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Molecular Epidemiology of Viral Hemorrhagic Septicemia Virus in the Great Lakes Region

emerald shiner

Viral hemorrhagic septicemia virus (VHSV) is considered by many nations and international organizations to be one of the most important viral pathogens of finfish (Office International des Epizooties 2007). For several decades following its initial characterization in the 1950s, VHSV was thought to be limited to Europe where it was regarded as an endemic pathogen of freshwater fish that was especially problematic for farmed rainbow trout, an introduced species (Wolf 1988; Smail 1999). Subsequently, it was shown that VHSV was present among many species of marine and anadromous fishes in both the Pacific and Atlantic Oceans where it has been associated with substantial mortality among both wild and cultured fish (Meyers and Winton 1995; Skall et al. 2005).

Beginning in 2005, reports from the Great Lakes region indicated that VHSV had been isolated from fish that had experienced very large die-offs in the wild (Elsayed et al. 2006; Lumsden et al. 2007; Groocock et al. 2007). By the end of 2007, VHSV had been isolated from more than 25 species of fish in Lake Michigan, Lake Huron, Lake St. Clair, Lake Erie, Lake Ontario, Saint Lawrence River and from inland lakes in New York, Michigan and Wisconsin (Figure 1). The Great Lakes strain of VHSV appears to have an exceptionally broad host range and significant mortality has occurred in muskellunge, freshwater drum, yellow perch, round goby, emerald shiners and gizzard shad.

Fisheries managers in the US and Canada are concerned about the spread of this highly virulent strain of VHSV from the Great Lakes region into new populations of native freshwater fish or into new geographic areas. Furthermore, the introduction of VHSV into the aquaculture industry could cause additional trade restrictions as well as direct losses from disease.

freshwater drum

In recent years, the tools of molecular biology have provided new insights into the ecology and epidemiology of many viruses of humans and animals. The purpose of this fact sheet is to review the results from molecular analyses that have added to our understanding about the distribution and spread of VHSV.

Figure 1. Locations in the Great Lakes region where VHSV has been isolated from infected fish. To date, more than 25 species have been found to be infected, but not all isolations of the virus have been associated with mortality. Photo by Rod Getchell, Cornell University

Genetics of Viral Hemorrhagic Septicemia Virus

Viral hemorrhagic septicemia virus is a member of the family of viruses known as rhabdoviruses. This family includes important pathogens such as the rabies virus. The VHSV particle is bullet-shaped with a single-stranded, negative-sense RNA genome approximately 11,000 nucleotides in length. The genome is organized into six genes that code for five structural proteins needed to construct the infectious virus particle and one non-virion protein of unknown function (Figure 2).

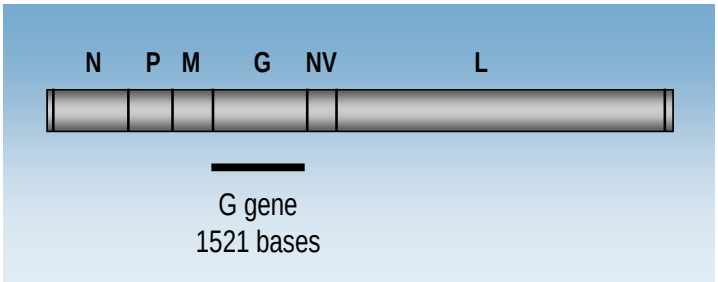

Figure 2. Diagram of VHSV RNA genome showing the six viral genes. Sequence analyses of the G and N genes has been used for genetic typing.

muskellunge

Sequence analysis of the glycoprotein (G) and nucleoprotein (N) genes of VHSV has shown that VHSV isolates can be divided into four genotypes that generally correlate with geographic location (Benmansour et al. 1997; Einer-Jensen et al. 2004; Snow et al. 2004). Isolates belonging to VHSV Genotypes I, II and III are present in continental Europe, the North Atlantic Ocean, the North Sea, the Baltic Sea and connecting waters (Table 1).

Table 1. Genotypes of VHSV and the types of fish and geographic region from which they are most commonly isolated.

Genotype	Where most commonly found
I	Trout farms, Continental Europe
II	Wild marine fish, Baltic Sea
III	Wild marine fish, North Sea near UK
IVa	Wild marine fish, west coast North America
IVb	Wild freshwater fish, Great Lakes region

Genotype I has several sub-groups and includes isolates from European rainbow trout farms. This genotype also includes isolates from marine fish in the Baltic Sea, supporting the hypothesis that the highly virulent strains affecting rainbow trout aquaculture in Europe had their origin in the marine environment and farmed trout may have acquired the virus through the former practice of using raw marine fish as feed (Dixon 1999; Skall et al. 2005).

Genotype II is a separate lineage of VHSV found among marine fish in the Baltic Sea. Genotype III contains marine VHSV isolates from the North Sea near the British Isles, and also includes isolates from British turbot mariculture, again revealing a likely epidemiological link between endemic marine isolates of VHSV and outbreaks affecting cultured fish.

Following the discovery of VHSV in anadromous and marine fish from the West Coast of North America beginning in 1988, sequence analysis showed the North American isolates were distinct from the European genotypes, causing them to be assigned to Genotype IV (Hedrick et al. 2003; Einer-Jensen et al. 2004; Snow et al. 2004; Figure 3).

Molecular Epidemiology of VHSV

While epidemiology refers to the traditional study of sources of outbreaks and patterns of spread for an infectious disease, molecular epidemiology refers to the application of new-generation tools such as gene sequencing or DNA/RNA fingerprinting to provide this information. Phylogenetic analysis uses sequence information and computer algorithms to construct a family tree that shows relationships and the evolutionary history of a pathogen.

round goby

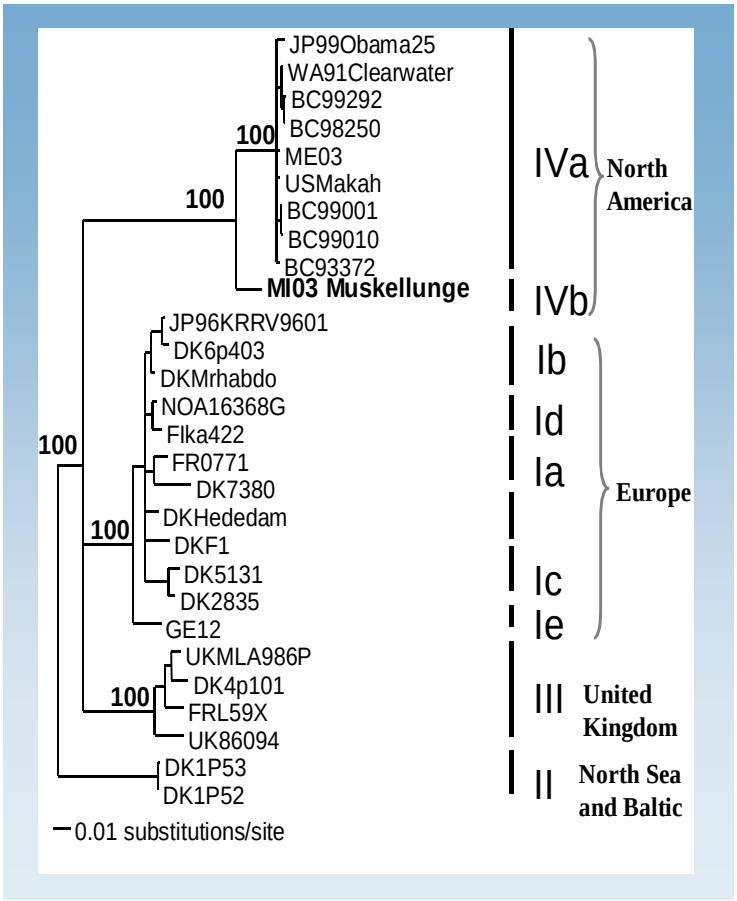

Figure 3. Phylogenetic tree (from Elsayed et al., 2006) showing the relationship of the first Great Lakes VHSV isolate, MI03 from muskellunge in Michigan, to VHSV genotypes, I-IV. MI03 was distinct, but closest to genotype IVa from the west coast of North America, so it was designated genotype IVb.

Genetic Analysis of VHSV Isolates from the Great Lakes Region

The new isolates of VHSV from fish in the Great Lakes were initially identified as being similar to members of VHSV Genotype IV (Canadian Cooperative Wildlife Health Centre 2005). Upon further analysis, the new isolates were sufficiently different from those on the West Coast to be assigned to a separate sub-lineage, now termed Genotype IVb, with the isolates of VHSV from the West Coast of North America forming Genotype IVa (Elsayed et al 2006).

Thus, genetic typing revealed that there were two distinct lineages of VHSV in North America and the introduction of VHSV into the Great Lakes was not from Europe where Genotypes I, II and III are present, but more likely from a source closer to North America. The Genotype IVb strain found in the Great Lakes region is the only strain outside of Europe that has been associated with significant mortality in freshwater species.

What is the Origin of the VHSV Isolates from the Great Lakes Region

At present, more than 30 isolates of VHSV from different host species or locations in the Great Lakes Basin have been analyzed. Genetic typing of many of the VHSV isolates from

US waters has been done at the USGS Western Fisheries Research Center (WFRC), while isolates from Canadian waters have been typed at the Pacific Biological Station (Department of Fisheries and Oceans Canada) in Nanaimo, British Columbia.

WFRC researchers work closely with Canadian and European colleagues to assure that information is shared to obtain a complete picture of the molecular epidemiology of VHSV in the Great Lakes. To date, all VHSV isolates from the Great Lakes region that were analyzed at the sequence level are members of Genotype IVb. In fact, the portion of the virus genome analyzed was identical, or nearly identical (less than 0.5% sequence difference), for many of the isolates from fish kills occurring among different species or in different lakes during 2006 and again in 2007.

yellow perch

Conclusions to date

These findings suggest that VHSV was relatively recently introduced into the Great Lakes, probably as a single event within the past 5-10 years, and there has not yet been sufficient time to observe the evolution of different strains in different hosts or geographic locations.

The lack of diversity also indicates that the fish kills occurring among different species and in different lakes can be considered as one large ongoing epidemic. These insights from the molecular analysis are also supported by the historic absence of VHSV in the region based upon the lack of large fish kills associated with the virus or isolation of the virus during routine fish health examinations prior to 2003.

While the molecular analysis has not revealed the exact origin of the virus or the mechanism of introduction, the Genotype IVb isolates obtained from fish in the Great Lakes are genetically most like isolates of VHSV recovered during 2000-2004 from mummichog and other diseased fish in rivers and near-shore areas of New Brunswick and Nova Scotia, Canada (Figure 4; Olivier 2002; Gagne et al. 2007). Thus, it appears likely that the VHSV strain in the Great Lakes may have had its origin among marine or estuarine fishes of the Atlantic seaboard of North America.

Predictions for the future

Due to increased fish health surveillance activities in the Great Lakes planned by both US and Canadian agencies, we anticipate that many more isolates of VHSV will be obtained from the region during the next several years. Molecular analysis of these isolates will increase our understanding of the epidemiology of VHSV infections among native populations of wild fish. In addition, there is recent evidence of some increase in genetic diversity among VHSV isolates from the Great Lakes, as would be expected for an introduced pathogen adapting to new host species or differing environments.

Because significant additional research is needed to assist managers in understanding the disease ecology of VHSV and its effects on the health of native fish populations in the region

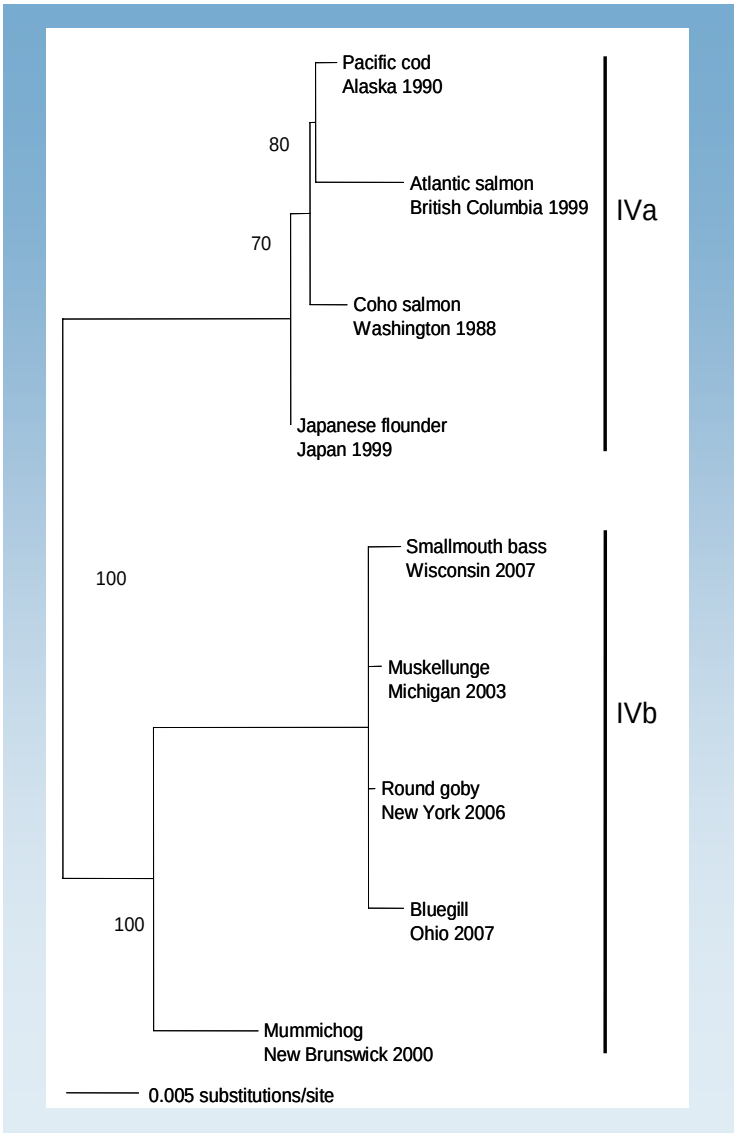

Figure 4. Phylogenetic analysis of Great Lakes isolates of VHSV compared with isolates representing Genotype IVa. The tree suggests the mummichog isolate from New Brunswick, Canada is related, and perhaps ancestral, to the more recent Genotype IVb isolates from the Great Lakes.

a second major area of VHSV research at the WFRC involves development of laboratory models in susceptible species from the Great Lakes (Figure 5).

Figure 5. Yellow perch experimentally infected at the WFRC with a Great Lakes strain of VHSV. These fish show high mortality with typical signs of disease. Such models will be useful to study features of the disease caused by VHSV and will aid in development of vaccines or other control methods.

Such models can be used to study the progress of infection in an appropriate host species and serve as a reference standard for virulence comparisons in other fish species. The model will also help investigate epidemiological features of the disease process including: virus shedding rate, formation of carriers, development of immunity and effects of environmental factors such as temperature on the disease process.

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Infectious disease is increasingly recognized as an important feature of aquatic ecosystems; however, the impact of disease on populations of wild fish has been difficult to study. While many of the viral, bacterial, protozoan and fungal pathogens of fish that were initially discovered in captive animals are actually endemic among wild populations, the introduction of exotic pathogens into aquatic systems can lead to explosive mortality and may be especially threatening to native stocks. At the WFRC, field and laboratory investigations, aided by the tools of molecular biology, have begun to provide information on the ecology of infectious diseases affecting natural populations of fish in freshwater and marine ecosystems.

Further Reading:

gizzard shad

A complete list of WFRC publications may be found at: http://wfrc.usgs.gov/pubs/pubs.htm

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