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Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination

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Abstract

Fish nodaviruses (betanodaviruses) are small, non-enveloped icosahedral single-stranded positive-sense RNA viruses that can cause viral encephalopathy and retinopathy (VER) in a number of cultured marine teleost species, including Atlantic halibut (*Hippoglossus hippoglossus*). A recombinant protein vaccine and a DNA vaccine were produced, based on the same capsid-encoding region of the Atlantic halibut nodavirus (AHNV) genome, and tested for protection in juvenile turbot (*Scophthalmus maximus*). Vaccine efficacy was demonstrated in the fish vaccinated with recombinant capsid protein but not in the DNA-vaccinated fish, despite the fact that in vivo expression of the DNA vaccine-encoded antigen was confirmed by RNA in situ hybridisation and immunohistochemistry. Combined DNA and recombinant vaccine administration did not improve the effect of the latter. Surprisingly, fish vaccinated with 50 μg recombinant protein demonstrated a threefold lower survival rate than the two groups that received 10 μg recombinant protein. Neither the recombinant protein vaccine nor the DNA vaccine induced anti-viral antibodies 9 weeks after immunisation, while antibodies reactive with the recombinant protein were detectable mainly in fish vaccinated with 50 μg recombinant protein. The study also demonstrates evidence of viral replication inside the myocytes of intramuscularly challenged fish.

Keywords: Fish nodavirus; DNA vaccine; Recombinant vaccine; Turbot; Antibodies; Vaccine dose

1. Introduction

Fish nodaviruses are neurotrophic, and cause viral encephalopathy and retinopathy (VER), also known as viral nervous necrosis (VNN), in a wide range of farmed marine teleosts, particularly during the larval and

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juvenile stages (reviewed in [1]). In Norway, one of the obstacles to successful cultivation of Atlantic halibut (*Hippoglossus hippoglossus*) has been repeated mass-mortality caused by nodavirus infections [2,3]. Other North Atlantic fish species such as turbot (*Scophthalmus maximus*) [4] (Johansen R. et al., unpublished data), cod (*Gadus morhua*, L.) [5,6] and spotted wolffish (*Anarhichas minor*) [7], are also susceptible to nodavirus. The nodavirus particle is non-enveloped and consists of a 25–30 nm icosahedral capsid containing two single-stranded positive-sense RNA segments, RNA1 (3.1 kb) and RNA2 (1.4 kb). RNA1 encodes the putative RNA-dependent RNA polymerase and RNA2 encodes the capsid protein. In marine fish the main infection route is believed to be by vertical transmission from infected broodstock to offspring. Vertical transmission of nodavirus has been demonstrated in striped jack (*Pseudocaranx dentex*) [8,9], barfin flounder (*Verasper moseri*) [10] and European sea bass (*Dicentrarchus labrax*) [11,12]. In addition, horizontal transmission has been demonstrated in European sea bass [13,14], and by cohabitation of sea bream (*Sparus aurata*), an asymptomatic carrier of fish nodavirus, and sea bass [15]. Different prophylactic strategies, such as nodavirus screening of broodstock fish by means of reverse transcription–polymerase chain reaction (RT–PCR) or enzyme-linked immunosorbent assay (ELISA) [9,10,16], ozonation of hatchery water [17,18] and limited stressing of broodstock fish have been recommended. Vaccination trials using *Escherichia coli*-expressed nodavirus capsid protein have been described by Húsgarð et al. [19] and Tanaka et al. [20], who reported enhanced survival and presence of virus-neutralising antibodies after immunisation. Such vaccination strategy could be useful for fish species affected by VER at grown-out stages, and in limiting the chance of nodavirus infection in pre-selected broodstock fish.

There are still a limited number of reports on DNA vaccines in fish, but high levels of protection in rainbow trout (*Oncorhynchus mykiss*) have been reported using DNA vaccines against viral hemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) [21–25]. A DNA vaccine that encodes the glycoprotein of spring viremia of carp virus (SVCV) has recently been shown to be protective [26], and DNA vaccination of channel (*Ictalurus punctatus*) against channel catfish herpes virus (IHV-1) has also shown promising results [27]. These efficacious DNA vaccines all have in common that the protective antigen is a glycosylated viral membrane protein. In the present study a DNA vaccine encoding the capsid protein of the Atlantic halibut nodavirus (AHNV) and a recombinant protein vaccine containing the *E. coli*-expressed AHNV capsid protein were tested. As the Atlantic halibut has proven difficult to use in challenge experiments, the vaccines were tested for protection against viral challenge using turbot as model fish.

2. Materials and methods

2.1. Cell culture and virus propagation

AHNV strain AH95NorA [28] was propagated in SSN-1 cells as described by Dannevig et al. [29] and a fourth passage of the virus was used in the challenge experiment. Virus titre was determined by 10-fold end-point titration using eight parallels, and $TCID_{50}$ was determined according to Reed and Muench [30]. Viral ELISA antigen was prepared by inoculating SSN-1 cells with a third passage of AHNV, and infected cells were harvested when a moderate cytopathogenic effect (CPE) was observed [31].

2.2. Expression of recombinant antigen (recAHNV-C) and vaccine preparation

A 1420 bp cDNA fragment corresponding to AHNV RNA2 [28] was subcloned into *Eco*RI and *Not*I digested pET30b(+) (Novagen), and verified by DNA sequencing. The recombinant plasmid was transformed into *E. coli* BL21 (DE3) for expression. Bacterial cells were grown in LB medium (containing 30 μg ml^{-1}kanamycin) at 37 °C, and protein expression induced at $OD_{600} = 1.0$ by the addition of IPTG (isopropyl-thio-β-D-galactoside) to a final concentration of 1 mM. Cells were harvested by centrifugation ($5000 \times g$) after a further 3 h of growth. The cells were resuspended in 0.1 volume TE-buffer (50 mM