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Research paper

Spatial and temporal heterogeneity of infectious hematopoietic necrosis virus in Pacific Northwest salmonids

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ABSTRACT

The aquatic rhaboviral pathogen infectious hematopoietic necrosis virus (IHNV) causes acute disease in juvenile fish of a number of populations of Pacific salmonid species. Heavily managed in both marine and freshwater environments, these fish species are cultured during the juvenile stage in freshwater conservation hatcheries, where IHNV is one of the top three infectious diseases that cause serious morbidity and mortality. Therefore, a comprehensive study of viral genetic surveillance data representing 2590 field isolates collected between 1958 and 2014 was conducted to determine the spatial and temporal patterns of IHNV in the Pacific Northwest of the contiguous United States. Prevalence of infection varied over time, fluctuating over a rough 5–7 year cycle. The genetic analysis revealed numerous subgroups of IHNV, each of which exhibited spatial heterogeneity. Within all subgroups, dominant genetic types were apparent, though the temporal patterns of emergence of these types varied among subgroups. Finally, the affinity or fidelity of subgroups to specific host species also varied, where UC subgroup viruses exhibited a more generalist profile and all other subgroups exhibited a specialist profile. These complex patterns are likely synergistically driven by numerous ecological, pathobiological, and anthropogenic factors. Since only a few anthropogenic factors are candidates for managed intervention aimed at improving the health of threatened or endangered salmonid fish populations, determining the relative impact of these factors is a high priority for future studies.

1. Introduction

Pacific salmonid fish are an important cultural and ecological resource in the Pacific Northwest of North America. Due to overfishing and habitat loss, all but one of the six species of Pacific salmonids are cultured to some extent in conservation hatcheries. Among hatchery populations, the rhabdoviral pathogen infectious hematopoietic necrosis virus (IHNV) is a particular burden (Wolf 1988; Bootland & Leong 1999). IHNV primarily infects sockeye and kokanee salmon (migratory and freshwater life history types of *Oncorhynchus nerka*, respectively), Chinook salmon (*O. tshawytscha*), and steelhead and rainbow trout (migratory and freshwater life history types of *O. mykiss*, respectively). In these hosts IHNV typically causes acute disease and mortality only in juvenile fish, while infection in adults is usually asymptomatic. Both juvenile and adult fish can transmit IHNV horizontally through water. Within hatcheries, transmission from infected adults to progeny via egg-associated virus is greatly reduced or eliminated by egg disinfection (Amend 1975; Meyers 1998). As observed in captive fish, acute IHN disease in juveniles is often associated with epidemic mortality, which can be up to 90% (Groberg 1983a; Groberg 1983b; LaPatra et al. 1993a; LaPatra et al. 1993b; Bootland & Leong 1999).

Established methods for IHNV molecular epidemiology are based on the sequence of a variable portion of the viral glycoprotein gene, the 303 nt midG region. Analysing virus isolate midG sequence genotype data together with epidemiological case data allows inference of probable transmission sources, and some estimates of secondary transmission risk. This method has been used to define recent emergence events and support effective control measures to mitigate losses due to IHN disease (Breyta et al. 2013; Breyta et al. 2016b). A previous molecular epidemiology study of 393 IHNV isolates collected throughout the entire range of IHNV in North America revealed three phylogenetic genogroups designated U, M, and L (Kurath et al., 2003). U genogroup viruses are found in Alaska, Western Canada, Puget Sound, Coastal Washington, and the large Columbia River basin that extends throughout much of Washington, Oregon, Idaho, and Montana. M genogroup viruses are also found in the Columbia River basin, as well as sporadic emergence events in coastal Washington (Breyta et al. 2013) and Oregon (this paper). L genogroup viruses have only ever been detected in California and the southern Oregon coastal region. Subsequent phylogenetic analyses have identified six subgroups within the M genogroup, designated MA-MF (Troyer and Kurath, 2003), two subgroups within the U

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genogroup, designated UC and UP (Black, 2015), and two subgroups within the L genogroup, designated L1 and L2 (Kelley et al. 2007).

U and M group IHNV have coexisted within the large complex Columbia River basin for decades (Garver et al. 2003). During that time, M group viruses caused serious disease impacts to conservation hatchery populations of steelhead trout and farm populations of rainbow trout (Garver et al. 2003; Troyer & Kurath 2003; Breyta et al. 2016b). These M group viruses are not uniformly distributed throughout the Columbia basin, despite the fact that the *O. mykiss* host species can be found in all parts of the watershed. Within one portion of the basin, dominant M virus types have been observed to exhibit serial emergence and displacement patterns, prompting investigations into possible mechanisms (Kell et al. 2014; Breyta et al. 2016a). Viruses in the U genogroup are also not uniformly distributed, and a recent study revealed population genetic structure (subgroups UP and UC) within the U group IHNV viruses in the Columbia River basin (Black, 2015). In this region the UC subgroup is mostly associated with Chinook salmon and steelhead trout, and the UP subgroup is associated with sockeye salmon (Black, 2015). While mortality in Chinook salmon due to UC viruses has not been reported at levels similar to that of steelhead trout infected with M group IHNV, disease outbreaks have occurred in juvenile Chinook salmon, and the impact of UC viruses in Chinook salmon conservation programs is not well characterized.

In order to determine the spatial and temporal distribution of IHNV subgroups and dominant genetic types in this region, an updated comprehensive molecular epidemiology analysis focusing on IHNV in the Columbia basin, coastal Oregon, coastal Washington, and Puget Sound was conducted. Compared to earlier studies, some IHNV subgroups were still detected, while others were no longer evident (MA, ME, MF) (Troyer & Kurath 2003). The analysis revealed that all subgroups exhibited heterogeneity in temporal and spatial occurrence, despite the availability of host species throughout the study area. Variation in host-specific patterns was observed for different virus subgroups, and both specialist and generalist viral strategies were observed. Several dominant virus genotypes were identified within the U and M genogroups, and specific focus sites with high levels of disease impacts were observed. Within this complexity there are novel insights with potential for contributions to targeted control strategies in the future.

2. Methods

2.1. Virus isolates

The molecular biology program at the US Geological Survey Western Fisheries Research Center (USGS WFRC) provides midG sequence analysis of IHNV field isolates as a technical assistance service to fisheries managers throughout the Pacific Northwest. The data generated is maintained in a database at WFRC. This database has a freely accessible internet version at http://gis.nacse.org/ihnv/.

IHNV isolates and diagnostic records were obtained from fish health laboratory staff from the United States Fish and Wildlife Service, Washington Department of Fish and Wildlife, Northwest Indian Fisheries Commission, Oregon Department of Fish and Wildlife, Idaho Department of Fish and Game, and from the archival collection at the WFRC. Samples were taken from dead, dying, or asymptomatic juvenile fish or from asymptomatic adult fish and processed for virus isolation in cell culture using standardized protocols (American Fisheries Society Fish Health Section Blue book). Virus culture supernatants of low passage number (≤2 passages for the majority of isolates) were sent to WFRC for analysis and archival storage at −80 °C. The 2590 IHNV isolates in this study were collected from a total of 252 different sites within the study region and represented 1469 distinct IHNV positive fish cohorts. These samples came from steelhead or rainbow trout (*O. mykiss*), Chinook salmon (*O. tshawytscha*), sockeye or kokanee salmon (*O. nerka*), coho salmon (*O. kisutch*), chum salmon (*O. keta*), Atlantic salmon (*Salmo salar*), or unknown hosts.

2.2. Bias and coding by detection events

Fish cohorts at hatcheries are tested by agency staff for IHNV infection primarily under three different testing schemes: routine screening of asymptomatic adult fish when they are spawned, occasional screening of asymptomatic juvenile fish, and diagnostic testing of juvenile fish suffering mortality or exhibiting signs of IHNV infection. When positive samples from these testing strategies are submitted for viral genetic typing, two kinds of sampling bias may occur. One is when few samples are submitted for sequence analysis from specific geographic areas because the presence of IHNV in those fish cohorts is not unusual, and a greater number are submitted from fish cohorts that are unexpectedly positive. This pattern creates a geographic surveillance-pressure bias: fish cohorts from areas with newly emergent IHNV are typed more heavily while fish in IHNV endemic areas are sampled and typed less intensely. The second form of bias is in the number of IHNV isolates submitted for typing. For example, one incident of juvenile epidemic IHN disease may be represented by only one or two isolates, while another may be represented by as many as thirty. If IHNV detections were reported by the raw number of typed isolates, these two sampling biases would mask important patterns. To correct this second form of bias, isolates have been coded into positive fish cohorts (by collection site, age, species, and seasonal timing, (Breyta et al. 2013)). After genetic typing, fish cohorts for which all virus isolates had identical sequence types were each assigned to one 'detection event'. If more than one sequence type was detected among multiple isolates from one cohort, a separate event was assigned for each sequence type detected (Breyta et al., 2013; Breyta et al., 2016a, 2016b). In the current report, cohorts with more than one virus type are described as having a 'dominant variant' and 'non-dominant variant(s)'.

2.3. Viral RNA extraction and sequence analysis

Viral genomic RNA was extracted from 200 to 500 μL of virus culture supernatant with TriReagent (Sigma) according to manufacturer's directions with tRNA (Promega) added to aid in RNA precipitation. Reverse transcription and amplification of a ~550 bp fragment containing the midG region was performed as previously described (Emmenegger et al. 2000) in one 50 μL reaction using avian myeloblastosis virus (AMV) reverse transcriptase, Taq polymerase, IHNV-specific primers and 30 cycles of amplification. The PCR product was purified away from amplification components using Strataprep PCR purification columns (Agilent Technologies), and 0.5 μL was used in each of two 10 μL Big Dye Terminator (Applied Biosystems) sequencing PCR reactions, using the same forward and reverse primers as above and 30 cycles as previously described (Emmenegger et al. 2003).

2.4. Phylogenetic analysis

Sequence data was assembled, edited, and trimmed to the established midG 303 nt fragment (Emmenegger et al. 2003; Garver et al. 2003; Kurath et al. 2003; Troyer & Kurath 2003) using Sequencher software v4.9. Consensus midG sequences for each virus isolate were aligned in Clustalx (Qt/QMake), and manually inspected and corrected for artefactual gap insertion. The term 'genotyping' is used to describe this process, and the term 'sequence type' or 'genetic variant' is used to describe individual sequences (haplotypes) as described previously (Breyta et al. 2013). Sequence types based on midG sequences are designated in the format mG###, where ### indicates a randomly assigned 3 digit number that is specific to a sequence, and U, M, or L is added at the end to indicate the major IHNV genogroup (e.g. type mG001U falls within the U genogroup in phylogenetic analyses). Newly generated sequences and all previously known USD sequences detected in North American IHNV were used for phylogenetic analysis. Taxa were individual sequences representing each midG haplotype, associated with the first year of its detection. Phylogenetic analysis was