Virus Research 214 (2016) 80–89

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Characterization of infectious dose and lethal dose of two strains of infectious hematopoietic necrosis virus (IHNV)

Douglas G McKenney[a,*], Gael Kurath[a], Andrew R Wargo[b]

[a] US Geological Survey, Western Fisheries Research Center, 6505 NE 65th St., Seattle, WA 98115, United States
[b] Virginia Institute of Marine Science, 1375 Greate Rd., Gloucester Point, VA 23062, United States

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
22 December 2015
Accepted 22 December 2015
Available online 2 January 2016

Keywords:
Infectivity
Virulence
Infectious hematopoietic necrosis virus
Infectious dose
Lethal dose
Independent action hypothesis

ABSTRACT

The ability to infect a host is a key trait of a virus, and differences in infectivity could put one virus at an evolutionary advantage over another. In this study we have quantified the infectivity of two strains of infectious hematopoietic necrosis virus (IHNV) that are known to differ in fitness and virulence. By exposing juvenile rainbow trout (*Oncorhynchus mykiss*) hosts to a wide range of virus doses, we were able to calculate the infectious dose in terms of ID_{50} values for the two genotypes. Lethal dose experiments were also conducted to confirm the virulence difference between the two virus genotypes, using a range of virus doses and holding fish either in isolation or in batch so as to calculate LD_{50} values. We found that infectivity is positively correlated with virulence, with the more virulent genotype having higher infectivity. Additionally, infectivity increases more steeply over a short range of doses compared to virulence, which has a shallower increase. We also examined the data using models of virion interaction and found no evidence to suggest that virions have either an antagonistic or a synergistic effect on each other, supporting the independent action hypothesis in the process of IHNV infection of rainbow trout.

Published by Elsevier B.V.

1. Introduction

The ability to infect a host is necessary for a virus to propagate, and if one virus strain can do so better, it will likely have a competitive advantage over other strains. Certainly, a variety of other parameters also contribute to the absolute fitness of a virus, such as replication, shedding, and duration of infection, all of which are shaped by virus and host factors (Wargo and Kurath, 2012). However, without the important first step of host entry and initiation of infection these other parameters cannot be realized and viral fitness is diminished to zero. In this paper, we are concerned with infectivity, defined here as the ability of a pathogen to enter a host and begin replication, and virulence, defined here as the ability of a pathogen to kill its host.

A long-standing question has been the relationship between virus infectivity and virulence. However, few studies have examined how the relationship between virulence and infectivity might depend on virus exposure dosage. The paucity of such studies is surprising considering that it is well known that for viruses, infection and mortality are heavily shaped by exposure dose. In fact, because of the strong effect of exposure dose on disease outcome, viral virulence has often been characterized across a range of dosages. Such studies often calculate the 50% lethal dose (LD_{50}), i.e., the virus dose at which fifty percent of exposed hosts die (Reed and Muench, 1938; Knittel, 1981; Engelking and Leong, 1989; LaPatra et al., 1993; Kim and Faisal, 2010). The LD_{50} is typically determined in a controlled experiment in which a range of exposure doses are administered to equivalent groups of hosts, and the resulting mortality at each dose is used to generate a dose-response curve and calculate the LD_{50} value. Such studies also make it possible to quantify the minimum lethal dose, the lowest dose at which mortality is observed (Kothary and Babu, 2001; Ward et al., 1986).

These LD_{50} studies are often used to make inferences about infectivity, assuming high virulence strains cause greater mortality because higher numbers of hosts become infected. However, this assumed relationship between virulence and infectivity has several limitations. For example, many viruses cause disease that does not result in host death. Viruses can also cause sub-clinical infections, where the host becomes infected but suffers no clinical disease. For example, a study of infectious pancreatic necrosis virus in Atlantic salmon found that at low challenge dosages a larger percentage of fish become infected than succumb to mortality (Urquhart et al., 2008). Quantification of actual infection is thus critical for an accurate assessment of infectivity, which is an essential component of overall viral fitness. Infectivity can be quantified in much the same way as virulence. For example a range of viral exposure dosages can be administered, after which hosts can be tested for infection

* Corresponding author.
E-mail addresses: dmckenney@usgs.gov (D.G. McKenney), gkurath@usgs.gov (G. Kurath), arwargo@vims.edu (A.R. Wargo).

http://dx.doi.org/10.1016/j.virusres.2015.12.020
0168-1702/Published by Elsevier B.V.

status at a specific time post-infection. The prevalence of infection at each exposure dose is then used to calculate the 50% infectious dose (ID_{50}), i.e., the dose at which fifty percent of exposed hosts are infected. Though the methods used to detect infection are different, the ID_{50} is determined in the same manner as the LD_{50} (Reed and Muench, 1938). As with lethal dose, minimum infectious dose, the lowest dose needed to cause an infection, can also be quantified. Interpretation of virus infection studies is heavily dependent on the methods used, which differ in their sensitivity and specificity for live virus, viral genetic material, or host responses to infection. In this study we define infection as the presence of viral RNA in the host as detected by real-time reverse transcriptase qPCR.

In general fewer studies have been conducted examining ID_{50} values compared to the number exploring LD_{50} values. Among studies that determine both ID_{50} and LD_{50} values for various host:pathogen systems, the relationship between infectivity and virulence is not always consistent. For example, a study of avian influenza virus in wild duck and poultry found large host species effects on infectious dose, but within a host species, LD_{50} values were tightly coupled with ID_{50} values, suggesting virulence was correlated with infectivity (Swayne and Slemons, 2008). However, in a study of Monkey B virus in mice, the relationship between LD_{50} and ID_{50} was less consistent, with some of the strains with the lowest ID_{50} values having the highest LD_{50} values, suggesting virulence may be decoupled from infectivity (Ritchey et al., 2005). Thus, investigation of infectivity and virulence for additional pathogens is of interest, and aquatic systems are ideal for experiments involving large numbers of hosts being exposed to a wide range of pathogen doses.

Examination of infectivity and virulence across a range of virus exposure dosages is a powerful method for comparing the traits of different virus strains. Such studies make it possible to reveal differences in virulence and infectivity that might not be apparent at single exposure dosages. This is because mortality and infectivity may saturate at the same levels for different virus genotypes, but the rate of increase in infection and mortality across exposure dosages may be different. In addition, the relationship between infectivity and exposure dose allows for an assessment of whether or not individual virions interact during the process of infection. For example, if there is a linear increase in the rate of infection as dose increases, this suggests that virions do not impact the infectivity of other virions. Here this is referred to as the independent action model, also sometimes referred to as the mass-action principle (Regoes et al., 2003; Schmid-Hempel, 2011). In contrast, if the rate of infection changes in a non-linear manner as the number of virions in the exposure dose increases, this would suggest that the virions interact with each other either in a synergistic or an antagonistic manner, here referred to as an interaction model. If there is a synergistic interaction, that could result in an invasion threshold, with a threshold dose (Regoes et al., 2003; Schmid-Hempel, 2011; Zwart et al., 2011). In this case, if the host receives less than the threshold dose it will not become infected, and infection can only occur when the dose meets or exceeds the threshold dose. Ultimately, whether infectivity follows the independent action or interaction model can influence epidemiological predictions about disease risk and spread (Schmid-Hempel, 2011; van der Werf et al., 2011).

Here we examined and compared prevalence of infection and mortality over a range of virus exposure doses to characterize the relationship between infectivity and virulence for an aquatic virus in fish hosts. We utilized a virus-host system that has been well-studied in vivo, infectious hematopoietic necrosis virus (IHNV; order *Mononegavirales*, family *Rhabdoviridae*, genus *Novirhabdovirus*) in rainbow trout (Wargo et al., 2010; Kell et al., 2013; Peñaranda et al., 2009; Bootland and Leong, 2011; Zhang and Gui, 2015). In the western United States, IHNV is endemic in salmonid fish throughout a range from Alaska to California, as well as inland via rivers to Idaho (Bootland and Leong, 2011). Within this range three main genogroups occur: U, M, and L, each of which exhibit some host specificity (Kurath et al., 2003). Relevant to this study, the M genogroup is hypothesized to have arisen in rainbow trout (Kurath et al., 2003). Under certain conditions IHNV causes disease epidemics in salmonid fish, with mortality due to necrosis of the hematopoietic kidney and spleen tissues (Bootland and Leong, 2011). Variation in virulence of IHNV strains has been reported in several studies, most often tested using a single high virus exposure dose (LaPatra et al., 1993; LaPatra, 1998; Garver et al., 2006; Wargo et al., 2010). However, infectious dose has not been previously quantified for IHNV.

We compared the infectious dose and lethal dose for two virus strains within the M genogroup of IHNV, previously characterized as having high virulence and low virulence in rainbow trout based on mortality caused to the host due to infection at a single, high challenge dose (Wargo et al., 2010). The rainbow trout used here were from an aquaculture stock that is not inbred, and thus provided a host background for testing viral traits that is relevant to field conditions. The two virus strains have been previously studied, and their virulence correlates positively with in-host viral replicative fitness, as well as host entry and shedding (Wargo et al., 2010; Wargo and Kurath, 2011). Here we exposed groups of juvenile rainbow trout to a range of doses of each genotype and then measured the infection prevalence, infection intensity, and daily mortality in order to quantify infectivity and analyze the relationship between exposure dose and both infection and mortality.

Five in vivo infection experiments were conducted using standardized one-hour batch immersion challenges to assure uniform, consistent virus exposure of fish within each group. Three of the experiments were independent infectious dose assays that determined ID_{50} estimates and provided a measure of the variability in those estimates. In these experiments fish were separated into isolated holding tanks after challenge to avoid cross-infection, and infection status was determined at 3 days post-exposure. The fourth experiment was a virulence assay that determined the lethal dose of each strain under the same isolation conditions used in the infectious dose assays, allowing direct comparison of ID_{50} and LD_{50} values for the two IHNV strains. Finally, as a secondary goal of this study we conducted a virulence assay using standard batch holding conditions, for comparison with the results of the virulence assay with fish held in isolation. This provided insight into how much of the mortality observed in standard batch challenge studies is due to holding conditions or secondary fish-to-fish infection. The combined data provide a comparison of the relationship between infectivity and lethality of two strains of a virus of differing virulence and expand upon the previous work done on the ecological parameters of various genotypes in the M genogroup of IHNV (Troyer et al., 2008; Wargo et al., 2010; Wargo and Kurath, 2011; Kell et al., 2013).

2. Materials and methods

2.1. Virus and host

For this study, we used two isolates of IHNV that differ in virulence. The more virulent strain is 220-90, referred to as HV for "high virulence"; the less virulent strain is WRAC (alternate name, 039-82), referred to as LV for "low virulence" (Wargo et al., 2010). Both strains were obtained from farmed rainbow trout in Idaho and have been previously characterized for virulence (LaPatra et al., 1994; Garver et al., 2006; Wargo et al., 2010). Over the glycoprotein gene of the virus there is 3.6% (58/1621 nucleotides) divergence between HV and LV; over the entire genome, the divergence is 2.8%