VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

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1. TNA Provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
Vaccination of Seabass against a lethal viral disease and characterization of protective immunity	Sofie Barsøe	DTU Aqua	DK	IZSVe aquarium	24/6/2019	26/9 2019	47
Role of rotifers in betanodavirus transmission to European sea bass larvae	Isabel Bandin	University of Santiago de Compostela	ES	IZSVe aquarium	04/11/2021	04/12/2021	34
Rainbow vacc Infectious Haematopoietic Necrosis virus	Niccolò Vendramin	DTU Aqua	DK	IZSVe aquarium	05/09/2022	30/09/2022	36

2. Final reports of each TNA provided

2.1 TNA 1: Vaccination of Seabass against a lethal viral disease and characterization of protective immunity

Betanodavirus, which causes viral nervous necrosis in several fish species including sea bass, is a big challenge for Mediterranean fish farmers who suffer several losses every year due to this disease. Betanodavirus is a small (37nm) icosahedral, non-enveloped RNA virus composed of two RNA strands, RNA 1 (RNA polymerase) and RNA 2 (capsid proteins). Four Betanodavirus strains have been described and recognized, with different host species and temperature preferences. The strain RGNNV causes the most problems in farmed sea bass.

There are currently two licensed vaccines against RGNNV in the EU, but they rely solely on virus inactivation, which is expensive and lacking of some information on the duration of the given immunity. A new innovative vaccine offering high protection is therefore still needed and needed and the objective of this study is to test such a candidate. The current vaccine is a VLP (virus like particle) produced by cloning the capsid protein (RNA 2) of the RGNNV virus into a yeast cell (Pichia pastoris). As the yeast grows, it produces the capsid strand which self-assembles into virus like particles (VLP) that has the same structure and size as the virus particle, but are unable to infect the host due to the lack of RNA1.

In the current study, three doses of the VLP vaccine were tested against RGNNV in juvenile European Sea Bass together in parallel with one of the commercial vaccines available in the European market in order to assess the level of protection.

The vaccination and challenge trial conducted during this TNA was successfully completed. Full sets of samples were collected for virological, serological, histological analysis to better understand the mechanisms of protection of the vaccinated European sea bass against NNV. The results obtained showed better protection of VLP based vaccine than the commercially available product, which encourages further research on this promising product. The study was published in Vaccines (Basel): https://doi.org/10.3390/vaccines9050447

2.2 TNA 2: Role of rotifers in betanodavirus transmission to European sea bass larvae

Nervous necrosis virus (NNV), a member of the Genus Betanodavirus, Family Nodaviridae, is the causative agent of Viral Encephalopathy and Retinopathy (VER), a neuropathological condition that causes high mortality in marine fish worldwide. The NNV genome is composed of two single-stranded positive-sense RNA molecules, RNA1 and RNA2, which encode for the RNA dependent RNA polymerase (RdRp) also known as protein A, and for the coat protein, respectively. A subgenomic RNA molecule (RNA3) is also transcribed during the infection process from the 3' termini of RNA1. On the basis of a small variable sequence of RNA2, known as T4 region, the Betanodavirus have been clustered into four genotypes: Barfin flounder nervous necrosis virus (BFNNV), Red spotted grouper nervous necrosis virus (RGNNV), Striped jack nervous necrosis virus (SJNNV) and Tiger puffer nervous necrosis virus (TPNNV). In addition, natural reassortants between RGNNV and SJNNV genotypes, in both RGNNV/SJNNV and SJNNV/RGNNV (RNA1/RNA2) forms, have been detected in southern Europe in recent years in wild and farmed fish.

Transmission of betanodaviruses occurs both horizontally and vertically. Horizontal transmission includes viral spreading through the water body during disease outbreaks, or by direct contact between infected fish or asymptomatic carriers and healthy susceptible animals. In addition, it has been suggested that marine invertebrates may serve as reservoirs or vectors of the virus.

Some of these invertebrates, such as the brine shrimp, *Artemia salina* and the rotifer *Brachionus plicatilis* are commonly used as life food for marine fish larvae, leading to the evaluation of their putative role as vectors of NNV. Both of these invertebrates have been shown to act as carriers of NNV infectious viral particles, and it has been recently demonstrated that the brine shrimp can transmit NNV to Senegalese sole larvae, causing disease symptoms and high mortality. However, to date, there are no data on the transmission of NNV from rotifers to fish larvae. Since rotifers are used as a primary food for marine fish larvae, such a mode of transmission would require the implementation of new appropriate prophylactic measures.

The results obtained clearly showed that rotifers do not allow replication of betanodavirus, but only act as passive vectors. However, we observed that fish fed with infected rotifers (single meal) contained an increasing load of NNV although the progression of the infection was slower than in the case of bath infection, and eventually developed the disease. These results suggest that rotifers, commonly used as starting feed for fish and mollusc larvae, can act as NNV vectors and may represent a risk for hatchery production. The study was published in Front Vet Sci: https://doi.org/10.3389/fvets.2022.932327

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2.3 TNA 3: Rainbow vacc Infectious Haematopoietic Necrosis virus

IHN is a viral disease caused by a Novirhabdovirus, an enveloped negative ssRNA bullet shaped virus belonging to the Rhabdoviridae family. On the basis of genotyping, five genotypes are recognized: U, M and L genotypes, located mainly in North America, cause severe mortality in different salmonid species. In particular, the U genotype has been associated with severe losses in farmed Atlantic salmon. Additionally, the J and E genotypes have been identified, the latter causing mortality in farmed Rainbow trout. In particular, recent studies have shown that strains circulating in Italy are characterized by an increasing virulence associated to a high evolutionary rate. Despite eradication efforts, the disease remains endemic in Europe. Vaccination is a valuable tool to reduce the impact of the disease on Rainbow trout production. While conventional vaccines have not been shown to be very protective against the disease, DNA vaccines have shown to be very effective in protecting rainbow trout against Novirhabdoviruses when tested both in controlled and in field conditions.

The aim of this TNA was to evaluate the efficacy of different doses of a new DNA vaccine in protecting rainbow trout against different strains of IHN. The new DNA vaccine (pVax-ihnG) used was developed by Technical University of Denmark (DTU) in collaboration with IZSVe. The two selected IHN strains belonging to the E genotype were the following: the IHNV strain 21-4070 isolated in 2021 in Denmark, which is the cause of the recent outbreaks of IHN in farmed Rainbow trout, and the IHNV strain IHNV/O.mykiss/I/TN/224-2/Mar18 (IHN-IT 224-2), isolated in 2018 from an infected farm in northern Italy.

The conducted trial provided additional data regarding the protection conferred by this prototype vaccine against different IHN strains that have recently caused outbreaks in Europe. The following conclusion were therefore drawn:

1- the IHNV isolated in 2021 in Denmark is highly virulent to rainbow trout, proportionally more so than other currently known isolates circulating in Europe (including the Italian strain of IHN).
2- The prototype vaccine conferred protection against IHNV isolated from rainbow trout in Denmark in 2021
3- Overall, it is challenging to test the efficacy of the prototype vaccine in rainbow trout due to the negative influence of fish size on IHNV pathogenicity (lower mortality is reported in fish over 10 g).

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