Vaccine 26 (2008) 6415–6421

Contents lists available at ScienceDirect

Vaccine

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

DNA vaccine protects ornamental koi (*Cyprinus carpio koi*) against North American spring viremia of carp virus

E.J. Emmenegger*, G. Kurath

U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115, USA

ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form 20 August 2008
Accepted 25 August 2008
Available online 21 September 2008

Keywords:
SVCV
DNA vaccine
North America
Koi

ABSTRACT

The emergence of spring viremia of carp virus (SVCV) in the United States constitutes a potentially serious alien pathogen threat to susceptible fish stocks in North America. A DNA vaccine with an SVCV glycoprotein (G) gene from a North American isolate was constructed. In order to test the vaccine a challenge model utilizing a specific pathogen-free domestic koi stock and a cold water stress treatment was also developed. We have conducted four trial studies demonstrating that the pSGnc DNA vaccine provided protection in vaccinated fish against challenge at low, moderate, and high virus doses of the homologous virus. The protection was significant ($p < 0.05$) as compared to fish receiving a mock vaccine construct containing a luciferase reporter gene and to non-vaccinated controls in fish ranging in age from 3 to 14 months. In all trials, the SVCV-G DNA immunized fish were challenged 28-days post-vaccination (546 degree-days) and experienced low mortalities varying from 10 to 50% with relative percent survivals ranging from 50 to 88%. The non-vaccinated controls and mock construct vaccinated fish encountered high cumulative percent mortalities ranging from 70 to 100%. This is the first report of a SVCV DNA vaccine being tested successfully in koi. These experiments prove that the SVCV DNA (pSGnc) vaccine can elicit specific reproducible protection and validates its potential use as a prophylactic vaccine in koi and other vulnerable North American fish stocks.

Published by Elsevier Ltd.

1. Introduction

Spring viremia of carp virus (SVCV) is a rhabdoviral pathogen that frequently decimates common carp (*Cyprinus carpio carpio*) stocks throughout Europe [1,2]. Carp populations in the European countries of Russia, Romania, Netherlands, Moldavia, Georgia, Germany, France, United Kingdom, and Denmark have the highest reported prevalence [3]. In fish species that succumb to infection by SVCV, the spleen, kidney, intestines, and air bladder are typically inflamed, hemorrhaging, or swollen. Disease progression leads to necrosis of the internal organs and eventually death. Outbreaks at common carp farms in Europe normally occur in the spring, as the water temperature begins to rise after a cold winter period. The highest fish mortalities due to SVCV infection occur between 11 and 17 °C [4,5]. Common carp belonging to the *Cyprinidae* family are the principal host species of SVCV [6]. Natural infections of SVCV have also occurred in other cyprinid fish including koi (*Cyprinus carpio koi*), goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), silver carp (*Hypopthalmichthys molitirix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), orfe (*Leucisucus idus*), and tench (*Tinca tinca*) [6,7]. Experimental infection of other cyprinid species included roach (*Rutilus rutilius*) [8], zebrafish (*Danio rerio*) [9], and fathead minnow (*Pimephales promelas*) [Emmenegger unpublished data]. Fish species from other families of *Poeciliidae*, *Esocidae*, *Centrarchidae*, *Siluridae*, and *Salmonidae* have also been infected by SVCV [2,10]. Due to the highly infectious nature of SVCV and potential impact this virus could have on susceptible fish populations globally, any detection of SVCV requires notification within 48 h to the Office of Internationale Epizootic (OIE), the organization charged with regulating world animal health. SVCV is one of only nine piscine viruses recognized worldwide by the OIE as a notifiable animal disease.

In April of 2002, at one of the largest koi production facilities in the United States, yearling koi in one pond began dying from SVCV [11]. Subsequently the virus was detected in other ponds at the facility, 15,000 fish died from SVCV and another 135,000 fish were euthanized from ponds located both in North Carolina and Virginia [12]. One month later in an apparently unrelated incident, dead wild carp began washing up on the shores of a Wisconsin lake. Mortalities reached 1500 and the causative agent of the epidemic was SVCV [13]. One year later the virus was isolated from a healthy common carp during a fish health screening in an Illinois water channel that is linked to Lake Michigan. In 2004 there were two outbreaks of SVCV, one at a private koi pond in Washington State

* Corresponding author. Tel.: +1 206 526 6282x276; fax: +1 206 526 6654.
E-mail address: eemmenegger@usgs.gov (E.J. Emmenegger).

0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2008.08.071

and the other at a commercial koi hatchery in Missouri [14]. In June 2006, SVCV was found for the first time in Canada in common carp from Lake Ontario [15]. These fish were scheduled for shipment to France, but the virus was detected during an exportation disease screening. Later in October 2006 the United States Department of Agriculture (USDA) instituted regulations restricting the importation of live fish, fertilized eggs, and gametes of specific fish species susceptible to SVCV. Until the first outbreak in 2002, SVCV had never been reported in North America. Six isolations of this exotic virus in the past 6 years and the import restrictions placed on SVCV susceptible fish are warnings of the potential invasiveness and impact SVCV could have on vulnerable fish stocks in North America.

Eradication of SVCV infected fish and hygiene measures are the standard methods used to combat SVCV [2,16,17]. Therapeutic and preventative strategies to control SVCV have been ineffective and as such there is no commercially available SVCV vaccine. There have been previous reports of inactivated SVCV vaccines using European strains providing limited protection [18–22]. However, continued research on the inactivated SVCV vaccine has not been pursued in part due to the risks associated with incomplete activation of the virus, cumbersome legal and marketing restrictions, prohibitively expensive production costs, and the lack of a quantitative assessment of the protection levels provided by the vaccine [17,23].

DNA vaccines targeted against viral pathogens are an attractive alternative to traditional vaccines (i.e., inactivated, attenuated or protein subunits) for a variety reasons: straightforward design and construction, heat stability, low production costs, and long-term storage capabilities [24,25]. In addition, there is no risk of reversion to a pathogenic form and they have virtually no chemical impurities [26]. Hurdles currently being addressed for DNA vaccines include regulatory approval, promoter selection, and delivery technologies. Despite these concerns research on DNA vaccines fighting fish pathogens has increased steadily for the last 10 years [27]. Previous SVCV DNA vaccines [28] designed against European SVCV isolates have demonstrated lower efficacy as compared to fish DNA vaccines against infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) [25]. All three viruses, SVCV, IHNV, and VHSV, belong to the rhabdovirus family, but are separated into two different genera [29]. IHNV and VHSV belong to the *Novirhabdovirus* genus of viruses whose genome contains five structural genes, N, M, P, G, and L, and a sixth nonvirion (NV) gene of indeterminate function. SVCV, which has tentatively been assigned to the *Vesiculovirus* genus, lacks the NV gene [29,30]. Phylogenetic analysis of a partial SVCV G-gene region divided isolates into four genogroups, enabling SVCV isolates from Europe (Genogroups Ib, Ic, and Id) to be distinguished from those originating from Asia (Ia) [44]. Further phylogenetic analyses revealed that all six SVCV isolates detected in North America clustered in the SVCV Ia genogroup, suggesting that the isolates were of Asian origin [14,15]. The G-gene of these fish rhabdoviruses codes for the surface glycoprotein, which is the primary antigen that the fish host mounts an immune response against and is the target gene used in the DNA vaccine constructs. The novirhabdovirus DNA vaccines have demonstrated long-lasting protection with small doses in a variety of salmonid species, against various viral strains [27,31,32]. In 2005 the IHNV DNA vaccine was licensed in Canada for use in the Atlantic salmon aquaculture industry and the VHSV DNA vaccine has undergone field trials at rainbow trout farms in Denmark [24,32].

To date attempts to develop an equally efficacious SVCV DNA vaccine have not been successful. Early reports with European genogroup SVCV DNA vaccines from a Russian laboratory suggested some efficacy, but have not been confirmed (reviewed in [25]). More recently mixtures of 10 SVCV DNA vaccine plasmids containing partial or complete G gene fragments from the European SVCV reference strain (Fijan-Genogroup Id) have been tested in carp [28]. The majority of treatment groups had little protection, with RPS values of −11 to 33%. One group of fish receiving a combination of three plasmids had an RPS of 48% in a single trial, but the specific plasmid responsible for protection was not identified.

The presence of SVCV in the US and Canada has renewed research efforts to develop an effective DNA vaccine to prevent the spread and establishment of SVCV in North America. In this project, a novel SVCV DNA vaccine utilizing the North Carolina (nc) SVCV G-gene was designed and tested in four trial experiments. In order to test the vaccine a reliable challenge model was developed by testing the susceptibility of different fish host species to the North American SVCV and devising challenge treatments that induced rapid and reproducible infections in the host.

2. Materials and methods

2.1. Fish stocks

Goldfish (*C. auratus*) less than 1 year old and 7–10 cm in length were shipped from a facility, that meets the OIE standard as a compartment free of SVCV, to the wet laboratory facility at the Western Fisheries Research Center (WFRC, Seattle, Washington). Fish were housed in tanks with flow-through sand-filtered and UV-treated fresh water at water temperatures of 16–18 °C. Fish were monitored daily and fed every other day Wardley Ten floating pellets (Hartz Mountain Co.). Goldfish were maintained in the stock tanks until commencement of the SVCV susceptibility challenges.

Koi (*Cyprinus carpio koi*) from a specific pathogen-free domestic stock were obtained from a local koi farm (Pan Intercorp., Kenmore, Washington). The koi distributor annually breeds his own koi stock. The breeder has voluntarily participated in the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) screening program for SVCV in ornamental fish. One-month-old specific pathogen-free domestic koi were transferred to the wet laboratory facility at the WFRC and reared under the same conditions described for the goldfish. Koi were also fed every other day, but received a mixed feed diet of moist and dry pellets consisting of Life Stage Diet Food (Oregon Biodiet), Wardley Pond 10 (Hartz Mountain Co.), and Hikari Gold (Kyorin Food Industries). The amount, pellet size, and type of feed varied as the koi aged. Koi were held in stock tanks until initiation of the susceptibility challenges, cold stress challenge experiment, or vaccine trial studies.

2.2. Virus propagation

The North American SVCV isolate from North Carolina (SVCVnc) was sent by Dr. Andy Goodwin, the first scientist to isolate and identify SVCV in North America [11]. The SVCVnc isolate was propagated in an *epithelioma papulosum cyprini* (EPC) cell line [33] at a constant temperature of 20 °C in minimum essential medium (MEM; Invitrogen Inc.) supplemented with 10% fetal bovine serum (Hyclone Inc.) and 2 mM L-glutamine (Invitrogen Inc.), and buffered to pH 7.0 with 7.5% sodium bicarbonate (Fisher Scientific Co.). Virus titers for the challenge inocula were determined by plaque assay following the procedure outlined by Batts and Winton [34] with a modified incubation temperature of 20 °C.

2.3. Development of the challenge model

2.3.1. Susceptible host species challenges

Prior to challenge fish were transferred to an aquatic biosafety level 3 (BSL-3) laboratory, housed in a separate building from the main WFRC wet laboratory. Due to OIE listing of SVCV and its exotic