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Fish Diseases: Viral Hemorrhagic Septicemia (VHS)

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Viral Hemorrhagic Septicemia (VHS) has been described as one of the most devastating fish diseases on a worldwide basis. VHS is caused by the Viral Hemorrhagic Septicemia Virus (VHSV), a member of the virus family *Rhabdoviridae*. Rhabdoviruses are bullet-shaped viruses that contain a single-stranded RNA genome. This is the same family of viruses that contains a distant relative to VHSV, the Rabies Virus. However, it is extremely important to realize that VHSV is a pathogen of fish and poses no health risk to humans.

Historically, VHS has been known for many years as a devastating disease of freshwater-reared rainbow trout on the European continent. Disease events known as early as the 1930's were thought to have a viral cause (a viral etiology), but it was not until the early 1960's, when the techniques of fish cell culture became available, that the virus was cultured and proven to be the cause of Viral Hemorrhagic Septicemia. A major event in the history of VHS occurred in 1988 and 1989 when VHSV was isolated from apparently normal returning sea-run chinook and coho salmon in the Puget Sound area of Washington, in the Pacific Northwest of the United States. There was great concern surrounding the apparent movement of this disease from the European continent to the Pacific Northwest by some unknown It was around this time that fish mechanism.

pathologists in Europe isolated VHSV from Atlantic cod. This was followed by the isolation of VHSV from Pacific cod and then from other marine fish species. Discovery of VHSV in marine fish made the fish health community think of VHSV as a pathogen of marine fish that somehow moved into the freshwater trout culture facilities of Europe. While it is not possible to prove the mechanism by which VHSV moved into the freshwater fish culture environment in Europe, one potential mechanism could have been the feeding of unpasteurized "rough fish", which were considered a by-catch of the marine harvest, to those rainbow trout reared in freshwater aquaculture.

The emergence of VHSV in the Great Lakes Basin of North America in 2005 marked another major milestone in the history of this virus and the disease it causes. Up to this time, VHSV was known to exist in various marine fish species in the Atlantic and Pacific Oceans. The confirmation of VHSV infections in several fish species in Lake St. Claire and Lake Ontario (Bay of Quinte) in the spring of 2005 marked the first time this pathogen was identified as a cause of mortality in freshwater fish in North America. As a pathogen that is listed by the OIE (World Organization for Animal Health) as reportable, the finding of VHSV in this new location has significant trade implications on a national and international level.

Because VHSV is an OIE reportable pathogen, the scientific community as well as fisheries managers and aquaculturists must be aware of some special regulatory requirements associated with this pathogen. If a USDA APHIS (United States Department of Agriculture Animal and Plant Health Inspection Service) accredited veterinarian or a laboratory official from an APHIS approved laboratory suspects that they have a diagnostic case in which VHSV will be found, they are legally obligated to report to their USDA APHIS Area Veterinarian-in-Charge. From that point, depending on the circumstances of the case (such as a new fish species being identified as a potential host), arrangements may be made to ship appropriate diagnostic materials to the USDA APHIS National Veterinary Services Laboratories, in Ames, Iowa, for confirmation of the finding.

As of the summer of 2007, appropriate diagnostic materials are understood to mean original fish tissues from the suspect animals. Requirements for veterinarians to report to state authorities will be based on legislation in the state where the veterinary has a license to practice. Reporting may also occur to the Competent Regulatory Authority in the state where the finding occurs as well as the State Veterinarian and the state natural resource agency, depending on whether the identification occurred in a wild or farmed fish and which state agency has authority within the state. Individuals with suspect cases of VHSV and who are not veterinarians, will typical report in a similar manner as a professional courtesy to USDA APHIS as well as other entitites and cooperate in efforts that will limit the adverse impact of important OIE notifiable pathogens, such as VSHV. Upon receipt of a VHSV report, APHIS and state authorities may take regulatory action to eradicate or prevent further spread of the virus. The action taken will depend on circumstances and may include anything from no action to quarantine and eradication of affected fish populations.

The Emergence of VHSV in the Great Lakes Basin

As noted above, VHSV in the Great Lakes Region was first identified in fish collected from the Bay of Quinte, Lake Ontario, Canada in the spring of 2005. The Bay of Quinte isolation of VHSV was the result of an investigation of a massive mortality event of freshwater drum *Aplodinotus grunniens* as well as other species. Subsequent investigation revealed that a virus

isolated from diseased muskellunge *Esox masquinongy* collected from Lake St Claire, Michigan in 2003 was, in fact, VHSV. The Lake St. Claire isolate was obtained when muskellunge were being examined for the presence of the piscirickettsia organism and is the earliest isolate from the Great Lakes Region to be identified to date. Until 2005, there were four genotypes of VHSV. Genotypes I, II, and III were known to occur in Europe and Japan. Genotype IV was known to occur in the marine environment of North America, Japan and Korea. The Lake St. Claire isolate of VHSV was most closely related to Genotype IV, which had only been found in the marine environment. However, there was enough difference between the marine VHSV Genotype IV and the freshwater VHSV Genotype IV, that the marine isolates were classified as belonging to Genotype IVa, while the freshwater isolate from the Great Lakes was classified as belonging to Genotype IVb. It is most interesting that all of the subsequent isolates (approximately 30) from the Great Lakes Basin that have undergone genetic evaluation have been found to belong to Genotype IVb and all are virtually identical to the original Lake St. Claire isolate. The value of this information is that it suggests that the emergence of VHSV IVb in the Great Lakes Basin is a relatively new occurrence. This can be said because VHSV is an RNA virus and RNA viruses have a much greater tendency to undergo genetic changes than do DNA viruses. If VHSV IVb had been present in the Great Lakes Basin for a considerable period of time, one would expect to find more genetic diversity in the isolates than has been observed to date (Table 1).

Table 1. Currently known Genotypes and Distribution Viral Hemorrhagic Septicemia Virus (VHSV).

VHSV Genotype I VHSV Genotype II VHSV Genotype III VHSV Genotype IVa VHSV Genotype IVb

Europe Europe Europe North America, Japan, Korea North America The spring and summer of 2006 were marked by a number of significant mortality events in a wide variety of fish species. Significant mortality events were documented in such species as freshwater drum, yellow perch, and smallmouth bass. Through disease investigations and surveillance efforts, by the end of 2006 VHSV IVb was found in the Great Lakes Basin in the St. Lawrence River, Lake Ontario, Lake St. Claire, and Lake Huron. The 2006 season also marked the first time that VHSV IVb was found in fish outside of the Great Lakes proper when the virus was isolated from walleye from Conesus Lake in New York State.

In 2007, VHSV IVb was found in Lake Michigan in the region of Green Bay, Wisconsin. Most disturbing was that the virus continued to be found in additional inland bodies of water when it was found in Skaneateles Lake, Little Salmon River, Cayuga-Seneca Canal, and a private pond in western New York State, the Lake Winnebago chain of lakes in Wisconsin and in Budd Lake, Michigan. The example of the private pond may stand as a particularly interesting lesson. The owners of the pond were hiking in the area and noted fish in "obvious distress" in a local stream. In a desire to do something they thought would be helpful, they "rescued the fish" and brought them home to be placed them in their private pond. Within approximately one month virtually all of the fish in the pond died and VHSV IVb was identified and confirmed in diagnostic specimens.

The 2008 season was marked by two significant events in the spread of VHSV IVb. The virus was isolated from wild muskellunge collected for use as broodfish from Clear Fork Reservoir, Ohio. This marked the first time that VHSV IVb was found in fish from a location outside of the Great Lakes drainage basin. Clear Fork Reservoir lies in the Mississippi River drainage. A second significant event was the isolation of VHSV IVb in sea lampreys from northern Lake Huron by fish health specialists from the U.S. Fish and Wildlife Service. Fish for which the isolation of VHSV has been confirmed by USDA APHIS are considered regulated species with regard to the VHSV interim rule. With the diversity of regulated fish species (Table 2), the

Table 2. List of Species Regulated	y the VHSV interim rule	(list effective to 9 September 2008)

Family Centrarchid	ae	<u>Family Salmoni</u>	<u>dae</u>		
Black Crappie	Pomoxis nitromaculatus	Brown Trout	Salmo trutta		
Bluegill	Lepomis macrochirus	Chinook Salmon	Oncorhynchus tshawytscha		
Largemouth Bass	Micropterus salmoides	Lake Whitefish	Coregonus clupeaformis		
Pumpkinseed	Lepomis gibbosus	Rainbow Trout	Oncorhynchus mykiss		
Rock Bass	Ambloplites ruprestris				
Smallmouth Bass	Micropterus dolomieu	Family Gadidae			
		Burbot	Lota lota		
<u>Family Cyprinidae</u>					
Bluntnose Minnnow	Pimephales notatus	Family Scianidae			
Emerald Shiner	Notropis atherinoides	Freshwater Drum Aplodinotus grunniens			
Spottail Shiner	Notropis hudsonius				
Shorthead Redhorse	Moxostoma macrolepidotum	Family Gobiidae			
Silver Redhorse	Moxostoma anisurum	Round Goby	Neogobius melanostomus		
		-			
			Family Clupeidae		
Family Ictaluridae					
Brown Bullhead	Amieurus nebulosus	Gizzard Shad	Dorosoma cepedianum		
	Amieurus nebulosus Ictalurus punctatus	Gizzard Shad	Dorosoma cepedianum		
Brown Bullhead Channel Catfish		Gizzard Shad Family Moronid	Dorosoma cepedianum lae		
Brown Bullhead Channel Catfish <u>Family Esocidae</u>	Ictalurus punctatus	Gizzard Shad Family Moronid White Bass	Dorosoma cepedianum <mark>lae</mark> Morone chrysops		
Brown Bullhead Channel Catfish <u>Family Esocidae</u> Muskellunge	Ictalurus punctatus Esox masquinongy	Gizzard Shad Family Moronid	Dorosoma cepedianum lae		
Brown Bullhead Channel Catfish <u>Family Esocidae</u>	Ictalurus punctatus	Gizzard Shad Family Moronid White Bass White Perch	Dorosoma cepedianum <mark>lae</mark> Morone chrysops Morone americana		
Brown Bullhead Channel Catfish <u>Family Esocidae</u> Muskellunge Northern Pike	Ictalurus punctatus Esox masquinongy	Gizzard Shad Family Moronid White Bass White Perch Family Percopsi	Dorosoma cepedianum <mark>lae</mark> Morone chrysops Morone americana		
Brown Bullhead Channel Catfish <u>Family Esocidae</u> Muskellunge Northern Pike <u>Family Percidae</u>	Ictalurus punctatus Esox masquinongy Esox niger	Gizzard Shad Family Moronid White Bass White Perch	Dorosoma cepedianum <mark>lae</mark> Morone chrysops Morone americana		
Brown Bullhead Channel Catfish <u>Family Esocidae</u> Muskellunge Northern Pike <u>Family Percidae</u> Walleye	Ictalurus punctatus Esox masquinongy Esox niger Sander vitreus	Gizzard Shad Family Moronid White Bass White Perch Family Percopsi	Dorosoma cepedianum <mark>lae</mark> Morone chrysops Morone americana		
Brown Bullhead Channel Catfish <u>Family Esocidae</u> Muskellunge Northern Pike <u>Family Percidae</u>	Ictalurus punctatus Esox masquinongy Esox niger	Gizzard Shad Family Moronid White Bass White Perch Family Percopsi	Dorosoma cepedianum <mark>lae</mark> Morone chrysops Morone americana		
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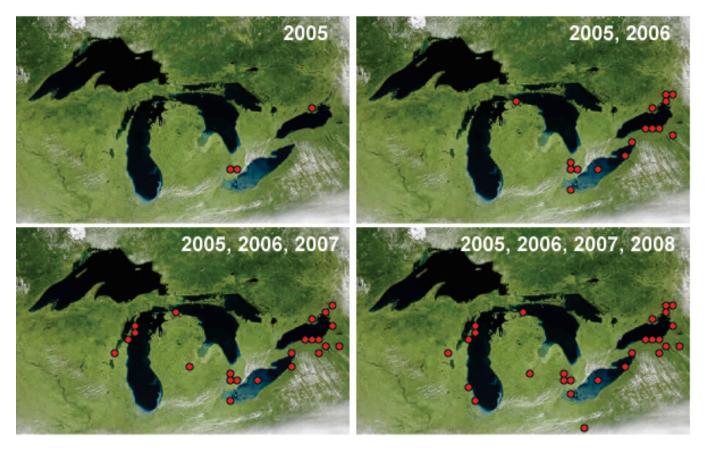


Figure 1. Map of the Great Lakes Region showing cumulative fish mortality events with confirmed isolations of VHSV IVb from 2005 through 2008. (basic map from http://www.coastwatch.msu.edu)

aquaculturist should exercise caution even when dealing with fish that are not on the regulated list. As of the end of the 2008 season, the "List of Fish Species Affected by the VHSV Federal Order" included 28 species from a variety of fish families.

(http://www.aphis.usda.gov/animal_health/animal_dis_s pec/aquaculture/ downloads/vhs_regulated_spp.pdf).

The Disease: Viral Hemorrhagic Septicemia

The name of the disease, Viral Hemorrhagic Septicemia, describes the typical pathology one might observe. Among the various types of lesions caused are those involving hemorrhage. The virus can destroy the endothelial cells, the cells lining the interior of the blood vessels. The vessels are then unable to retain blood and hemorrhage occurs. While hemorrhage is a common lesion associated with VHS, hemorrhage can also occur as a result of fish diseases caused by a wide variety of other viral, bacterial, parasitic diseases and can be caused by water quality, toxic or even mechanical injuries. A further complication in the disease diagnostic picture is that not all fish species and not all fish in a group of the same species may show what are described as the most common or "typical signs" of VHSV. The bottom line is that visible signs of disease are used by the fish pathologist or veterinarian as supportive information in reaching a diagnosis, but a confirmed diagnosis can only be reached when supportive laboratory-based information is also available.

Diagnosis of VHS

A diagnosis of the presence of VHSV must be based on laboratory testing of appropriate samples from infected fish. In that VHSV is an OIE reportable pathogen, there are specific laboratory techniques that have been designated by the international community as acceptable for the diagnosis of VHSV. The accepted diagnostic techniques have undergone a rigorous evaluation prior to their acceptance as the methods of choice.

The current accepted method for the diagnosis of VHSV is a two-stage procedure. The first stage is a screening step where the virus is cultured in fish cell cultures grown in the laboratory. If the virus is present in

The current accepted method for the diagnosis of VHSV is a two-stage procedure. The first stage is a screening step where the virus is cultured in fish cell cultures grown in the laboratory. If the virus is present in the cultured fish cells, a visible change or destruction of the cells can be seen with a microscope. To determine the specific identity of the virus (i.e. is it VHSV or some other virus?) the second stage of the process involved using testing methods that are specific for VHSV. In this second stage, material from the infected cell culture is prepared and assayed by a Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) that is specific for VHSV. There are two documents that are generally accepted as providing the specific details of thses procedures. These documents are the OIE Aquatic Manual and the American Fisheries Society, Fish Health Section "Blue Book."

Diagnostic methods are continually being modified and improved upon as the biomedical sciences move forward. New and rapid tests that can detect the genetic material of VHSV directly from fish tissues are being developed. While such methods show great promise, they are not currently listed in either the OIE Aquatic Manual or the "Blue Book" and thus are not currently a method that can be used to provide a confirmed diagnosis of any genotype of VHSV for regulatory purposes.

VHSV – Preventing Further Spread

To date there have been no isolations of VHSV from fish reared in any aquaculture facility, government or private, in North America. All individuals who have a stake in fisheries and aquaculture in North America have a stake in maintaining the VHSV-free status of aquaculture on this continent. Preventing the movement of VHSV to locations where it is not currently found can only be accomplished by a multi-faceted effort. That effort must include an understanding of how the virus can be transmitted as well as how it might be transmitted.

While much has been stated in the popular press regarding how VHSV gained entrance into the Great Lakes Basin, there is no definitive proof that demonstrates the method by which that event occurred. The genetic similarities of the Great Lakes Basin and the marine VHSV suggest that the virus moved from the marine environment of the Atlantic coast into the Great Lakes, but this is speculation and not fact. If VHSV did move from the ocean to the Great Lakes and then move within the Great Lakes, some of the methods by which such a movement could have occurred include:

- 1. Natural movement of an infected fish up the St. Lawrence River.
- 2. Movement of an infected fish as a food item by a non-human animal (note: investigations have shown that the virus can withstand the acid nature of the gut of animals for approximately 120 minutes).
- 3. Movement of an infected fish through human intervention (e.g. movement of an infected adult fish for the purpose of "ad hoc stocking."
- 4. Movement of infected bait.
- 5. Movement of infected water by recreational boating (e.g. live well water).
- 6. Movement of the virus on equipment (e.g. fishing equipment, nets, boat hulls).
- 7. Movement of the virus in ballast water.

Concerns over the spread of VHSV in the United States resulted in Emergency Regulations being implemented in October, 2006 by USDA APHIS. These regulations required that listed live fish that were destined to be shipped interstate within the 8 states bordering the Great Lakes, or entering the United States from the provinces of Ontario or Quebec, Canada, must undergo a fish health inspection and found to be free of VHSV. In addition, certain states have implemented fish health regulations in addition to those of USDA APHIS. At the time of this writing, the Emergency Rule is still in effect, but it is logical to expect that it will some day be supplanted by a more detailed and permanent rule.

The emergence of VHSV in the Great Lakes Basin has heightened the awareness of the aquaculture community of the need for practicing effective biosecurity on their facility. Commercial aquaculturists who have not already done so, should investigate opportunities to receive formal training in biosecurity measures for aquaculture. An effective biosecurity program will include the establishment of the following procedures:

- 1. Training for all personnel on the aspects of the facility biosecurity program.
- 2. Maintenance of training records for all personnel.
- 3. Development of written Standard Operating Procedures (SOPs) for fish health and biosecurity practices.
- 4. Limit the movement of new fish onto the facility property.
- 5. Only allow the entrance of new fish, or,

preferably, only disinfected fish eggs into the facility after they have undergone a fish health inspection and were found to be free of VHSV and other important fish pathogens.

- 6. Evaluation of water used on the facility (use only well water or spring water–water that is devoid of other fish and fish pathogens).
- 7. Establishment of a professional relationship with a fish health program that can provide fish disease diagnostic expertise to the facility.
- 8. The maintenance of records for all disease events, corrective action(s) taken and success of those actions.
- 9. Evaluation of the various facility systems, the need for back-up electricity, water and back-up equipment.

Summary

The emergence of VHSV IVb in the Great Lakes Basin has had a significant impact on the wild fish populations of the Great Lakes Basin. Massive mortality events have occurred in some fish species. Because VHSV is one of the few fish pathogens to be listed as a reportable organism by the OIE, the impact of VHSV has also extended into commercial aquaculture in the form of regulations designed to limit the spread of VHSV beyond its current geographic distribution. To date VHSV has not been found in any commercial aquaculture facility in the Great Lakes States. In that VHSV is considered to be the most serious fish disease on a world wide basis, it goes without saying that it is critical that aquaculturist become familiar with and practice sound biosecurity on a day-to-day basis to prevent this devastating pathogen from entering their production facility. As a disease caused by a virus, VHS is not treatable. Currently the only practical means to avoid losses is to avoid the pathogen.

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General Web Sites of Interest

Aquatic Animal Health Program, College of Veterinary Medicine, Cornell University–VHSV Web Page. http://www.vet.cornell.edu/Public/FishDisease/Aquatic Prog/highlights/VHSV/VHSV%20NYS%20Public.htm

Focus on Fish Health–VHS web site.

http://www.focusonfishhealth.org/

USDA APHIS Newsroom. Viral Hemorrhagic Septicemia. (updated periodically).

http://www.aphis.usda.gov/newsroom/hot_issues/vhs/vhs.shtml

Acknowledgments

This work was conducted with the support of the Northeastern Regional Aquaculture Center, through grant number 2006-38500-17065 from the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not neccessarily reflect the view of the U.S. Department of Agriculture. This fact sheet was prepared with assistance from the Connecticut Sea Grant College Program.





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