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Characteristics of cyprinid herpesvirus 3 in different phases of infection: Implications for disease transmission and control

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ABSTRACT

Koi herpesvirus disease (KHVD) is an emerging and highly contagious viral disease of koi and common carp (*Cyprinus carpio*), causing mass mortalities and huge economic losses to the carp aquaculture industry. The disease has spread rapidly to 28 countries worldwide. However, mechanisms of koi herpesvirus (species *Cyprinid herpesvirus 3*; CyHV-3) transmission remain unclear. A potential experimental model of CyHV-3 infection in carp was used to characterise CyHV-3 in different phases of infection and to demonstrate that CyHV-3 persists in survivor fish and has the capacity to reactivate and transmit the disease to healthy fish. During acute infection, which occurred when fish were maintained at 22 °C, viral genes were abundantly expressed and infectious virus was produced in association with tissue damage, clinical disease and mortality. In fish maintained at a lower temperature (11 °C), viral DNA was present but viral gene expression was absent or greatly restricted, infectious virus was not recovered and there was no evidence of disease. Productive replication was re-initiated following an increase in water temperature to 22 °C, resulting in 45% mortality. Shedding of reactivated virus killed 75% of cohabitating naïve fish, suggesting a potential risk for disease transmission.

1. Introduction

Koi herpesvirus (species *Cyprinid herpesvirus 3*, CyHV-3) is a large double-stranded (ds) DNA virus of fish which has been assigned to the family *Alloherpesviridae* within the order *Herpesvirales* (Davison, 2010). Koi herpesvirus disease (KHVD) has caused huge economic losses and significant negative social impact in many countries around the world (Pearson, 2004). For example, in the early years of the epidemic, disease outbreaks have been estimated to have cost Israeli and Indonesian aquaculture US$3 million year^{-1} (Perelberg et al., 2003) and US$10–15 million year^{-1} (Sunarto and Cameron, 2005), respectively. CyHV-3 has been included in the list of notifiable diseases by the World Organisation for Animal Health (OIE, 2012), and of threats for aquaculture and food resources (FAO, 2010).

Since the first outbreak of KHVD in Germany in 1997 (Bretzinger et al., 1999), the disease has been reported from at least 28 countries in Europe, Asia, Africa and America (OIE, 2012). It is likely that the virus is present in many more countries but has not yet been identified or reported. Outbreaks of KHVD generally occur during spring and autumn but not when water temperatures are high in summer or low in winter (Hedrick et al., 2000; Perelberg et al., 2003; Sano et al., 2004; Yuasa et al., 2008). However, it is unclear where the virus persists between seasons (Ilouze et al., 2011).

If a host survives an acute primary infection, viruses may establish persistent infections which have been categorised as either latent non-productive or chronic productive (Boldogh et al., 1996; Kane and Golovkina, 2010). The latter are characterised by continued production of low numbers of infectious virions over an extended period of time between episodes of recurrent disease (Buchmeier et al., 2007; Goff, 2007; Imperiale and Major, 2007; Lindenbach et al., 2007). Latent infections, by contrast, may be characterised by the presence of viral genome in the absence of

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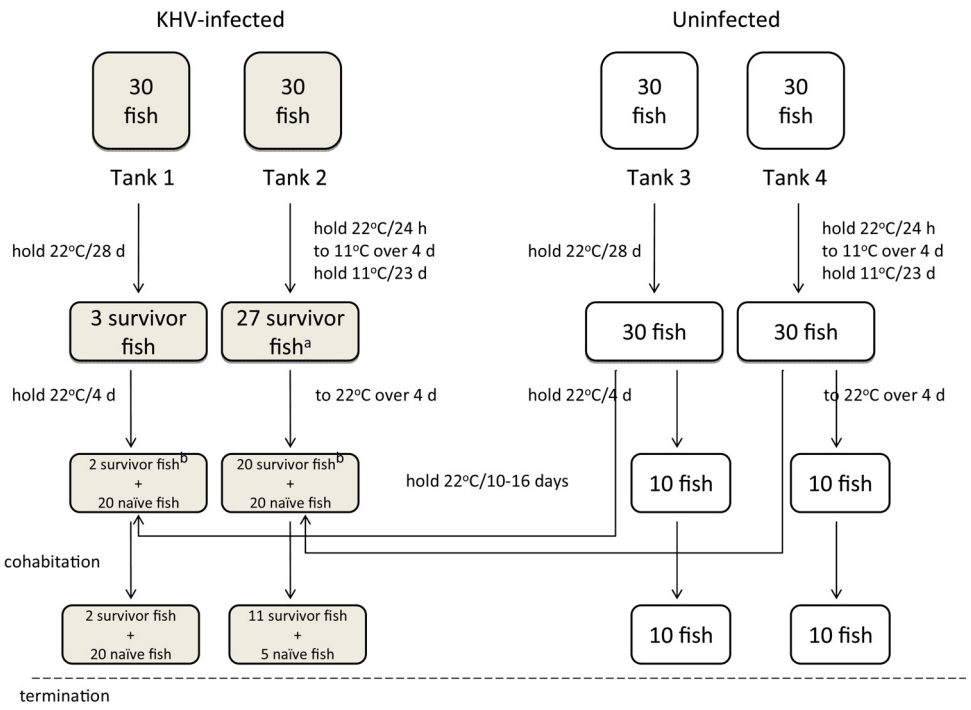

Fig. 1. Diagrammatic representation of the different treatment groups, and the number of surviving fish in each group. (a) Three survivor fish from Tank 2 were euthanized for sample collection at 10 dpi and (b) one survivor fish from Tank 1, and seven from Tank 2 were also euthanized at 28 dpi for sample collection.

infectious virus in various tissues of the host. In addition, there is the capacity for reactivation under the influence of specific stimuli (e.g., stressors) to the host that activate viral gene expression, recurrent disease and virus transmission (Roizman et al., 2007; Sinclair and Sissons, 2006; Stevens, 1989). The ability to establish a life-long latent infection has been assumed to be the hallmark of all herpesviruses. However, a gene encoding a latency-associated transcript, often the only viral gene abundantly expressed during latency in alphaherpesviruses (Preston, 2000), has not been found in any fish herpesvirus (Stingley et al., 2003). Thus, the nature of CyHV-3 persistence in carp is not clearly understood.

CyHV-3 has been presumed to establish a latent infection in survivor fish and various aspects of latency, including sites of persistence (Eide et al., 2011; Gilad et al., 2004) and reactivation (Baumer et al., 2013; St-Hilaire et al., 2005), and gene expression in vitro (Dishon et al., 2007) and in vivo (Sunarto et al., 2012), have been investigated. However, there are no studies that provide a comprehensive and fully-integrated description of virus activity in all three phases of infection. In this paper, we report a potentially reproducible experimental model of CyHV-3 infection in carp that allows us to characterise the presence of viral genomic DNA, viral RNA transcripts, viral proteins (antigens), infectious virus, tissue damage, clinical signs of disease and mortality of fish during the acute, persistent and reactivation phases of infection. We also demonstrate that, in survivor fish persistently infected with CyHV-3, virus may be reactivated and transmitted to in-contact healthy fish.

2. Materials and methods

2.1. Cell culture and virus isolate

The Indonesian CyHV-3 (C07) isolate used in this study was obtained from common carp suffering mass mortality in West Java, Indonesia in 2007 (Sunarto et al., 2011). The virus was grown in cultures of koi fin cell-line (KF-1) kindly provided by Professor R. P. Hedrick (University of California, Davis, USA). The cells were maintained in Leibovitz L-15 medium (Life Technologies) supplemented with 10% foetal bovine serum (FBS) (Thermo Trace), 2 mM L-glutamine (Invitrogen), 100 IU ml^{-1} penicillin (JRH Biosciences), 100 μg ml^{-1} streptomycin (Sigma), and incubated at 25 °C. The virus used for these experiments was derived from passage 3. The concentration of virus was estimated by determining the 50% tissue culture infective dose ($TCID_{50}$) (Reed and Muench, 1938).

2.2. Potential experimental model

All work was conducted at the microbiologically-secure CSIRO Australian Animal Health Laboratory (CSIRO-AAHL), and fish experiments were approved by the CSIRO-AAHL Animal Ethics Committee. Juvenile common carp (mean length ± standard deviation: 12.1 ± 1.0 cm) were wild-caught fish supplied by Fisheries Victoria in Australia, which is recognised as a CyHV-3-free country. Upon arrival at the laboratory, the carp were acclimatised for 8 days, and treated with formalin to remove adventitious ectoparasites (Lahnsteiner and Weismann, 2007). A sample of gills from each fish was tested by a CyHV-3-specific PCR (Bercovier et al., 2005) prior to all experiments. The fish were held in 80 l aquaria on a 12 h/12 h day/night cycle, and fed with commercial feed at a rate of 1% bodyweight per day throughout the experiment.

One hundred and twenty carp were distributed into two equal groups. Sixty carp were infected experimentally with CyHV-3 by immersion using a dose of 100 $TCID_{50}$ ml^{-1} for 2 h at 22 °C. After exposure, fish were briefly rinsed twice in freshwater and transferred to clean 80 l aquaria. The infected fish were then separated into two groups (30 fish each, Figs. 1 and 2): Tank 1, in which fish were held at a water temperature of 22 °C; and Tank 2, in which fish were held initially at 22 °C for 24 h and then the water temperature was decreased to 11 °C over a period of 4 days. At 28 days post-infection (dpi), reactivation of CyHV-3 was attempted with the fish in Tank 2 by increasing the water temperature from 11 to 22 °C in increments of 2 to 3 °C per day. When the temperature reached