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Viral fitness does not correlate with three genotype displacement events involving infectious hematopoietic necrosis virus

Alison M. Kell [a,b,*], Andrew R. Wargo [a,b,1], Gael Kurath [a,b]

[a] University of Washington, Seattle, WA, USA
[b] U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA, USA

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ABSTRACT

Viral genotype displacement events are characterized by the replacement of a previously dominant virus genotype by a novel genotype of the same virus species in a given geographic region. We examine here the fitness of three pairs of infectious hematopoietic necrosis virus (IHNV) genotypes involved in three major genotype displacement events in Washington state over the last 30 years to determine whether increased virus fitness correlates with displacement. Fitness was assessed using in vivo assays to measure viral replication in single infection, simultaneous co-infection, and sequential superinfection in the natural host, steelhead trout. In addition, virion stability of each genotype was measured in freshwater and seawater environments at various temperatures. By these methods, we found no correlation between increased viral fitness and displacement in the field. These results suggest that other pressures likely exist in the field with important consequences for IHNV evolution.

Introduction

Infectious hematopoietic necrosis virus (IHNV) is a single-stranded, negative-sense RNA virus and a member of the Rhabdovirus family. IHNV, endemic within both wild and cultured host populations, establishes acute, lethal infection in juvenile Pacific salmonids and results in significant losses to hatchery programs and aquaculture industries every year. Over the last 15 years genetic characterization of over 2000 virus isolates from western North America has led to a greater understanding of the movement and evolution of this virus throughout the region (Troyer and Kurath, 2003; Troyer et al., 2000; Kurath et al., 2003; Garver et al., 2003; Emmenegger et al., 2000; Breyta et al., 2013). Three major genogroups of IHNV in North America have been defined: U, M, and L, with a maximum of 8.6% nucleotide diversity between groups (Kurath et al., 2003). Isolates are assigned a major genogroup and a specific genotype within that genogroup, based on the sequence of a 303 nucleotide region within the viral glycoprotein gene. The three North American genogroups have well-defined spatial and host ranges with some overlap (Kurath et al., 2003).

Genogroup M causes disease predominantly in rainbow and steelhead trout (*Oncorhynchus mykiss)*, in the Columbia River basin, which is a large, complex watershed that extends through much of Washington state, central Idaho, and northern Oregon (Fig. 1). Within the M genogroup of IHNV, a series of virus genotype displacement events has been observed within populations of steelhead trout in the Columbia River basin (Garver et al., 2003), and more recently on the Washington state coast (Breyta et al., 2013) (Fig. 1). Displacement events are characterized by replacement of a previously dominant virus genotype with a novel genotype in a given region. Dominant IHNV genotypes were defined as those genotypes associated with the highest number of viral "events" for a given period of time (season or year), where events are defined by features such as collection site, host species, host age, and seasonal timing (Breyta et al., 2013). Therefore, a genotype that was isolated from more sites, individual hosts, and over more years was determined to be the dominant genotype for that time period. Here we address three historical displacement events of previously dominant IHNV genotypes.

From 1980 to 1994, genotype mG007M (hereafter referred to as 007) was dominant in the Columbia River basin at multiple locations, with the last known detection in 1994 (Kurath et al., 2003). Genotype 007 was displaced by genotype mG111M (hereafter referred to as 111), first detected in the lower Columbia River basin in 1994 and then remained the dominant genotype until 1999. Genotype 111 was then displaced by genotype mG110M (hereafter referred to as 110), which was first detected in the Columbia River basin in 2002, and remains the dominant M

* Corresponding author at: University of Washington, Seattle, WA, USA. Tel.: +1 206 685 8289.
E-mail address: kella@uw.edu (A.M. Kell).
[1] Present address: Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA, USA.

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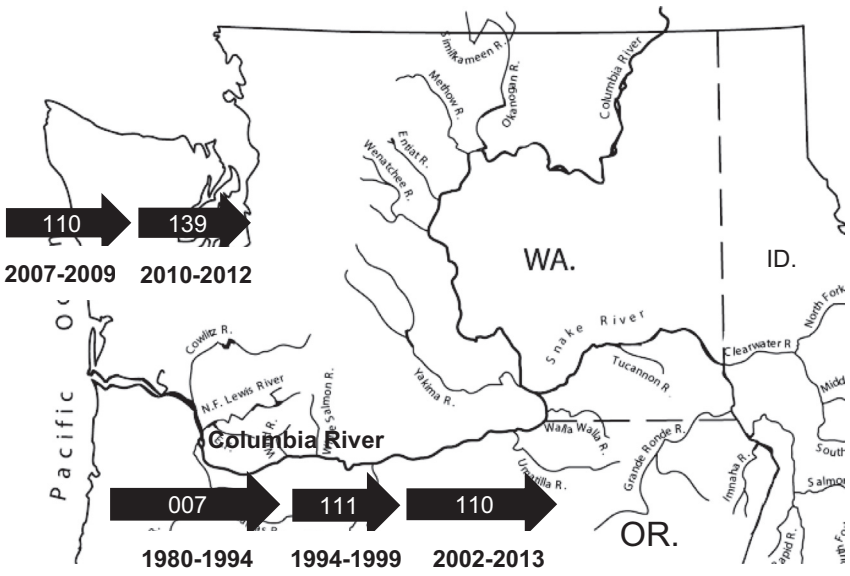

Fig. 1. Genotype displacement events in the lower Columbia River basin and Washington state coast. The Columbia River basin located in Washington (WA), Idaho (ID), and Oregon (OR). Arrows represent dominant genotypes present in each region. Dates of dominance are shown below arrows.

genogroup virus, despite the detection of other IHNV M genogroup variants (Breyta et al., 2013). Genotype 110 was also responsible for a major emergence event of IHNV M in previously naïve steelhead trout on the Washington coast from 2007 to 2009 (Breyta et al., 2013). In 2009, genotype 110 was displaced on the Washington coast by genotype mG139M (hereafter referred to as 139), which became the dominant coastal genotype from 2009 to 2011 (Breyta et al., 2013).

These displacement events observed for M genogroup IHN viruses are similar to displacement events reported for other vertebrate RNA viruses, including dengue, rabies, measles, West Nile, and chikungunya viruses (Carrillo-Valenzo et al., 2010; Davis et al., 2005; Tsetsarkin et al., 2011; Davis et al., 2013; Nojiri et al., 2008; Li et al., 2010; Vu et al., 2010). Numerous theories have been postulated to explain such events including, but not limited to, genetic drift resulting from stochastic processes, bottlenecks or founder events, antigenic variation to escape host immunity, changes in host specificity, and changes in viral replication fitness or transmission fitness. While some groups have found positive correlations between displacement events and viral fitness (Armstrong and Rico-Hesse, 2003; Hanley et al., 2008; Lambrechts et al., 2012; Moudy et al., 2007; Tsetsarkin and Weaver, 2011), others have determined that differences in fitness could not explain these events (Myat Thu et al., 2005; Lourenco and Recker, 2010; OhAinle et al., 2011). As such, there is a need to investigate additional host–pathogen systems to determine the range of factors that may be driving viral displacement events.

Here we examined what role viral fitness may have played in three IHNV displacement events observed in the field. We measured a suite of fitness traits for four virus isolates representative of the four genotypes involved. These fitness traits included in vivo quantification of the replication kinetics, host interferon-induced Mx-1 expression during single infections, and the ability to replicate in co-infection and superinfection contexts. All in vivo fitness assays were conducted in the natural host, steelhead trout. We also measured the environmental stability of each genotype outside the host in freshwater and seawater at three temperatures. Differences between the four genotypes were assessed as three pairs representing the three known displacement events, in order to determine whether differences in overall viral fitness correlate with the sequential displacements observed in the field.

Results

In-host virus replication kinetics and host Mx-1 gene expression

To determine the in-host replication kinetics of each genotype, we measured total viral load in individual fish infected with single genotypes over seven days post-infection (Fig. 2a). The four genotypes were quantified independently but the data is presented in Fig. 2(a) as genotype pairs. Genotypes in displacement pairs 1 (007 and 111) and 3 (110 and 139) showed comparable 7 day replication profiles. However, fish exposed to the genotypes in displacement pair 2 (111 and 110) had significantly different viral loads during the first four days following infection ($p=0.0306$), with the displaced genotype 111 reaching peak levels more quickly than genotype 110. Peak viral load in fish exposed to genotype 111 was 7.5 log virus copies/g of fish on day 3, whereas genotype 110 reached this same peak value on day 6.

Because the kinetics of viral growth could be affected by the level of innate immune stimulation induced by each genotype, we examined the induction of the type I interferon stimulated gene, Mx-1, in the same fish for which viral load was quantified (Fig. 2b). For IHNV infection in rainbow trout, it has been thoroughly documented that, within the first few days of infection, the host response is dominated by a strong induction of numerous interferon-stimulated genes, including Mx-1 (Purcell et al., 2012). In addition, pre-treatment of rainbow trout with injection of Poly(I:C), a TLR-3 agonist, offers significant protection from infection and mortality following challenge with IHNV (Kim et al., 2009). For displacement pair 1, Mx-1 expression in fish exposed to genotype 007 was significantly higher than that in fish exposed to genotype 111 at day four post-infection, but not on any other day. For displacement pair 2, fish exposed to genotype 111 demonstrated a stronger Mx-1 induction at days 1–3 than fish exposed to genotype 110. For displacement pair 3, Mx-1 induction following infection with genotype 110 or 139 did not differ over the 7-day period. Thus Mx-1 expression generally tracked with viral load profiles as previously observed in this host:virus system (Penaranda et al., 2011; Purcell et al., 2009, 2011).

Co-infection fitness

To determine the competitive in-host replication fitness of displacement pair isolates, we challenged groups of fish by