Viral Hemorrhagic Septicemia and Spring Viremia of Carp: Threats to Aquaculture

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Overview

• Introduction
  – Aquaculture in Northeastern US
  – VHSV, SVC and the OIE
• Viral Hemorrhagic Septicemia
  – Etiology and pathogenesis
  – 2006 Great Lakes Outbreak
  – 2006 surveillance efforts
  – Work in progress
• SVC
  – Etiology and pathogenesis
• Summary
Aquaculture

- 1998 National Aquaculture Survey
  - Northeastern Region total sales: $127,393,000
    - Foodfish: $85,558,000
    - Baitfish: information withheld
    - Ornamental fish: $5,130,000
  - Southern region: $637,394,000
  - Western Region: $166,565,000
- Estimated 2003 total US production: $960,973,000
Importance of VHSV and SVC

- Viral Hemorrhagic Septicemia virus (VHSV) and Spring Viremia of Carp (SVC) are reportable diseases to the OIE.
- Northeastern US currently faced with an outbreak of VHSV in wild freshwater fish in the Great Lakes and surrounding waterways.
Viral Hemorrhagic Septicemia Virus (VHSV)

- *Rhabdoviridae*, genus *Novirhabdovirus* (VHSV, IHNV, HIRRV)
- Readily transmissible to fish of all ages.
- Colder water disease, mortalities greater at lower water temperatures.
- Survivors of the virus can be lifelong carriers.

Essbauer and Ahne 2001
VHSV - Genotypes

- **Genotype I**
  - Europe, Japan
- **Genotype II**
  - Europe, Japan
- **Genotype III**
  - Europe, Japan
- **Genotype IV**
  - North America, Japan, Korea

0.01 substitutions/site

Elsayed et al. 2006
History of VHSV

- Probably in Europe for many years as a non-problematic pathogen
- 1879 Rainbow trout introduced to France
- 1882 Rainbow trout introduced to Germany
- Naive host and new pathogen
  - DISEASE
History of VHSV

- Early name: Egtved Disease (Denmark)
- First described in Europe by Schaperclaus (1938) as “Kidney Swelling” (Nierenschwellung)
- 1950’s: Thought to be of viral etiology
- 1959: Transmission by injection of fish with a bacteria-free filtrate
- 1963: Isolation of a virus (Jensen)
- Low pathogenicity marine isolates.
VHSV in US

- 1988 and 1989: First known isolations in North America
  - Returning Chinook and Coho Salmon in Pacific NW, clinically normal fish.
  - Very concerning due to proximity to the US Rainbow Trout Industry (Hagerman Valley, Idaho)
- 1990’s:
  - Genotype IV found in multiple marine species (cod, herring) off the Pacific and Atlantic coasts, low mortalities.
- 2005:
  - Lake St. Claire, Michigan (MSU): Muskellunge (some submitted over several years to 2003)
  - Bay of Quinte, Lake Ontario, Canada (U of Guelph): Muskellunge, Freshwater Drum
- 2006:
  - Lake Erie (USFWS, LaCrosse, WI): Freshwater Drum “Windrows of fish” along the beach piled up 10’ wide and 4’ high.
  - New York: Round goby, muskellunge, smallmouth bass, walleye, emerald shiners, bluegill.
  - Lake Huron, MI: walleye, chinook salmon and whitefish 22 miles from the entrance to Lake Michigan.
Clinical signs

• Disease signs (early descriptions in Europe):
  – Primarily young fish
  – Initiate when water temperature reaches 10C
  – Can cause significant losses

• External signs
  – Fish take on darker coloration
  – Exophthalmia ("pop eye")
  – Gills may be pale with some petechiation
  – Hemorrhage in orbits and base of fins

• Impossible to diagnose VHS based on clinical signs alone, some fish may have severe signs of the disease, others may have no signs.
The Disease

• Internal signs
  – Some fish with severe internal hemorrhage; some with petechial hemorrhage
  – Petechial hemorrhage in muscle
  – Visible changes in
    • Liver – pale, mottled with hyperemic areas
    • Kidneys – more intensely red, swollen
    • Spleen – enlarged
  – Digestive tract may be devoid of food
VHSV – Gross Pathology

• Causes a hemorrhagic disease

• Multiplies in endothelial cells of blood capillaries, leucocytes, hematopoietic and nephron cells
Diagnosis of VHSV- Histopathology

• Histopathological changes are generally confined to the liver, kidneys, spleen, and skeletal muscle.
  – In the liver, kidneys, and spleen, focal to extensive necrotic changes can occur
  – The hematopoietic areas of the kidney and spleen are the initial foci of infection.
  – In skeletal muscle, blood cells accumulate in muscle bundles and fibers but little damage occurs.
VHSV - Histopathology

Liver: pyknotic nuclei

Kidney: necrosis

Spleen: necrosis

Gill: thickened lamellae
Diagnosis of VHSV- Virology

- Virology is required to diagnose VHSV.
- Standard cell culture methods used to initially detect the presence of a virus.
  - Cell lines recommended are FHM, RTG-2, CHSE, and EPC.
  - Inoculate cell monolayers with filtered homogenate of tissues from suspect fish.
  - Incubate at 15\(^\circ\)C, inspecting for cytopathic effect (CPE) regularly.
  - CPE positive samples should be re-inoculated on cells (passaged) and exhibit CPE again on new monolayers.
VHSV - Detection

- VHSV isolates were cultured on fathead minnow cells.
- Cytopathic effects was noted after 3 days at 15°C.
- Cells rounded up with a granular appearance.

Negative control

CPE 4 days PI
Diagnosis of VHSV- Virology

• Confirm VHSV (from the 2003 OIE Manual of Diagnostic Tests for Aquatic Organisms):

• rt-PCR
  – Sequence a specific unique area (eg: N gene) of the VHSV genome.
  – Extract mRNA from suspect tissue or CPE-positive cell cultures.
  – Use specific markers from the VHSV sequence to detect the viral mRNA.
  – Need specific sequence of virus.
  – Faster turn-around.
VHSV - New York isolations

- In May 2006, VHSV was isolated during an extensive die-off of round gobies *Neogobius melanostomus* from the St. Lawrence River near Cape Vincent, NY and from Lake Ontario near Rochester, NY.
- Testing of moribund muskellunge from the St. Lawrence River also showed the presence of VHSV by tissue culture.
VHSV - New York isolations

• Subsequent testing of moribund smallmouth bass (*Micropterus dolomieu*) from two locations on Lake Ontario also demonstrated the presence of VHSV by tissue culture.

• Finally, a moribund burbot (*Lota lota*) from the St. Lawrence River near Clayton, NY was found infected with VHSV by culture.
VHSV - Sequencing

- A region of the G gene was sequenced at the USGS Seattle Laboratory.
- VHSV isolates from the Lake Ontario freshwater drum, the Lake St. Clair muskellunge, and the round goby were found to be essentially identical.
- Significantly different from other North American VHSV isolates- Genotype IVb.

Elsayed et al. In Press
2006 VHSV Testing

- Surveillance for VHSV in New York State
- Approximately 1300 samples (fish or pools of fish) received for VHSV testing.
- Cell culture on all samples according to FHS and OIE standards.
- Quantitative rt-PCR on all samples.
- Samples received from:
  - Fish kill investigations.
  - St. Lawrence River healthy fish survey.
  - NYSDEC healthy fish survey.
  - Baitfish survey.
2006 VHSV Testing

- Fish kill investigations:
  - Fish submitted as part of ongoing mortality event investigations.
  - 21 different cases from 16 locations, mostly Great Lakes and St. Lawrence.
- Confirmed VHSV cases:
  - Round goby: Cape Vincent, St. Lawrence
  - Burbot: Clayton, St. Lawrence
  - Round goby: Irondequoit Bay, Lake Ontario
  - Smallmouth bass: Sodus Bay, Lake Ontario
  - Muskellunge: Clayton, St. Lawrence
  - Smallmouth bass: Tibbitts Point, Lake Ontario
  - Walleye: Lake Conesus
2006 VHSV Testing

• St. Lawrence healthy fish survey.
• All fish were collected from St. Lawrence River by SUNY-ESF during late May to early June 2006.
• Samples frozen and transported to Cornell for processing.
• Approximately 300 fish from 18 locations along the St. Lawrence River near TIBS.
• VHSV confirmed species:
  – Brown bullhead: Garlock
  – Bluegill: Garlock
  – Smallmouth bass: Rose Outer
2006 VHSV Testing

• NYSDEC Healthy fish survey
  – Survey of predator and prey species from priority water bodies in New York State.
  – Goal to have 30 prey and 30 predator samples from each location.
  – Samples delivered ASAP to Cornell for testing to reduce loss of diagnostic value.
  – 16 locations, 865 fish.
  – No cell culture positive samples at present time!
Baitfish Survey

• Healthy baitfish minnows collected.
• Pooled samples for VHSV testing (5 fish each)
• Confirmed VHSV:
  – Bluntnose minnows: St. Lawrence River
  – Emerald shiners: Niagara River
  – Emerald shiners: Lake Erie
• Control: Van Camp Pond, private baitfish pond with no new additions for 3+ years.
  – Cell culture negative, 1 pool was qRT-PCR positive.
VHSV - Summary

- VHSV is an emerging disease of freshwater fish in North America.
- VHS has been detected in Lake Ontario, Lake Erie, Lake St. Clair, Conesus Lake, and the St. Lawrence River.
- A type IV strain of VHSV has been isolated from varied species including round goby, muskellunge, freshwater drum, smallmouth bass, yellow perch, bluegill, walleye, emerald shiners.
- Sequence of the round goby and muskellunge isolates are significantly different from other North American VHSV marine isolates, designated Genotype IVb
Spring Viremia of Carp

- *Rhabdoviridae*, specifically *Rhabdovirus carpio*.
- Affects common carp, koi carp, grass carp, silver carp, bighead carp, crucian carp, goldfish, tench, and sheatfish.
- Common carp are the most susceptible species.
  - considered to be the principal host.
- Very young fish of various species are also susceptible.
  - Eg: pike, perch

History of SVC

• First described in Yugoslavia in 1971.
• Present in Europe for at least 50 years.
• Substantial impact on carp production in Europe:
  – Estimated 10-15% of 1 year old fish.
  – Natural mortalities can reach 70%
Spring Viremia of Carp in USA

- **2002:** First cases in USA were confirmed
  - North Carolina, Virginia: Koi hatchery.
  - Wisconsin: Cedar Lake, 10-ton fish kill.

- **2003:**
  - Illinois: carrier carp detected in the Cal-Sag channel near Chicago

- **2004:**
  - Washington: common carp
  - Missouri: Pike County, ornamental koi.
SVC 2006

• June 2006:
  – 150 common carp from Hamilton Harbor, Lake Ontario screened for VHSV prior to shipment.
  – VHSV was not detected in these fish, but Spring Viremia of Carp virus was isolated.
  – Virus was isolated from 18 of 30 five fish tissue pools on EPC cells. CPE was not evident on CHSE cells.
  – The virus was sent to CEFAS, Weymouth, UK for confirmation testing, confirmed as SVCV.
  – Phylogenetic analysis grouped the Canadian isolate in SVCV genogroup together with isolates from the United States and Asia.
  – This isolation represents the first detection of SVCV in Canada.
Spring Viremia of Carp

• Optimal temperature 16-17°C
  – 90% mortalities in experimental studies.
• Mortality reduced at higher and lower temperatures.
• Outbreaks occur during spring with warming temperatures.
• Horizontal transmission through water.
SVCV Summary

• Highly contagious disease of yearling carp.
• Present in the Great Lakes, however no mortality events confirmed to date.
• Affects other species, including endangered wild minnows and commercial species such as perch.
VHSV and SVCV – future work

- Continued co-operation with the New York State Department of Environmental Conservation.
- Dead or dying fish that are suspected to be infected with VHSV or SVC should be immediately submitted for evaluation to regional agencies.
VHSV and SVCV - ongoing work

• Development of a quantitative RT-PCR test for VHSV.
  – Cloned regions of the N and G gene specific to the Great Lakes type IVb genotype of VHSV.
  – Allows for very sensitive detection of low levels of VHSV in fish and water.
  – Results give an absolute quantity of viral particles or genes detected in the sample.

• Development of a QRT-PCR test for SVCV.
VHSV – ongoing work

• Validation of qRT-PCR test.
  – Official procedure outlined by the OIE (World Organization of Animal Health).
  – Statistics on 2006 results.
  – Test becomes officially sanctioned as a valid test for the initial detection of VHSV.
    • Advantages: cheap and fast results.
    • Disadvantages: expensive equipment and need highly trained laboratory personnel.
VHSV and SVC: Concerns

- Historically VHSV and SVCV are known as an extremely serious viral pathogens of economically important fish species.
- Recent isolations signal an early invasion of VHSV and SVC in freshwater fish that inhabit the Great Lakes.
- VHSV had not been previously known in the freshwater environment of the western hemisphere.
- Current host and geographic range of VHSV and SVC must be determined to manage the spread of this disease.
- The full significance to aquaculture is not known at this point.
VHSV and SVC: Questions

• Are the Lake Ontario salmonids at risk?
• By what mechanisms can VHSV be transmitted to new fish species and to new locations?
  – Can forage fish facilitate transmission to predatory fish?
  – Can commercial and/or recreational boat traffic serve as vectors?
  – Can sport fishing activities serve as vectors?
  – Can other animals serve as vectors (e.g. aquatic birds)?
  – What is the impact of movement of fish?
    • Stocked fish
    • Baitfish
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