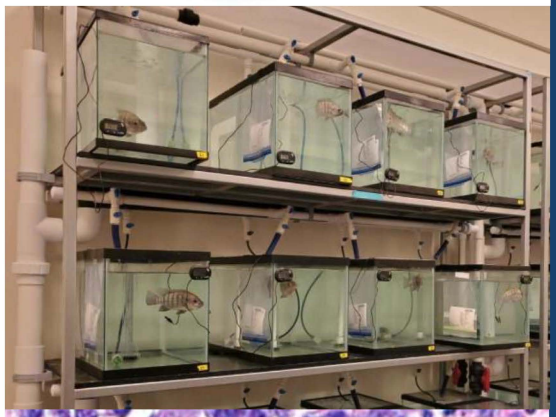
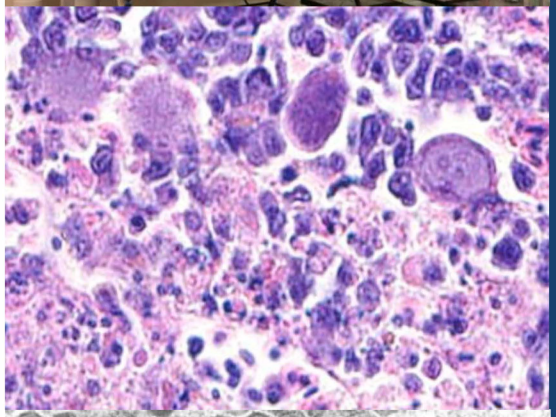
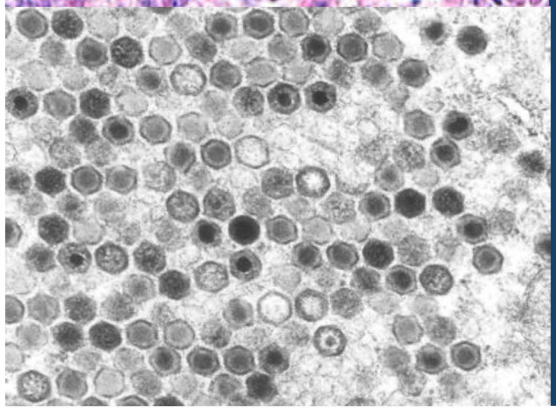






Updates to the Taxonomy, Diagnostics, and Regulations of Megalocytiviruses

Thomas B. Waltzek

(MS, DVM, PhD, DACVM)

McLeary Distinguished Professor

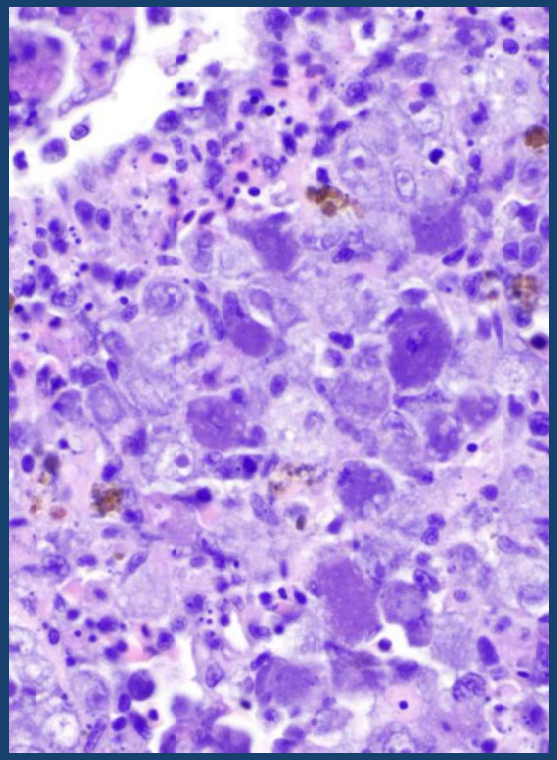
Washington Animal Disease Diagnostic Laboratory

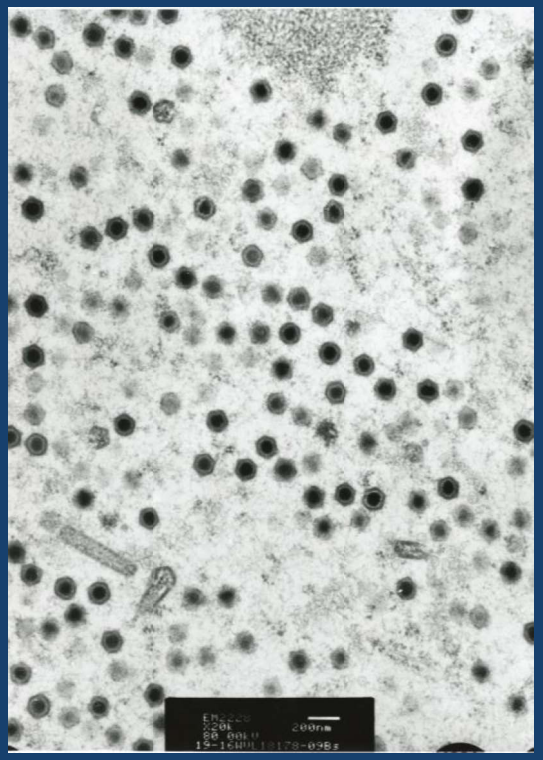
Department of Veterinary Microbiology & Pathology

College of Veterinary Medicine,

Washington State University

WASHINGTON STATE UNIVERSITY





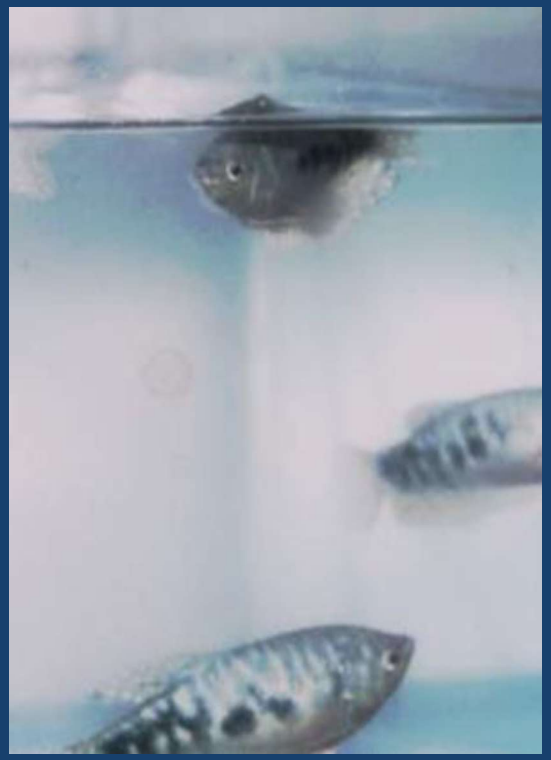
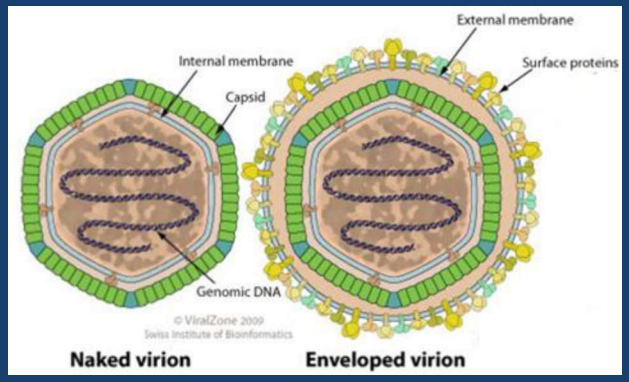


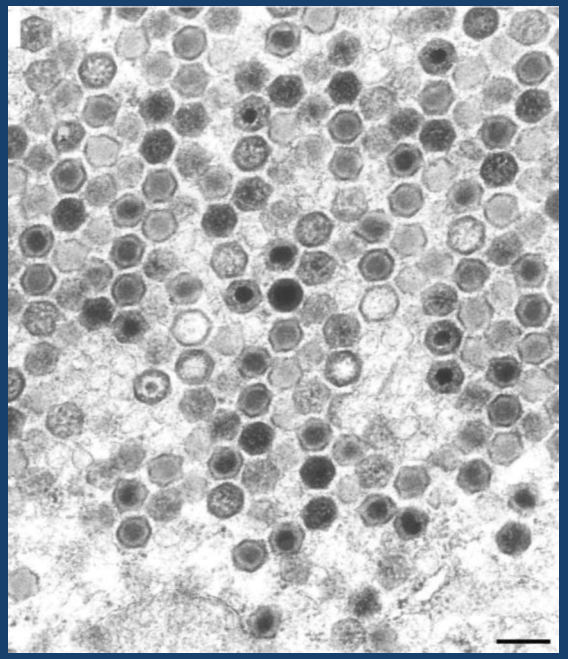
Photo credit: Dr. Roy Yanong

Introduction to ISKNV

ISKNV Morphology



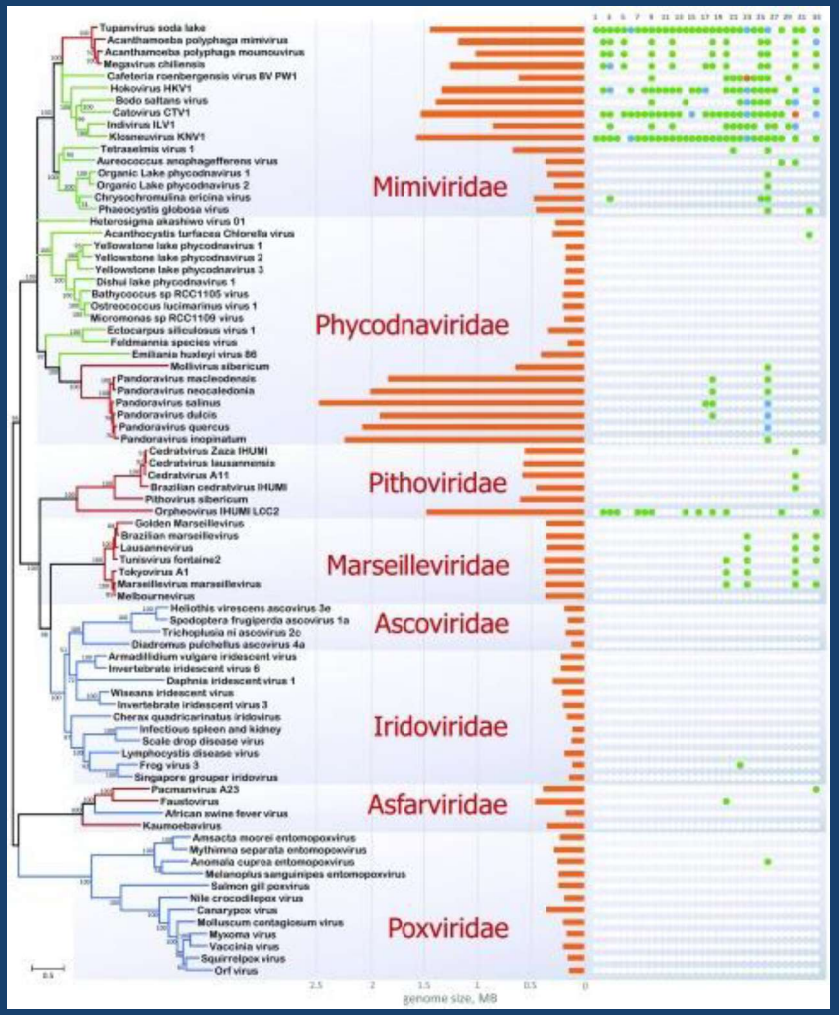
Cartoon showing the basic virion structure of iridoviruses. ICTV.



Transmission electron micrograph showing megalocytivirus particles from infected spleen tissue. Photo courtesy of Dr. Popov.

- Nucleocytoplasmic large DNA virus (NCLDV)
- 120-200nm in diameter
- DNA core surrounded by internal membrane & viral capsid
- Hexagonally-shaped nucleocapsid

ISKNV Taxonomy



Phylogenetic tree of NCLDVs (Koonin & Yutin 2018)

- Family *Iridoviridae* taxonomy
  - Subfamily *Alphairidovirinae*
    - Genus *Lymphocystivirus*
    - Genus *Ranavirus*
    - **Genus *Megalocytivirus***
      - **Species *Infectious spleen and kidney necrosis virus* (ISKNV)**
      - **Species *Scale drop disease virus* (SDDV)**
        - **Unclassified species *European chub iridovirus* (ECIV)**
        - **Unclassified: *Three spine stickleback iridovirus* (TSIV)**

ISKNV Disease

Fish showing abnormal position in water column & lethargy

Fish with petechial hemorrhages on body

Fish displaying coelomic distension

- Behavioral
  - Anorexia
  - Abnormal position in water column
  - Lethargy
  - Increased gilling
- External
  - Darkened or lightened body
  - Pale gills (anemia)
  - Petechial hemorrhaging
- Internal
  - Splenomegaly
  - Coelomic distension (ascites)

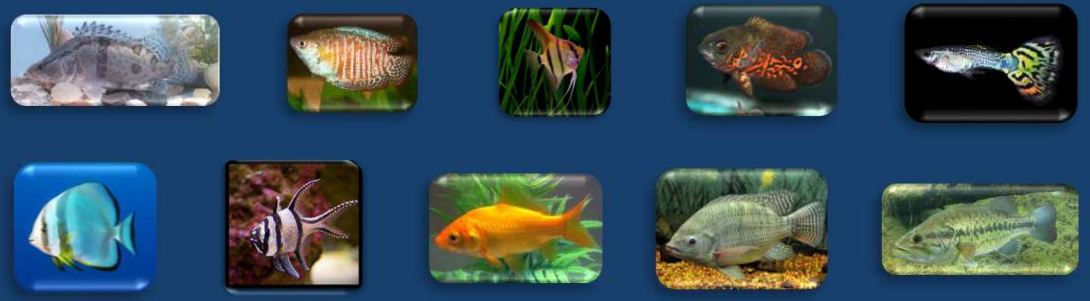
Infectious spleen and kidney necrosis virus

RSIV genotype

Red seabream iridovirus

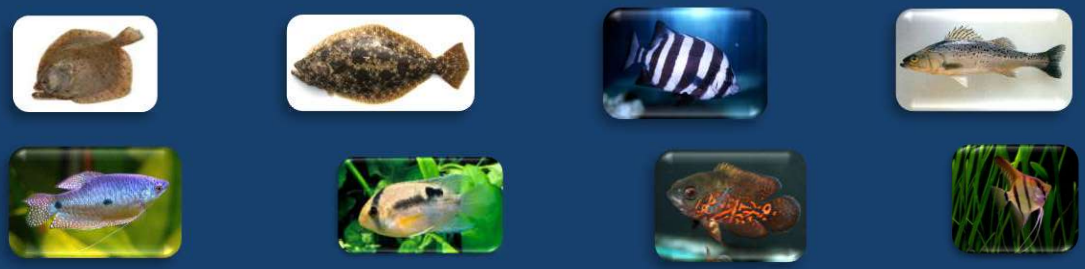
ISKNV genotype

Infectious spleen and kidney necrosis virus



TRBIV genotype

Turbot reddish body iridovirus



>16 Orders, 53 Families, 165 fish species

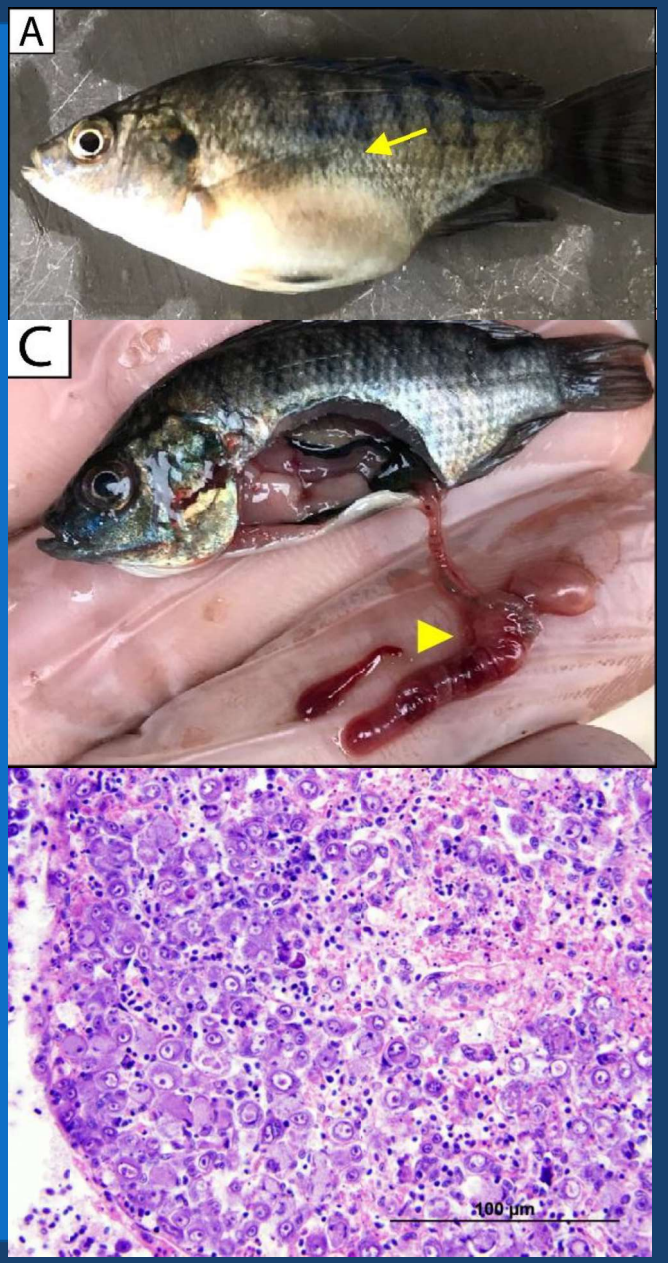
Geographic Range of ISKNV Reports

22 countries
6 continents

- Australia
- Canada
- South Korea
- United States
- China
- Denmark
- Germany
- India
- Singapore
- Thailand
- Indonesia
- Japan
- Israel
- Malaysia
- Taiwan
- **Brazil (Nile tilapia)**
- United Kingdom
- Vietnam
- **Ghana (Nile tilapia)**
- Spain
- Central America
- Dominican Republic

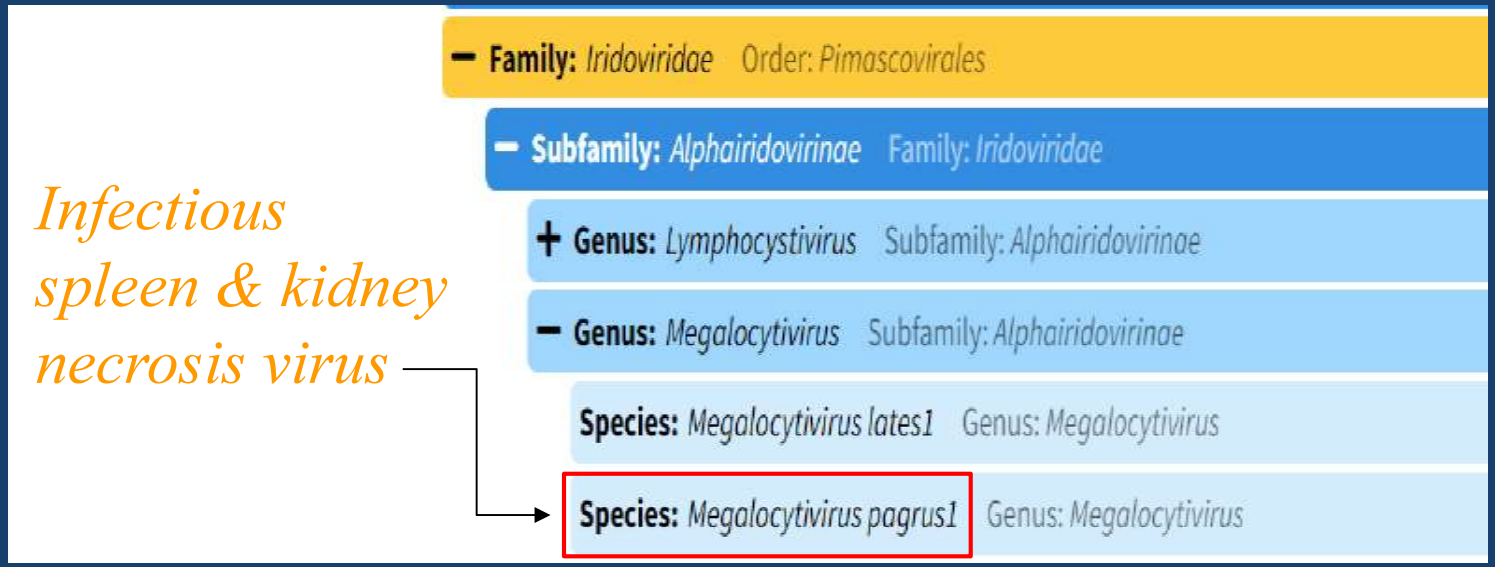
Global Outbreaks of ISKNV in Farmed Nile Tilapia

- United States & Canada (Smith et al. 1997, McGrogan et al. 1998, Subramaniam et al. 2016)
  - Elevated mortality, darkening, lethargy, organ pallor, ascites
  - Histopathology: IC inclusions, TEM: icosahedral iridovirus-like particles
- Thailand (Dong et al. 2015, Suebsing et al. 2016)
- **Ghana 2018-19** (Ramírez-Paredes et al. 2020, Viadanna et al. 2024)
- **Brazil 2019-2024** (Figueiredo et al. 2021, Viadanna et al. 2024, Ferreira et al. 2024)



ICTV Binomial Mandate (Spring 2024)

Infectious spleen & kidney necrosis virus



ICTV

WOAH Listed Fish Diseases (May 2024)

- Infection with *Aphanomycesinvadans* (epizootic ulcerative syndrome)
- Infection with epizootic haematopoietic necrosis virus
- Infection with *Gyrodactylus salaris*
- Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
- Infection with infectious haematopoietic necrosis virus
- Infection with koi herpesvirus
- Infection with *Megalocytivirus pagrus 1*
- Infection with salmonid alphavirus
- Infection with spring viraemia of carp virus
- Infection with tilapia lake virus
- Infection with viral haemorrhagic septicaemia virus.

World Organisation for Animal Health
Founded as OIE

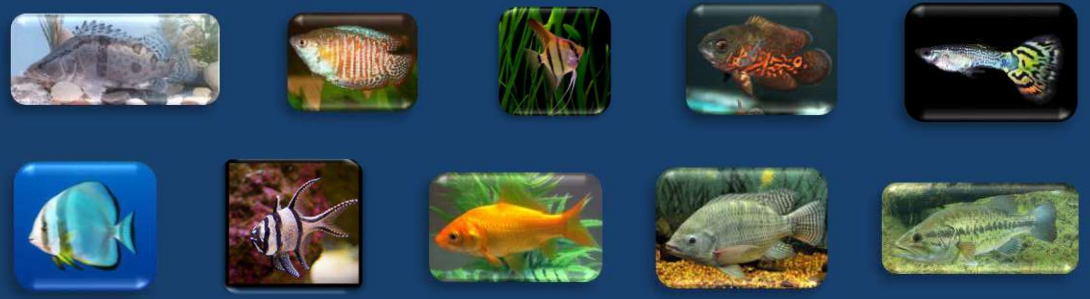
Megalocytivirus pagrus1

RSIV genotype

Red seabream iridovirus

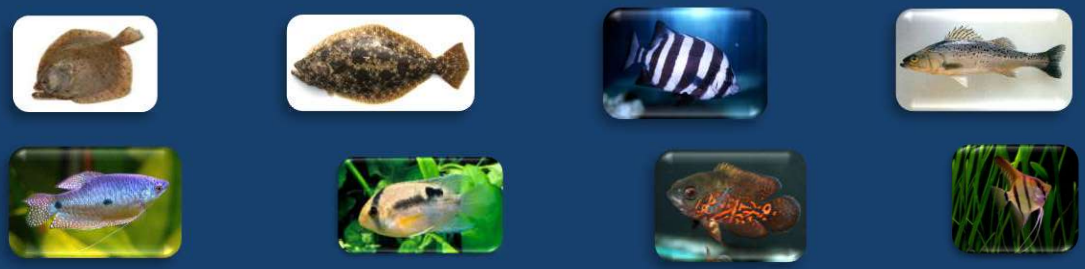
ISKNV genotype

Infectious spleen and kidney necrosis virus



TRBIV genotype

Turbot reddish body iridovirus



Problem 1: **>16 Orders, 53 Families, 165 fish species**

Problem 2: WOAH Manual Chapter 2.3.7 written primarily for the RSIV genotype

RSIV genotype

Red seabream iridovirus

ISKNV genotype

Infectious spleen and kidney necrosis virus

X

TRBIV genotype

Turbot reddish body iridovirus

X

CHAPTER 2.3.7.

RED SEA BREAM IRIDOVIRAL DISEASE

1. Scope

For the purpose of this chapter, red sea bream iridoviral disease (RSIVD) (Inouye *et al.*, 1992) is caused by infection with red sea bream iridovirus.

RSIVD is a significant cause of mortality in farmed red sea bream (*Pagrus major*) and more than 30 other species of cultured marine fish (Kawakami & Nakajima, 2002; Matsuoka *et al.*, 1996) belonging mainly to the orders Perciformes and Pleuronectiformes. The first outbreak of RSIVD was recorded in cultured red sea bream in Shikoku Island, Japan in 1990 (Inouye *et al.*, 1992). Since then, the disease has caused mass mortalities in cultured fish populations in the western part of Japan, mainly among juvenile red sea bream. Affected fish become lethargic, exhibit severe anaemia, petechiae of the gills, and enlargement of the spleen (Inouye *et al.*, 1992; Jung *et al.*, 1997; Nakajima & Maeno, 1998). The disease is characterised by the appearance of enlarged cells stained deeply with Giemsa solution in the histopathological observations of the spleen, heart, kidney, intestine and gill of infected fish (Inouye *et al.*, 1992).

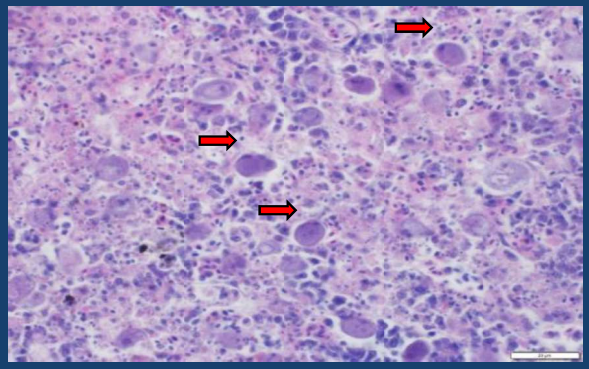
Recently, it has been proved that the disease is caused not only by RSIV (Inouye *et al.*, 1992; Jeong *et al.*, 2003; 2006; Kurita *et al.*, 2002; Kusuda *et al.*, 1994; Nakajima & Kurita, 2005; Nakajima & Sorimachi, 1994) and its synonyms (Chou *et al.*, 1998; Do *et al.*, 2004; 2005; Gibson-Kueh *et al.*, 2004; Jung *et al.*, 1997; Jung & Oh, 2000; Kim *et al.*, 2002; Kurita *et al.*, 2004; Miyata *et al.*, 1997; Nakajima & Kurita, 2005; Sudthongkong *et al.*, 2002b), but also by infectious spleen and kidney necrosis virus (ISKNV) (He *et al.*, 2001; Oseko *et al.*, 2004). The disease is found not only in Japan but also widely in East and South-East Asian countries (Chou *et al.*, 1998; Do *et al.*, 2004; 2005; Gibson-Kueh *et al.*, 2004; Jeong *et al.*, 2003; 2006; Jung *et al.*, 1997; Jung & Oh, 2000; Kim *et al.*, 2002; Kurita *et al.*, 2004; Miyata *et al.*, 1997; Nakajima & Kurita, 2005; Oseko *et al.*, 2004; Sudthongkong *et al.*, 2002b).

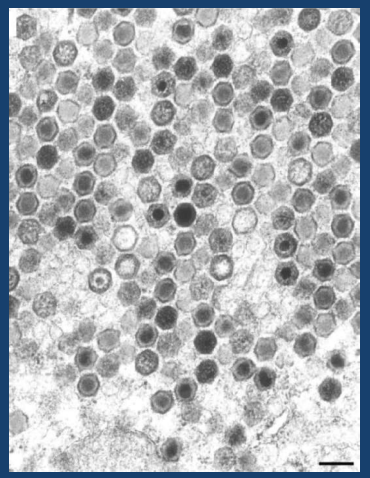
A monoclonal antibody (MAb) against RSIV (Nakajima & Sorimachi, 1995) can detect both RSIV and ISKNV, whereas it does not recognise fish ranaviruses (family: Iridoviridae) by immunofluorescent antibody tests (IFAT) (Nakajima *et al.*, 1998; Oseko *et al.*, 2004).

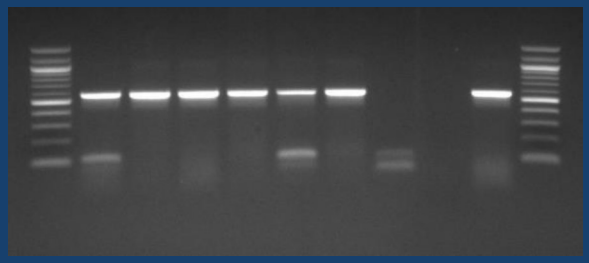
A number of useful diagnostic methods are in use, such as the observation of stained impression smears or tissue sections, an IFAT using a MAb, and polymerase chain reactions (PCR) (Jeong *et al.*, 2004; Kurita *et al.*, 1998; Nakajima *et al.*, 1995; Nakajima *et al.*, 1998; Oshima *et al.*, 1996; 1998).

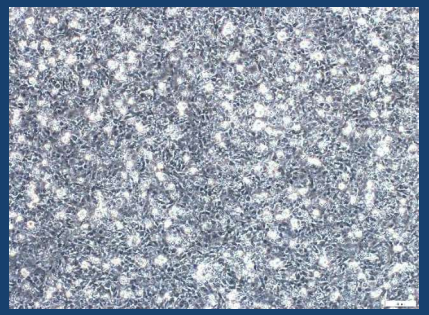
Megalocytivirus pagrus1 Diagnostics

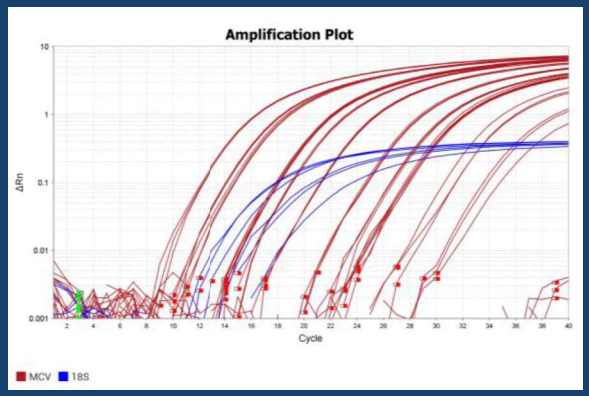
- Microscopic & ultrastructural pathology
  - ISKNV, RSIV, TRBIV
- Virus isolation
  - ISKNV, RSIV, TRBIV
- Immunofluorescence (IFAT)
  - ISKNV, RSIV, **TRBIV**
- Conventional PCR
  - ISKNV, RSIV, **TRBIV**
- Quantitative PCR
  - **No approved assay!**



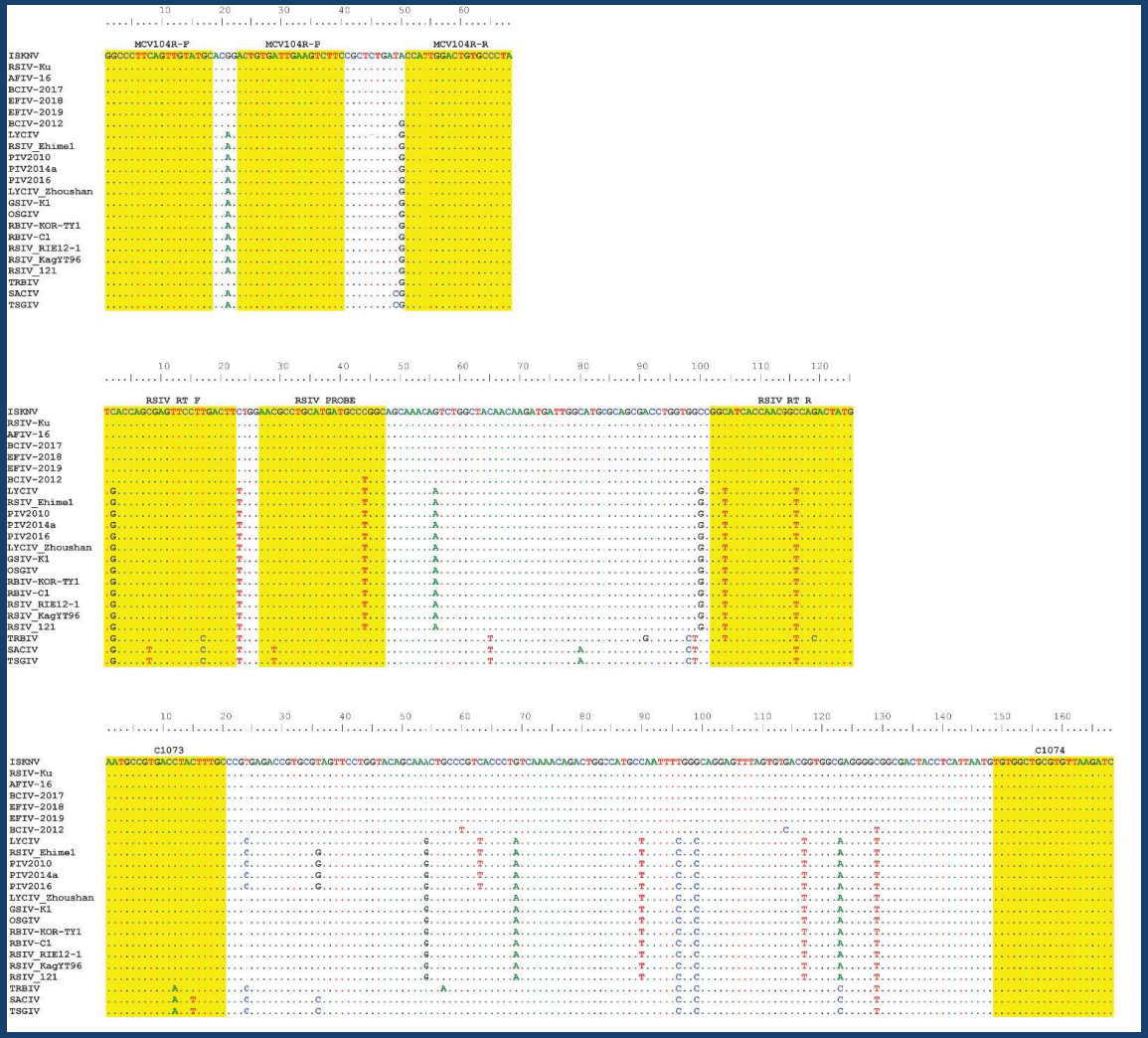








Megalocytivirus pagrus1 qPCR



- SYBR and TaqMan assays developed
- Pan-*Megalocytivirus pagrus1* qPCR assay (Koda et al. 2020)
- **Problem 3: No WOAH recommended qPCR assay**

`

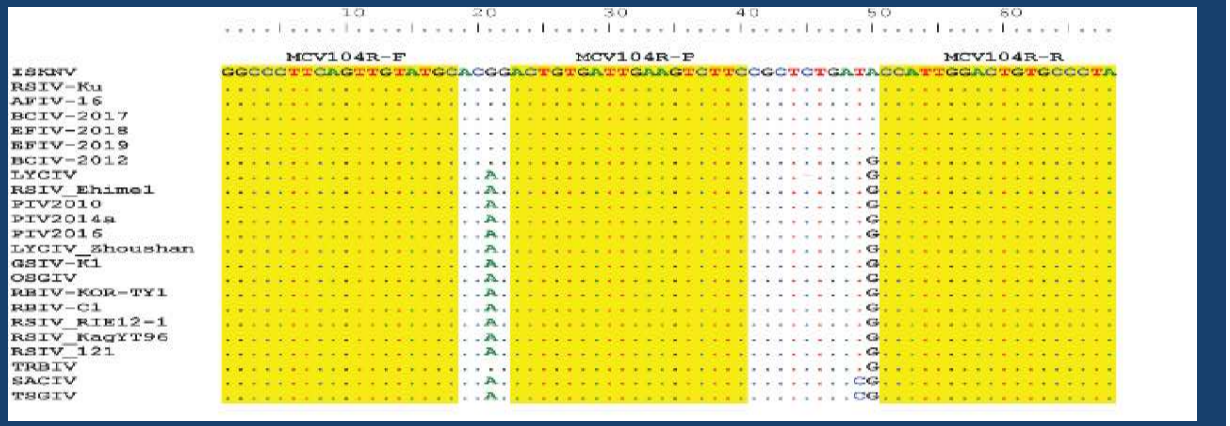
Megalocytivirus pagrus1 qPCR

PLOS ONE

RESEARCH ARTICLE

Partial validation of a TaqMan quantitative polymerase chain reaction for the detection of the three genotypes of *Infectious spleen and kidney necrosis virus*

Samantha A. Koda[1,2], Kuttichantran Subramaniam[1,2], Paul M. Hick[3¤a], Evelyn Hall[3], Thomas B. Waltzek[1,2¤b]*, Joy A. Becker[4]*



Assay	Gene Target	Diagnostic Sensitivity	Diagnostic Specificity	LOD (copy#)
TaqMan	ORF104R	84%	94.4%	10

Megalocytivirus pagrus1 qPCR Assay Validation

STAGE 1

Analytical characteristics ✓

STAGE 2

Diagnostic characteristics ✓

STAGE 3

Reproducibility

STAGE 4

Implementation

World Organisation for Animal Health
Founded as OIE

WOAH ad hoc Working Group

- Lead an interlaboratory comparison of relevant diagnostic methods (stage 3)
  - cPCR & qPCR
- Draft an Aquatic Manual Chapter

2.2. Host factors

2.2.1. Susceptible host species

In the case of RSIV infection: red sea bream (*Pagrus major*), black porgy (*Acanthopagrus schlegeli*), yellowfin sea bream (*Acanthopagrus latus*), crimson sea bream (*Evynnis japonica*), Japanese amberjack (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), yellowtail amberjack (*Seriola lalandi*), hybrid of yellowtail amberjack and Japanese amberjack (*S. lalandi* × *S. quinqueradiata*), striped jack (*Pseudocaranx dentex*), northern bluefin tuna (*Thunnus thynnus*), Japanese Spanish mackerel (*Scomberomorus niphonius*), chub mackerel (*Scomber japonicus*), Japanese jack mackerel (*Trachurus japonicus*), Japanese parrotfish (*Oplegnathus fasciatus*), spotted knifejaw (*Oplegnathus punctatus*), cobia (*Rachycentron canadum*), snubnose pompano (*Trachinotus blochii*), chicken grunt (*Parapristipoma trilineatum*), crescent sweetlips (*Plectorhinchus cinctus*), Chinese emperor (*Lethrinus haematopterus*), spangled emperor (*Lethrinus nebulosus*), largescale blackfish (*Girella punctata*), rockfish (*Sebastes schlegeli*), croceine croaker (*Pseudosciaena crocea*), Hong Kong grouper (*Epinephelus akaara*), convict grouper (*Epinephelus septemfasciatus*), Malabar grouper (*Epinephelus malabaricus*), longtooth grouper (*Epinephelus bruneus*), orange-spotted grouper (*Epinephelus coioides*), yellow grouper (*Epinephelus awoara*), greasy grouper (*Epinephelus tauvina*), brown-marbled grouper (*Epinephelus fuscoguttatus*), giant grouper (*Epinephelus lanceolatus*), Japanese sea perch (*Lateolabrax japonicas*), *Lateolabrax* sp., barramundi or sea bass (*Lates calcarifer*), hybrid of striped sea bass and white bass (*Morone saxatilis* × *M. chrysops*), largemouth bass (*Micropterus salmoides*), bastard halibut (*Paralichthys olivaceus*), spotted halibut (*Verasper variegatus*), and torafugu (*Takifugu rubripes*), are known to be susceptible. In the case of ISKNV infection: Chinese perch (*Siniperca chuatsi*), red drum (*Sciaenops ocellatus*), flathead mullet (*Mugil cephalus*), and *Epinephelus* sp. are known to be susceptible.

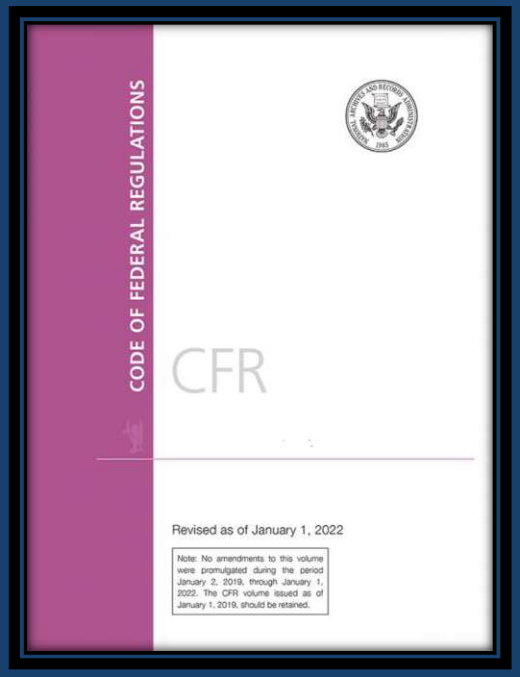
Conclusions

- ***Megalocytivirus pagrus1*** **disease, caused by infection with** ***Megalocytivirus pagrus1*****, is a WOAH listed disease**
  - **Impacts FW/SW fishes reared for food/ornamental purposes**
- **Strains can be grouped into 1/3 genotypes (RSIV, ISKNV, TRBIV)**
  - **All genotypes notifiable (>165 fish species across 16 Orders & 53 Families)**
  - **WOAH Manual Chapter 2.3.7 written primarily for RSIV**
  - **No WOAH recommended qPCR assay**
- **WOAH ad hoc Working Group**
  - ILC of relevant diagnostic methods
  - Draft an Aquatic Manual Chapter

World Organisation for Animal Health
Founded as OIE

Future Import/Export Regulations?

- Will *Megalocytivirus pagrus1* import/export regulations negatively impact US trade?
  - Food/ornamental
- Will US labs be prepared for export testing of ornamental spp.?



USDA APHIS

Lessons Learned: Emergence of TiLV in the US

- Collaboration of private/university/government labs lead to the rapid confirmation of TiLV in Nile Tilapia in the US (2019)
  - USDA response prevented TiLV from becoming established
  - TiLV deemed a significant threat to US tilapia aquaculture
  - USDA Federal Order imposed testing requirements on imports
  - Underscored need for robust/reproducible molecular assay
    - Interlaboratory (6) comparison of TiLV RT-qPCR assay

Wildlife & Aquatic Veterinary Disease Laboratory

BRONSON ANIMAL DISEASE DIAGNOSTIC LABORATORY

College of Veterinary Medicine
Discovery. Detection. Prevention.
WADDL
Washington Animal Disease Diagnostic Laboratory

KENNEBEC RIVER BIOSCIENCES

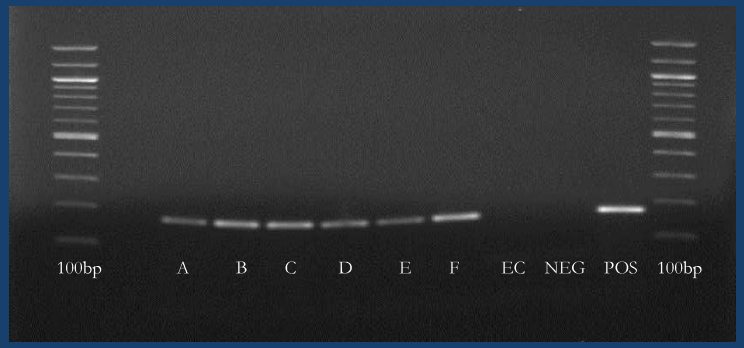
USDA-NVSL
United States Department of Agriculture-National Veterinary Services Laboratory

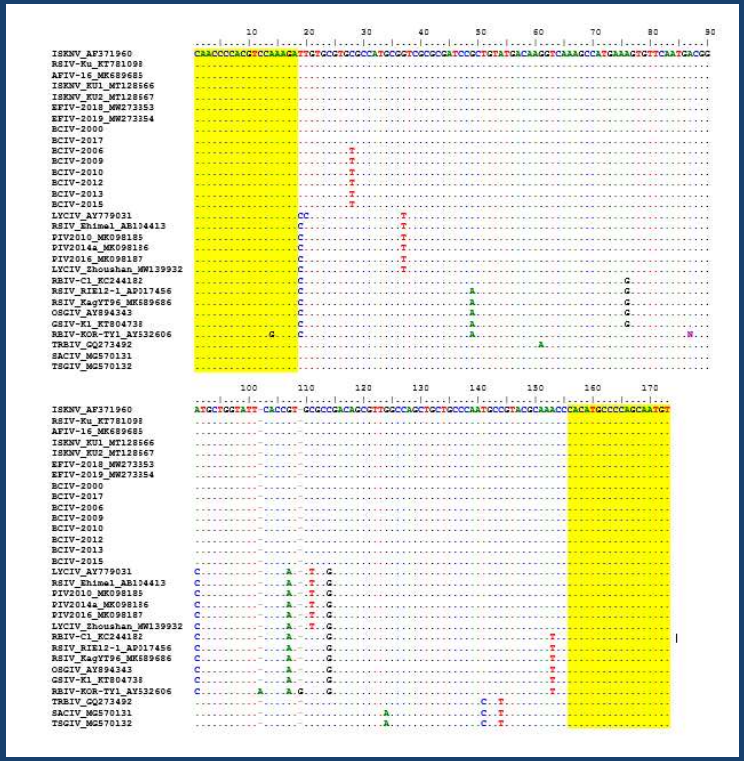
State of New Jersey
Department of Agriculture
Animal Health Diagnostic Laboratory

Thanks for Your Attention!

Questions?

Megalocytivirus pagrus1 Conventional PCR





- 2 OIE primer sets RSIV1-F/R & RSIV4-F/R (Kurita et al. 1998)
- Multiple single, semi-nested, nested PCR assays
- Major capsid protein (MCP) the most targeted gene for ISKNV detection
- Developed pan-ISKNV PCR assay MCV-F/R (Koda et al. 2018)
  - Based on partial of myristlyated membrane protein
  - 173 bp amplicon size
  - Genotyping