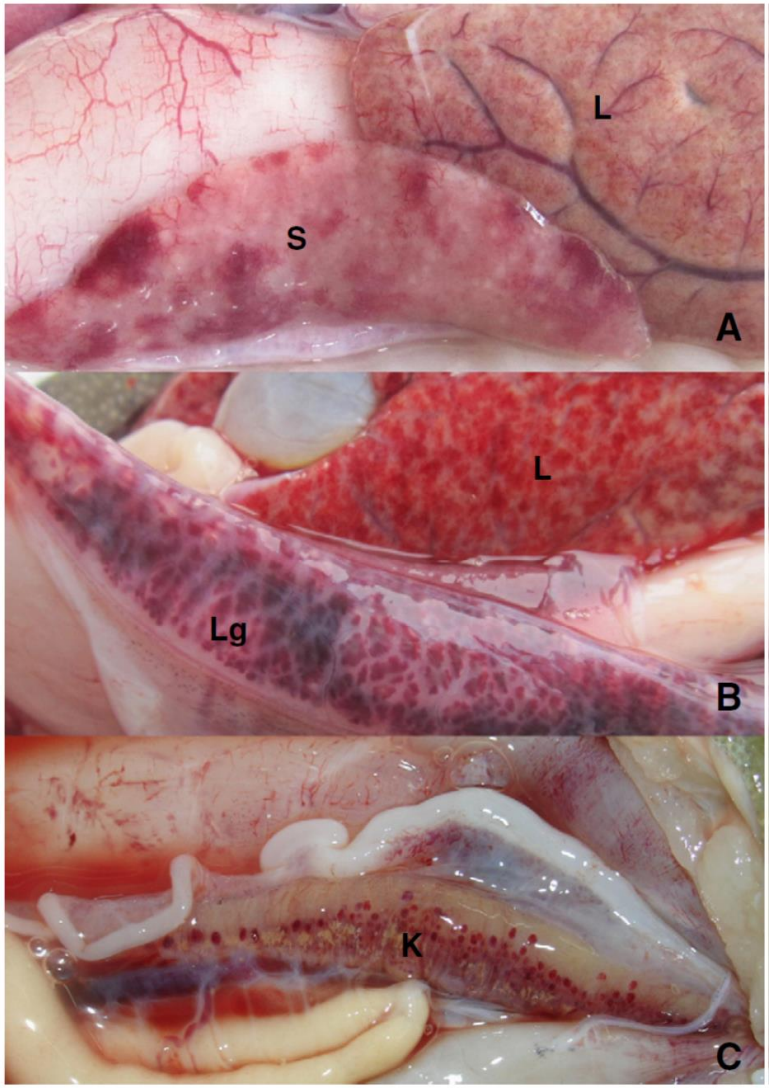


Symptoms & Histopathology

Clinical signs

- **swollen head and neck,**
- **skin and subcutaneous hemorrhage on the dorsal and ventral surfaces**
- **ulceration of limbs.**
- **The liver, spleen and kideney were fragile and hemorrhagic spots.**
- **The intestinal tract contained yellow mucus and hemorrhagic.**

Symptoms & Histopathology

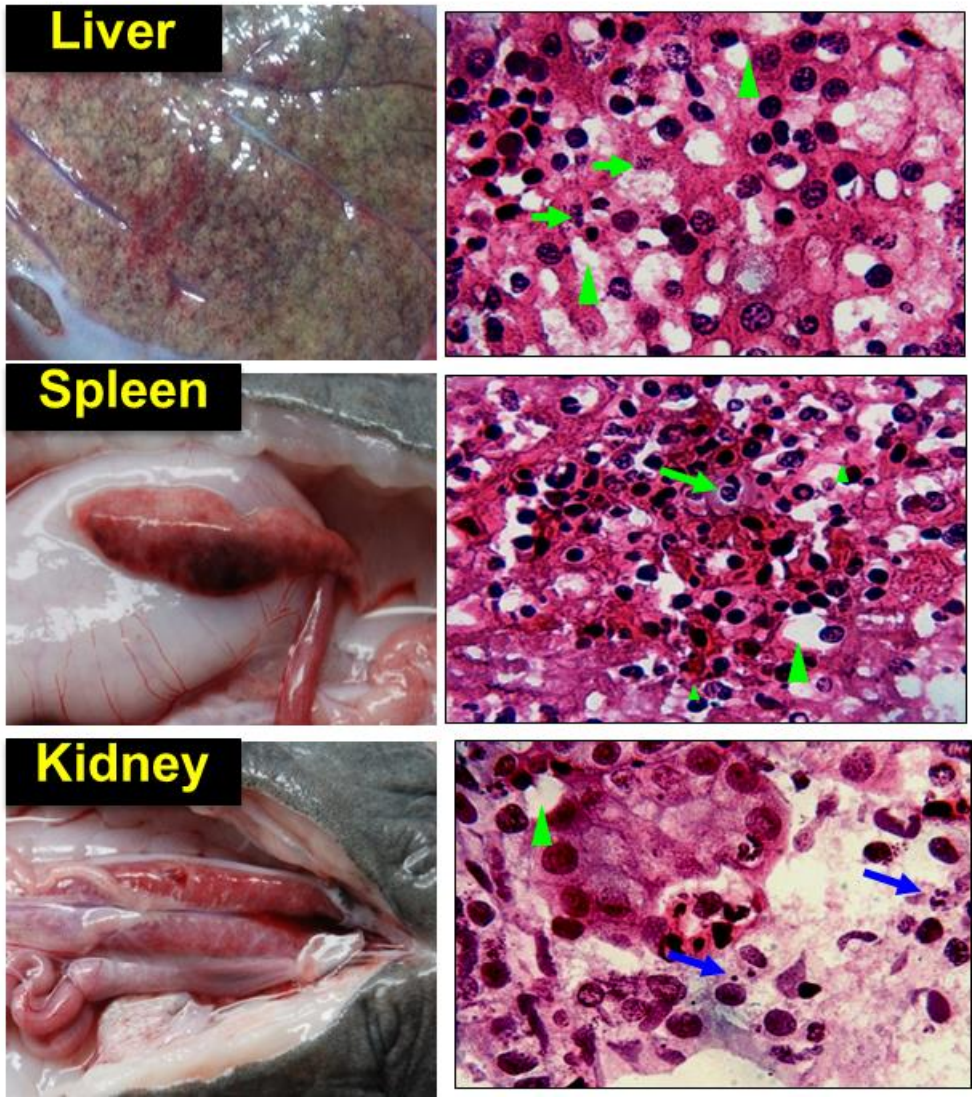


Gross lesions in infected Chinese giant salamanders. (A) The liver and spleen were pale, with the diffused ecchymosis. (B)The liver and lung were swollen with multifocal hemorrhages. (C) The diffused ecchymosis and necrosis in the Kindey (L: liver, S: spleen, Lg: lung, K: kidney).

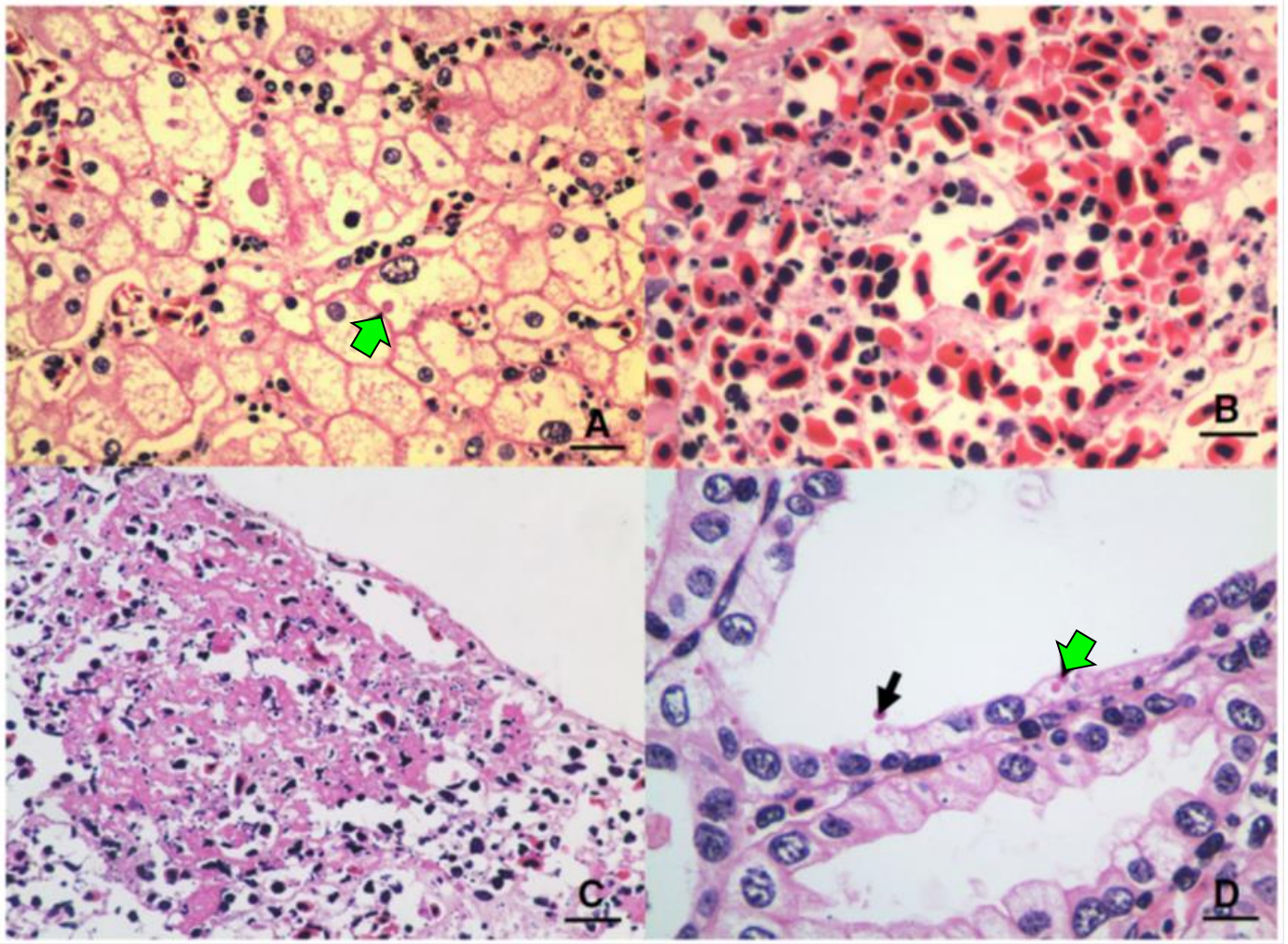
Zhou et al. Aquaculture 384–387 (2013) 66–73

Symptoms & Histopathology

- Lesions consisted of scattered areas of single cell necrosis and variably sized areas of focal necrosis.
- In necrotic cells, the cytoplasmic inclusions were observed after staining with H.E .
- Nuclear Fragmentation
- vacuolar degeneration



Histopathology



Zhou et al. Aquaculture 384–387 (2013) 66–73

Diagnosis/Detection

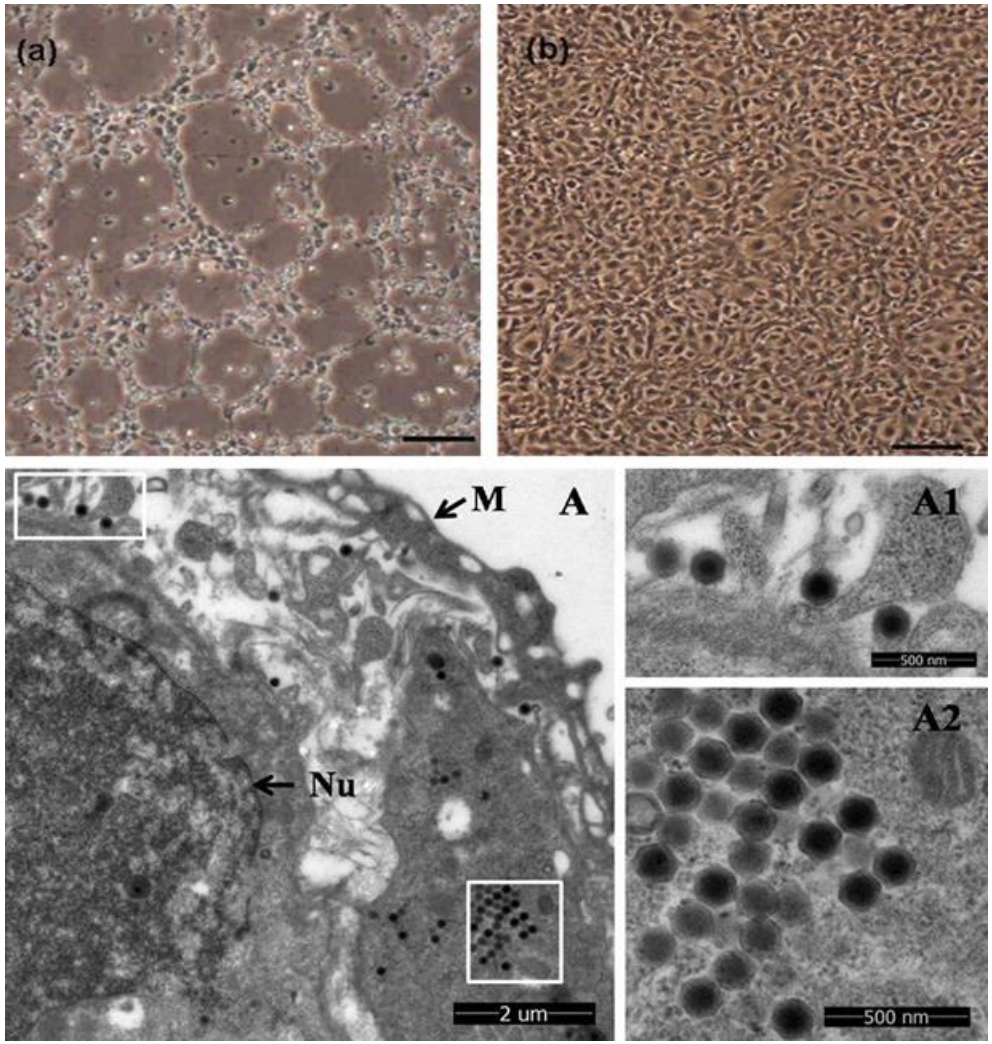
- **Cytopathic effect (CPE)**

  two days post infection of *Epithelioma papulosum cyprini* (EPC) cell line

- **EM observation**

  Typical particle size at

  140-180 nm



Diagnosis/Detection

Table 1 Different fish cell lines were infected with ADRV.

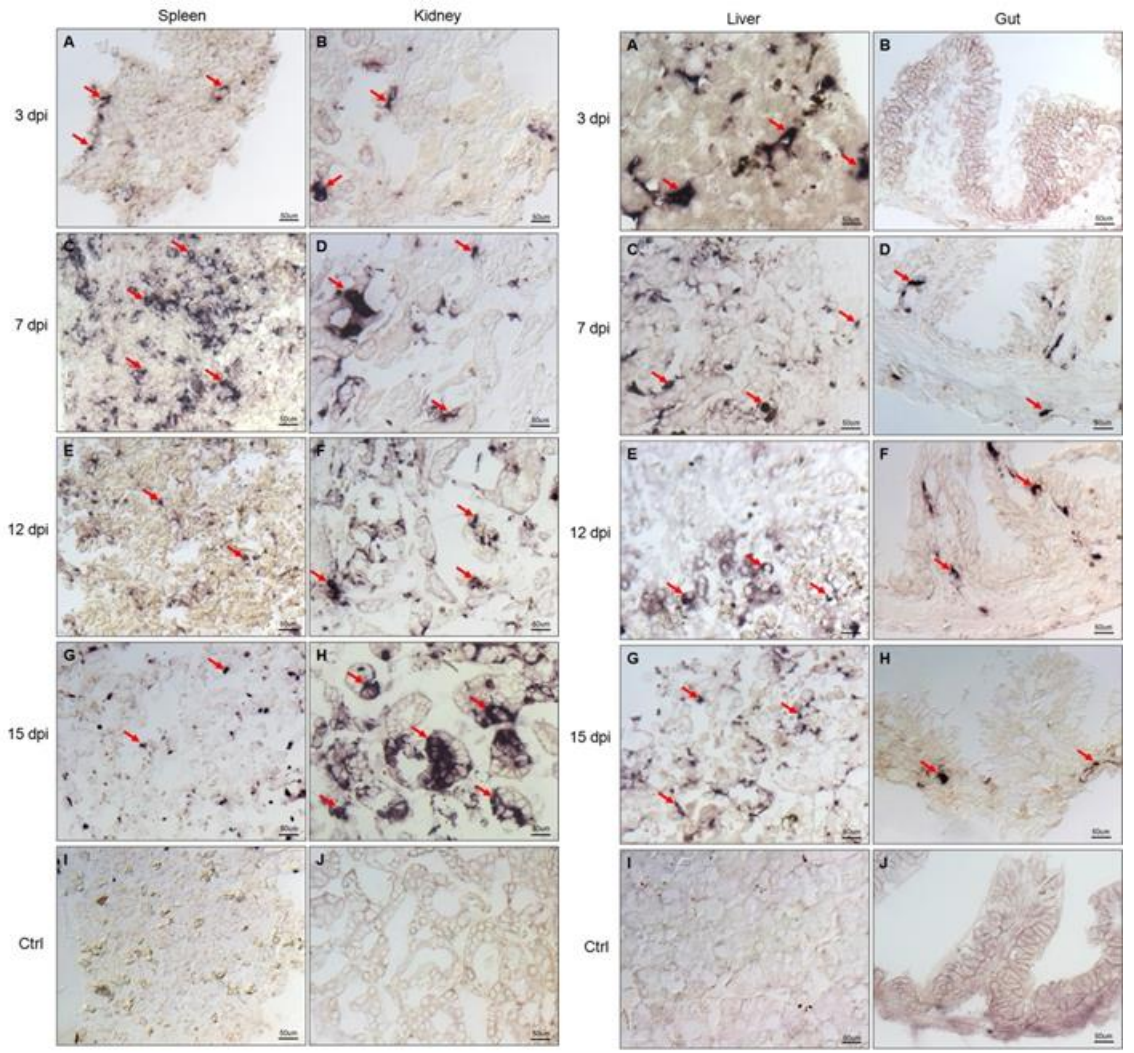
Fish cell line	Time (h) of first appearance of the cytopathic effect	Viral titer ($TCID_{50}mL^{-1}$)
Epithelioma papulosum cyprini (EPC)	24	$10^{6.5}$
Chinook salmon embryo (CHSE)	24	$10^{6.5}$
Bluegill fry (BF-2)	24	$10^{6.0}$
Grass carp fins (GCF)	36	$10^{6.0}$
Grass carp ovary (GCO)	36	$10^{5.5}$
Fathead minnow (FHM)	48	$10^{4.5}$

Chen et al. Veterinary Research 2013, 44:101
http://www.veterinaryresearch.org/content/44/1/101

Diagnosis/Detection

***In situ* hybridization** with a digoxigenin (DIG)-labeled RNA probe specific to the gene encoding the major capsid protein (MCP)

MCP probe was
5'-TCA CCA AGC TGC CGT CTC TG-3'
The reverse primer
•5'-GAG GTC CTG GAT GGC CCT CA-3'



Diagnosis/Detection

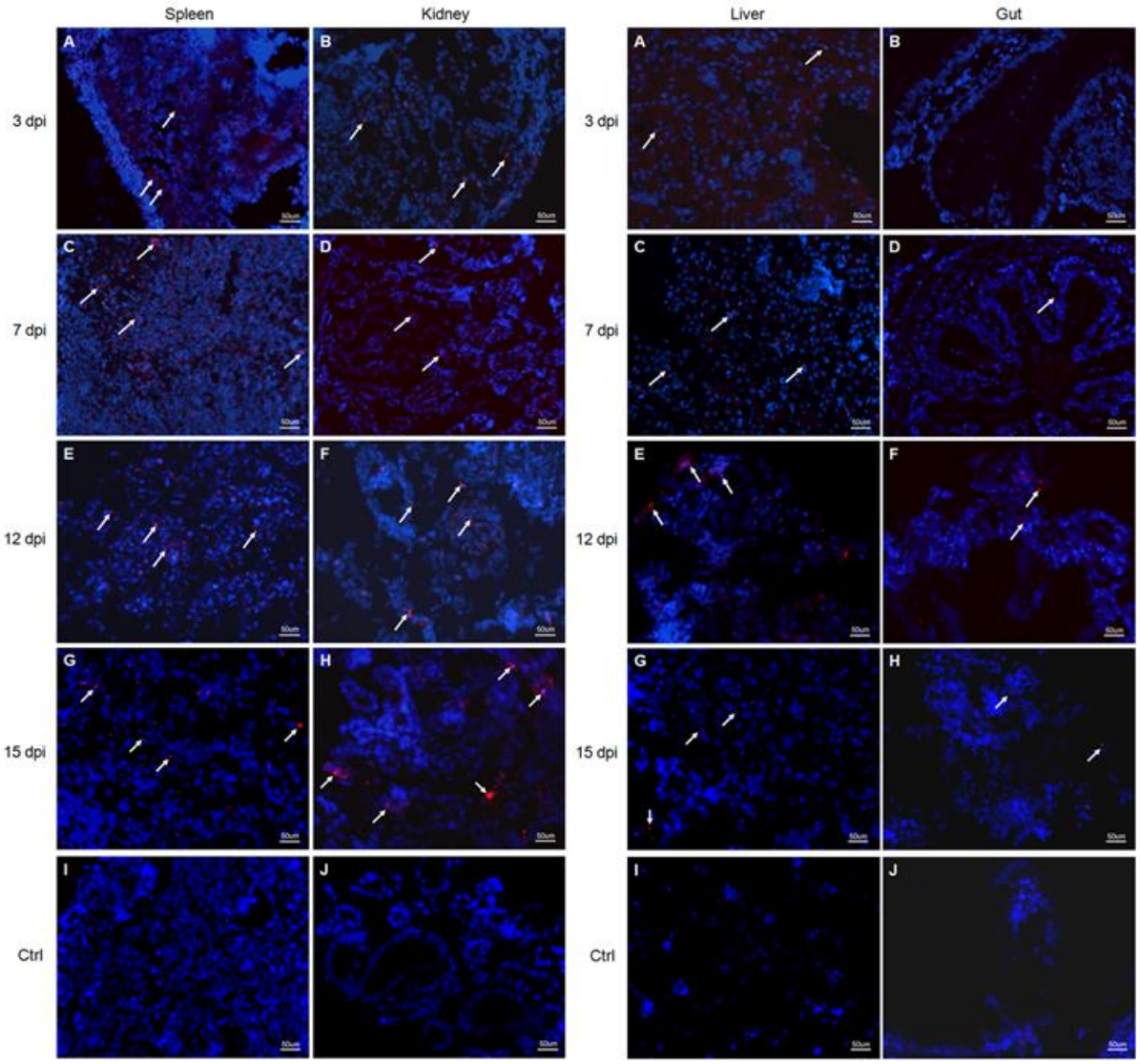
Tissue Tropism

The percentage of positive cells in tissue sections detected by *in situ* hybridization

Days post-infection	Spleen (%)	Kidney (%)	Liver (%)	Gut (%)
3	4.1 ± 0.3	5.8 ± 0.3	10.7 ± 0.2	0
7	66.3 ± 6.9	16.4 ± 2.4	18.1 ± 1.7	4.7 ± 0.4
12	22.5 ± 1.6	48.3 ± 4.2	19.4 ± 2.1	7.7 ± 1.0
15	20.5 ± 1.2	68.5 ± 6.4	17.4 ± 1.8	8.3 ± 1.2

Diagnosis/Detection

Immuno-fluorescence *detection* with monoclonal antibody against the major capsid protein (MCP)



Diagnosis/Detection

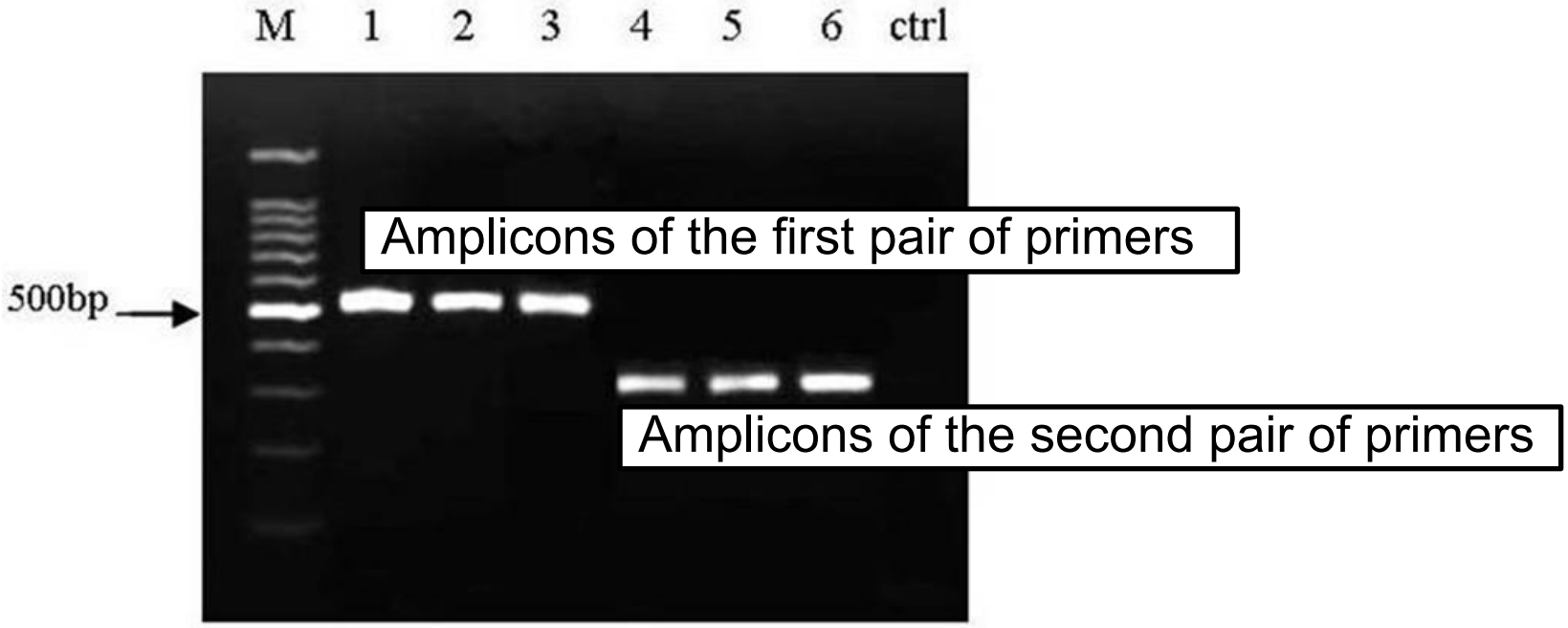
Tissue Tropism

The percentage of positive cells in tissue sections detected by *immuno-fluorence*

Days post-infection	Spleen (%)	Kidney (%)	Liver (%)	Gut (%)
3	1.5 ± 0.2	13.4 ± 4.5	2.1 ± 0.3	0
7	69.6 ± 6.7	24.8 ± 3.1	15.2 ± 2.2	2.6 ± 0.3
12	41.2 ± 4.3	53.8 ± 4.2	13.4 ± 1.8	8.4 ± 1.1
15	44.6 ± 3.8	56.2 ± 4.3	13.8 ± 2.0	8.1 ± 1.0

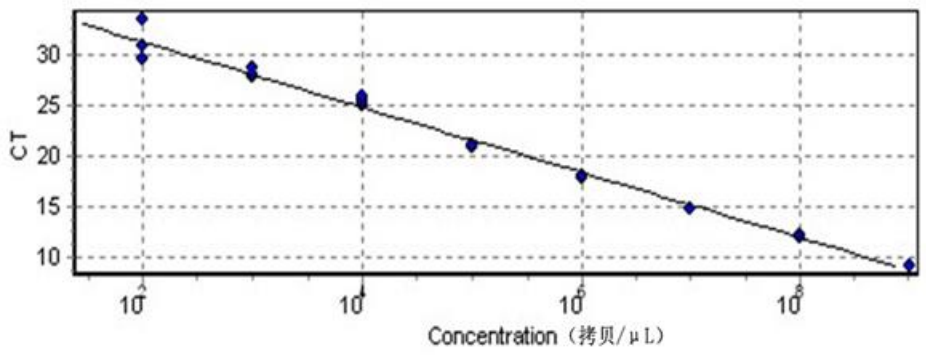
Diagnosis/Detection

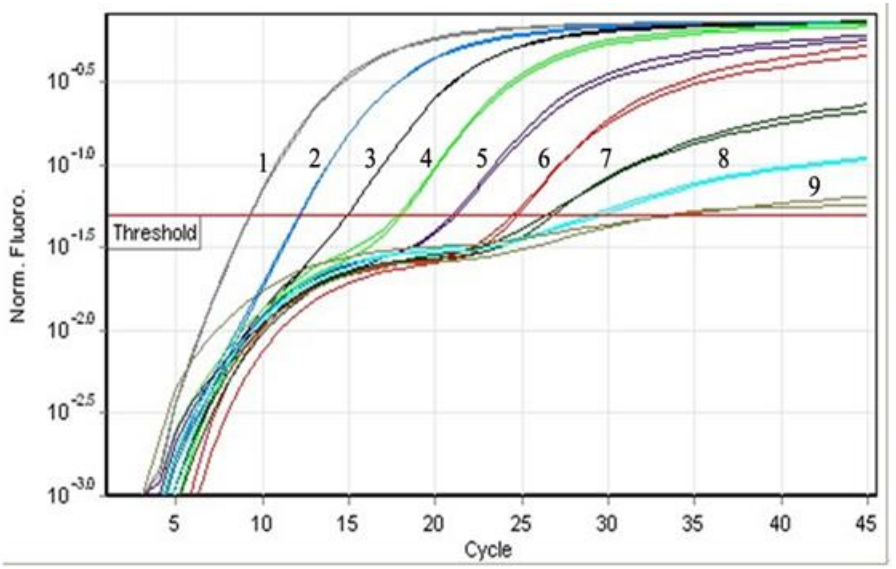
Based on the sequence of major capsid protein gene (MCP), five methods were established to detect GSIV, including conventional PCR, fluorogenic quantitative PCR, nested PCR and loop-mediated isothermal amplification assay (LAMP)

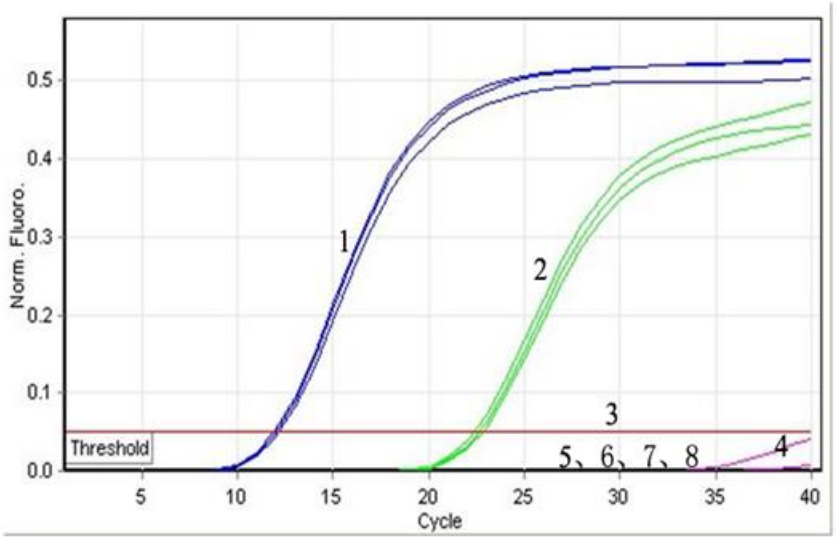


Conventional PCR and nested PCR

Diagnosis/Detection



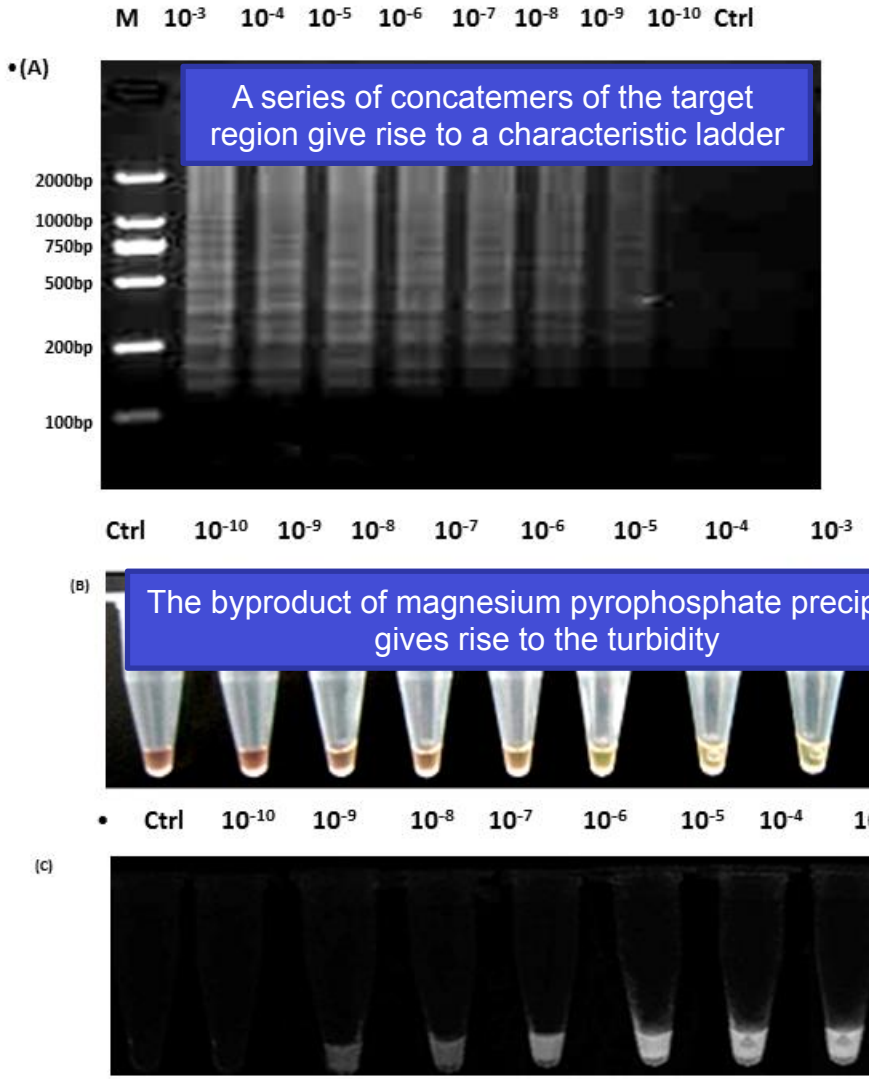




Fluorescent quantitative PCR

Able to detect 10 copies of viral DNA

Diagnosis/Detection

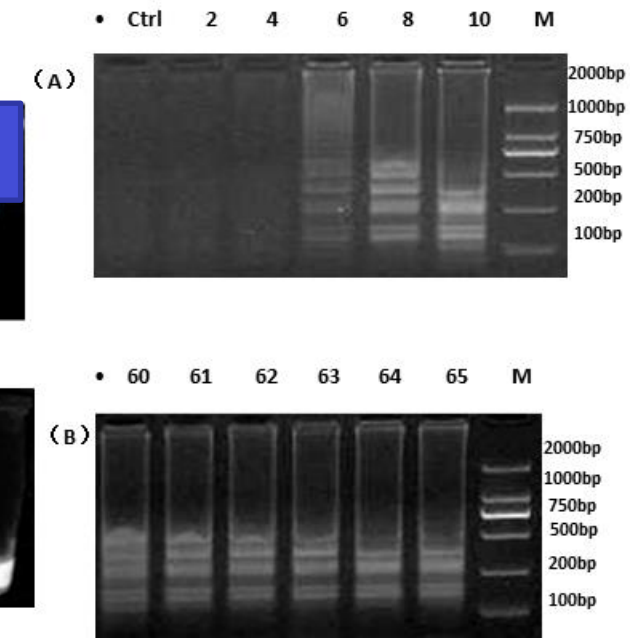


Loop-mediated isothermal amplification assay (LAMP)

Reaction condition：

Mg^{2+} 8 mM, 65℃, 60 min;

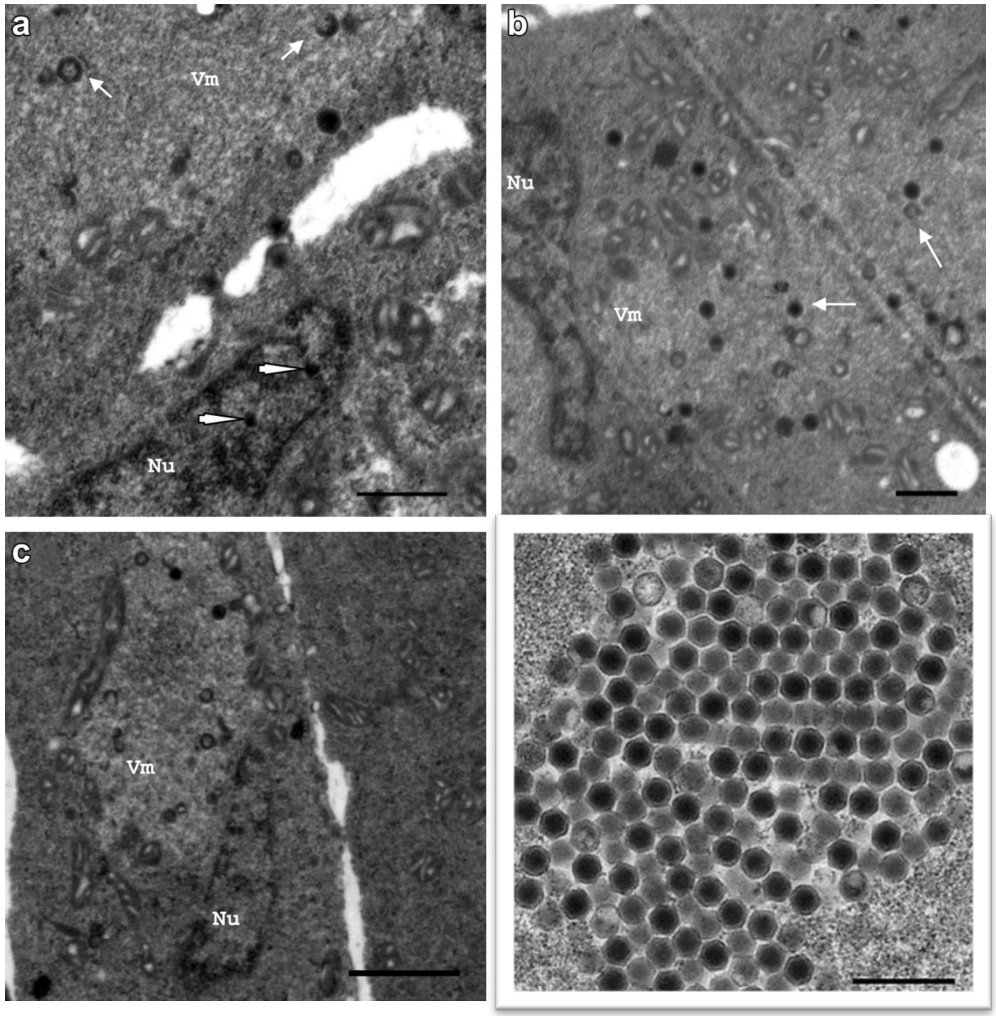
Higher detection efficiency than nested PCR



Basic Features of GSIV

Morphogenesis

- Icosahedral shape with a diameter of 140 nm
- Morphogenesis ocurs in viromatrix in cytoplasm
- Matured viral particles form paracrystalline or budding out via cytoplasm membrane



Basic Features of GSIV

Sensitivity of GSIV to different treatments

Physicochemical treatments	$TCID_{50}$/0.1 ml	
	Before treatment	After treatment
Chloroform	$10^{4.8}$	0
pH 3	$10^{5.0}$	$10^{1.5}$
pH 10	$10^{5.0}$	$10^{1.5}$
0.5% trypsin solution	$10^{5.3}$	$10^{2.6}$
56 °C, 30 min	$10^{5.0}$	0
BrdU	$10^{5.4}$	$10^{1.0}$

- All tests were carried out with EPC cells
- 5-bromouracil deoxyriboside (5-BrdU), is a nucleoside that substitutes for thymidine in DNA and thus acts as an antimetabolite. It causes breaks in chromosomes and has been proposed as an antiviral agent.

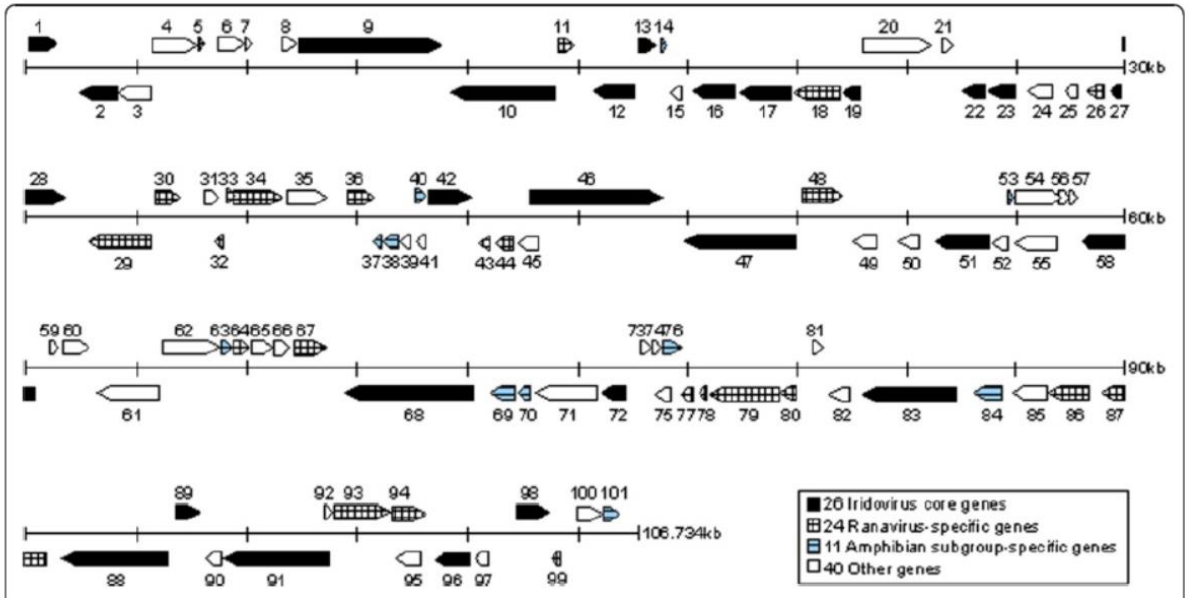
Zhou et al. Aquaculture 384–387 (2013) 66–73

Basic Features of GSIV

Genome structure

The *Andrias davidianus* ranavirus (ADRV) genome has a full length of **106,719 bp**, with a GC content of 55.04% and **101 ORFs** encoding putatively expressed proteins ranging in size from 44 to 1294 amino acids

GenBank no.
KF033124



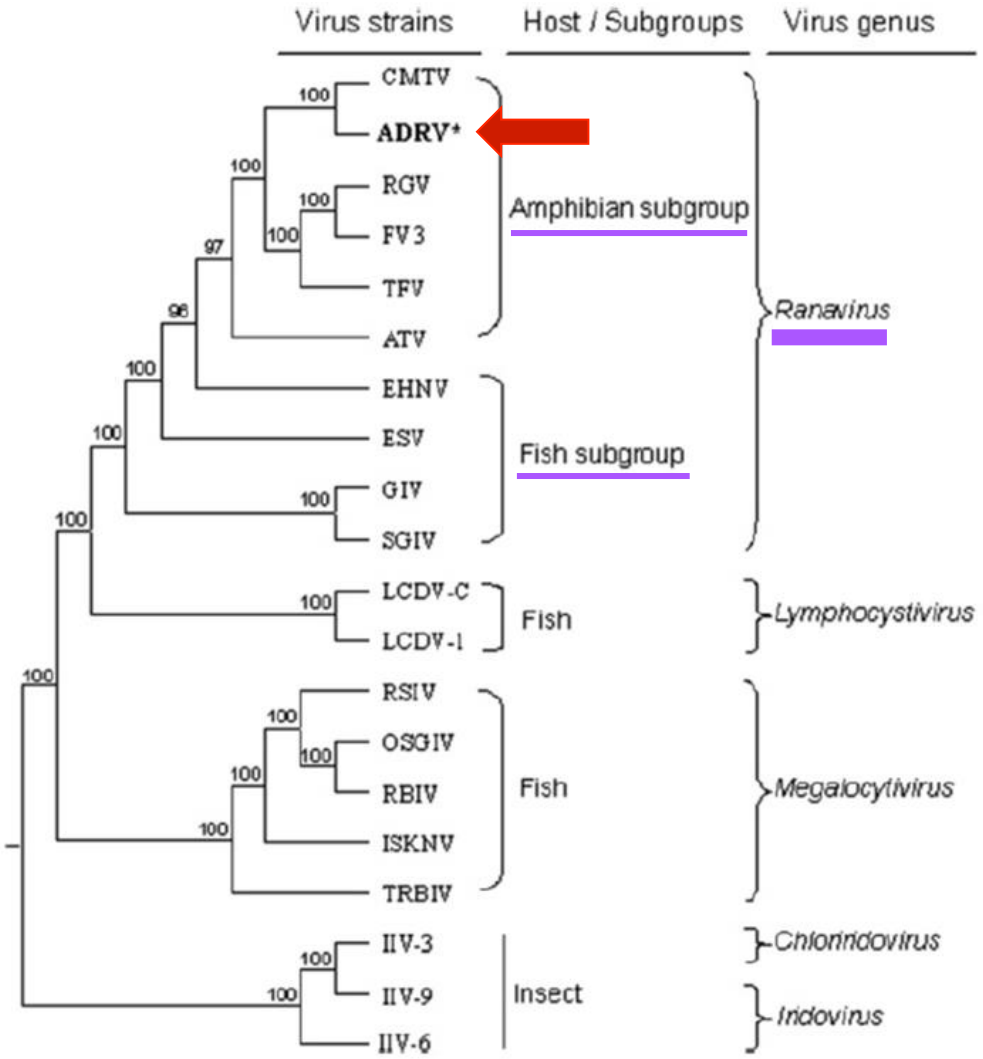
Chen et al. Veterinary Research 2013, 44:101

Basic Features of GSIV

Phylogenetic Tree

Constructed based on 26 iridoviral core genes from 20 completely sequenced iridoviruses

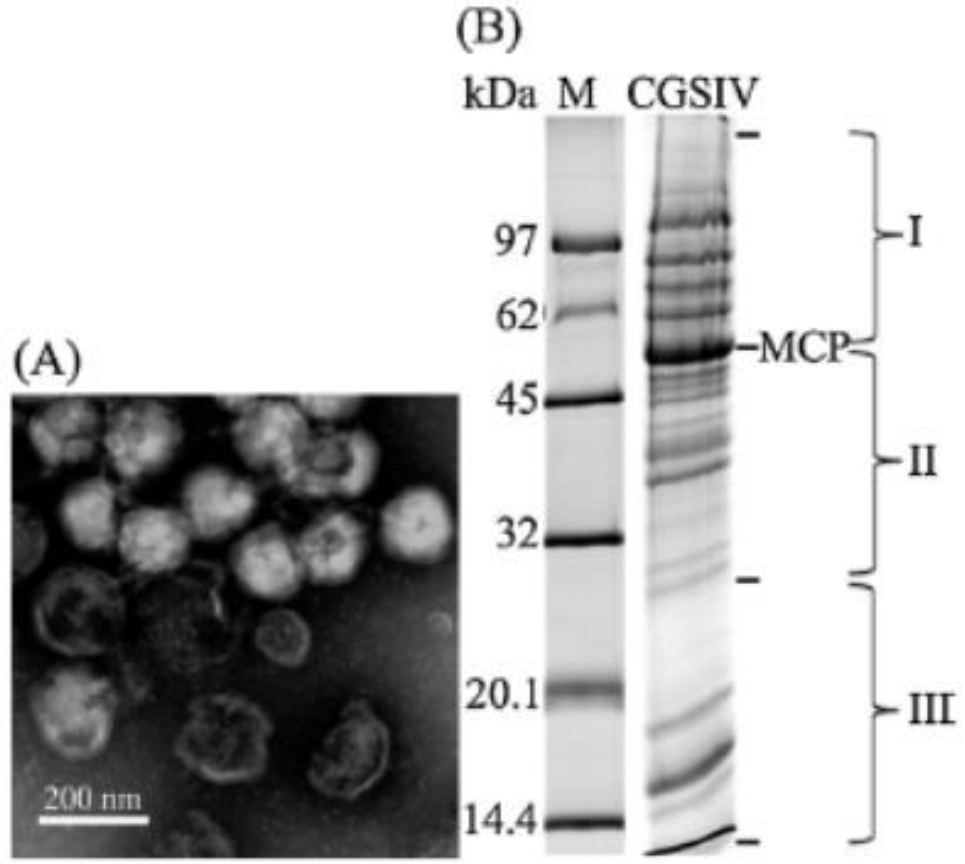
Chen et al. Veterinary Research 2013, 44:101



Basic Features of GSIV

Viral Proteins

- Viruses purified by sucrose gradient centrifuge,
- Proteins were identified by mass spectrometry
- A total of 40 proteins identified

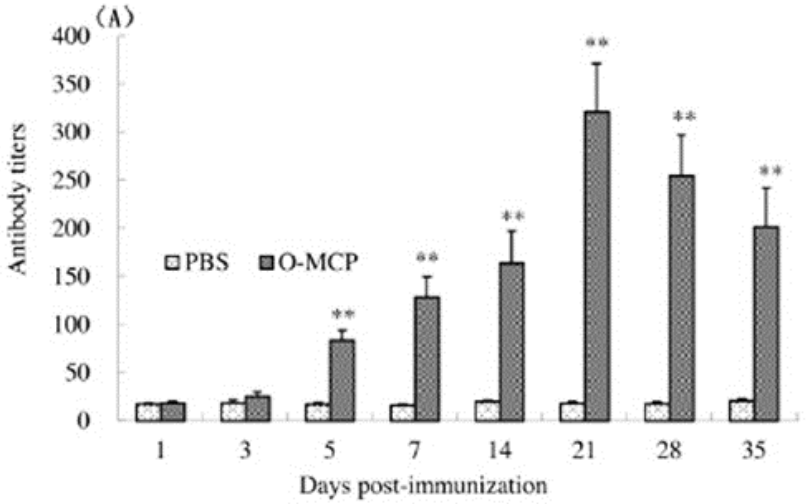


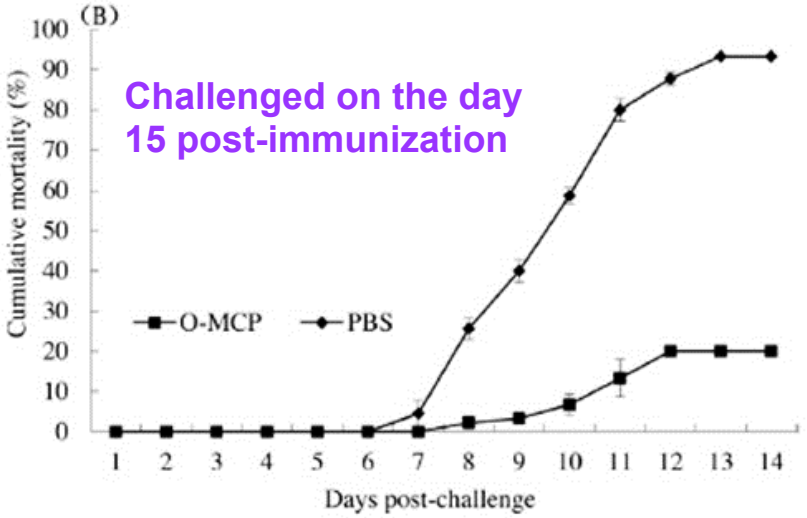
Wei et al. Veterinary Microbiology 172 (2014) 129–139

Prevention & Intervention

Immunization with MCP

- Optimized MCP gene (O-MCP) was expressed in yeast *P. pastoris*
- inoculated intramuscularly with 0.1 ml of 20 ug
- Serum neutralization antibody assay and GSIV challenge test

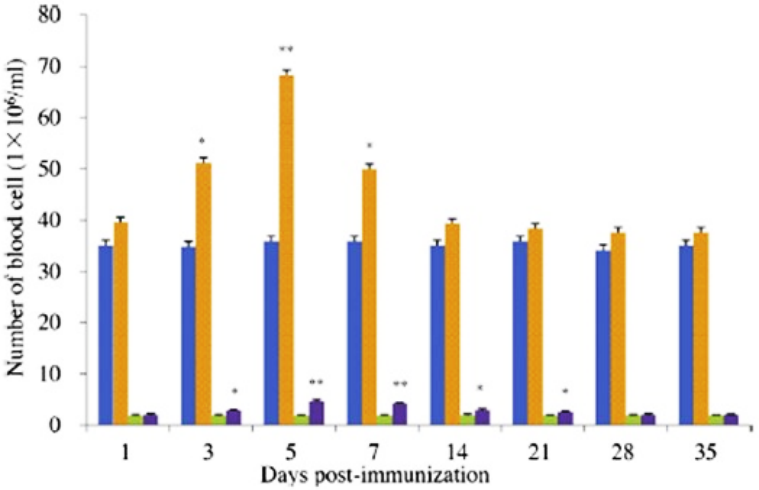




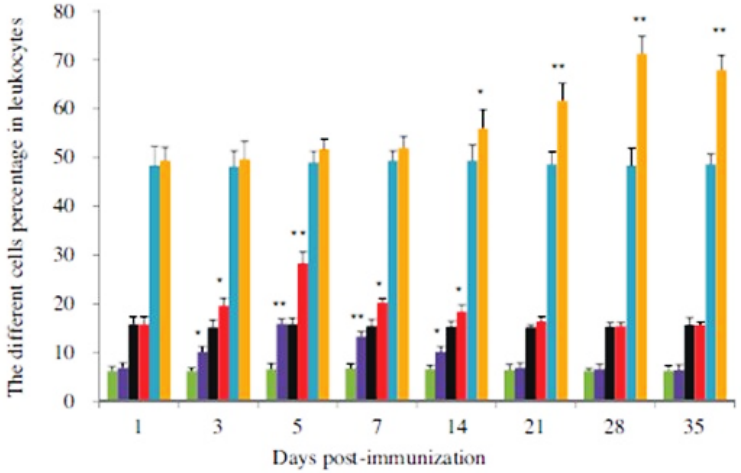
Zhou et al. Vaccine 33 (2015) 5662–5669

Prevention & Intervention

Immunization with MCP



The changes of the erythrocyte and leukocyte numbers



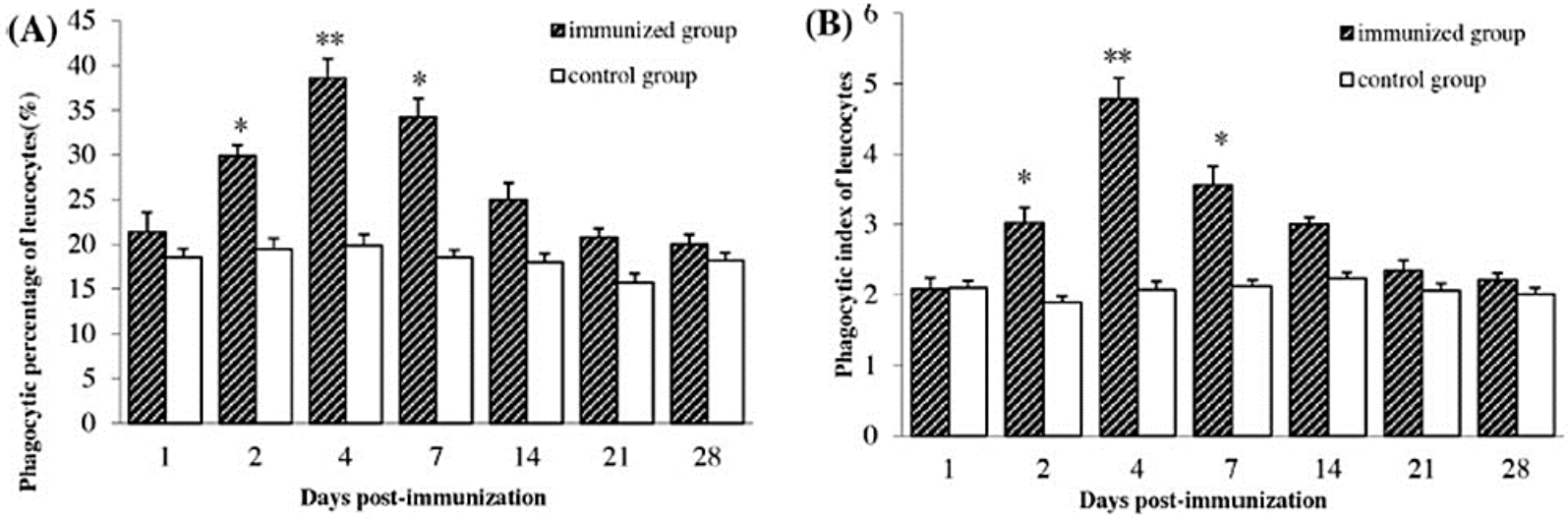
The changes in the differential leukocyte counts of monocytes, neutrophils and lymphocytes

Zhou et al. Vaccine 33 (2015) 5662–5669

Prevention & Intervention

Immunization with inactivated GSIV

The challenge test conducted at day 30 post-injection demonstrated that the immunized group produced a relative survival of 72%.

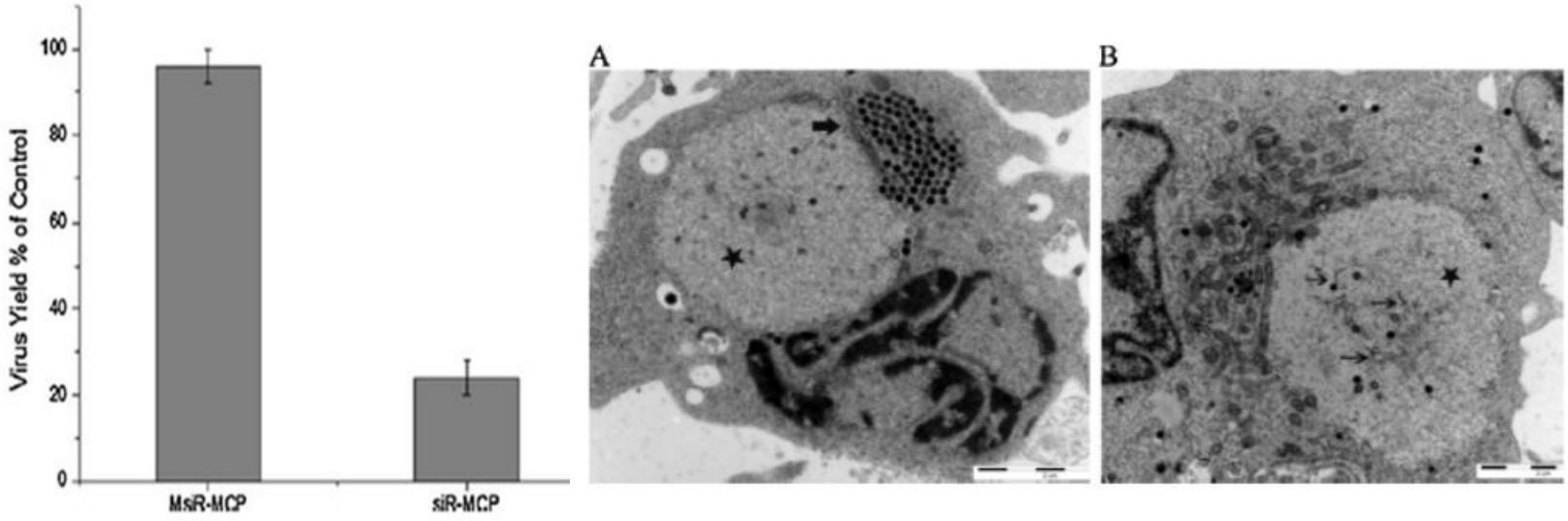


Changes in the phagocytic percentage (PP) and phagocytic index (PI) of the leucocytes

Liu et al. Veterinary Microbiology 174 (2014) 382–390

Prevention & Intervention

siRNA-based knockdown



Effect of viral replication after siRNA-mediated silencing. FHM cells were transfected with MsiR-MCP and siR-MCP and followed infection with CGSIV at an MOI of 0.4 at 24 h post-transfection.

Wei et al. Veterinary Microbiology 172 (2014) 129–139

Protein database of host

- Type I IFN

Initiate host innate immune responses against viral infection

Chen et al. Molecular Immunology 65 (2015) 350–359

- The toll-like receptor 7 (TLR7)

Involved in innate immune responses,

Huang et al. Comparative Biochemistry and Physiology, Part B 184 (2015) 52–57

- Ranavirus-induced thymus cDNA library

Play roles in both cell-mediated and humoral immunity

Zhu et al. Developmental and Comparative Immunology 46 (2014) 413–422

- Proteomic analysis of the skin

Organ acting in defense and cutaneous respiration

Geng et al. J O U R N A L O F P R O T E O M I C S 1 1 9 (2 0 1 5) 1 9 6 – 2 0 8

Future Study

❖ Investigation of wild distribution

The host and virus, systematic assess its ecological impacts

❖ Transmission routes, host sizes/ages

Current research revealed the virus is spread via horizontal transmission, but details remain unknown such as the susceptibility and host sizes/ages, dosage, etc

❖ Viral Life cycle

It has been extensively studied in SGIV, Frog virus 3, but not GSIV

❖ Host immuno-response upon GSIV infection

It is still at very preliminary stage, vaccination, duration and specificity

Thank you for your attention