Veterinary Microbiology 177 (2015) 53–61

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Experimental susceptibility of European sea bass and Senegalese sole to different betanodavirus isolates

S. Souto [a,2,*], B. Lopez-Jimena [b,1,2], M.C. Alonso [c], E. García-Rosado [c], I. Bandín [a]

[a] Universidad de Santiago de Compostela, Instituto de Acuicultura, Constantino Candeira C.P.: 15705, Santiago de Compostela, A Coruña, Spain
[b] IFAPA Centro El Toruño, Junta de Andalucía, Ctra N.IV, Camino de Tiro Pichón, C.P.: 11.500, El Puerto de Santa María, Cádiz, Spain
[c] Universidad de Málaga, Departamento de Microbiología, Facultad de Ciencias, Campus de Teatinos, 29.071 Málaga, Spain

ARTICLE INFO

Article history:
Received 6 November 2014
Received in revised form 18 February 2015
Accepted 23 February 2015

Keywords:
Betanodavirus
Genotypes
Natural reassortant
Experimental infection
European sea bass
Senegalese sole

ABSTRACT

The susceptibility of juvenile European sea bass and Senegalese sole to three VNNV isolates (a reassortant RGNNV/SJNNV, as well as the parental RGNNV and SJNNV genotypes) has been evaluated by challenges using two inoculation ways (bath and intramuscular injection). The results demonstrate that these two fish species are susceptible to all the VNNV isolates tested. In European sea bass, RGNNV caused the highest cumulative mortality, reaching maximum values of viral RNA and titres. Although the SJNNV isolate did not provoke mortality or clinical signs of disease in this fish species, viral production in survivor fish was determined; on the other hand the reassortant isolate did cause mortality and clinical signs of disease, although less evident than those recorded after RGNNV infection. These results suggest that the changes suffered by the SJNNV RNA2 segment of the reassortant isolate, compared to the parental SJNNV, may have involved host-specificity and/or virulence determinants for European sea bass. Regarding Senegalese sole, although the three isolates caused 100% mortality, the reassortant strain provoked the most acute symptoms, and more quickly, especially in the bath challenge. This was also the isolate showing less difference between the number of RNA copies and viral titre, reaching the highest titres of infective viral particles in nervous tissue of infected animals. The RGNNV isolate produced the lowest values of infective viral particles. All these results suggest that the RGNNV and the reassortant isolates are the most suited for infecting European sea bass and Senegalese sole, respectively.

1. Introduction

Viral nervous necrosis (VNN), or viral encephalopathy and retinopathy (VER), is a serious emerging disease affecting a wide range of marine farmed and wild fish species (Munday et al., 2002), freshwater fish (Maltese and Bovo, 2007; Bigarré et al., 2009; Vendramin et al., 2012), and invertebrates (Gomez et al., 2006) worldwide.

The etiological agent is the viral nervous necrosis virus (VNNV, *Betanodavirus* genus, *Nodaviridae* family), which is responsible for high mortalities, particularly in larvae and juveniles, with deleterious economic consequences in the aquaculture industry. Affected fish display lesions in retina, brain and spinal cord. The clinical signs associated to this pathology include abnormal swimming, loss of appetite, changes in pigmentation and hyperinflation of

* Corresponding author at: Instituto de acuicultura, C/ Costantino Candeira Bloque C 15705, Santiago de Compostela, A Coruña, Spain. Tel.: +34 881 816 091/087.
E-mail address: sandra.souto@usc.es (S. Souto).
[1] Current address: Institute of Aquaculture, University of Stirling, FK9 4LA, Scotland, United Kingdom.
[2] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.vetmic.2015.02.030
0378-1135/

the swim bladder (Maltese and Bovo, 2007). Betanodaviruses are small non-enveloped icosahedral viruses with a genome composed of two single-stranded positive sense RNA molecules (RNA1 and RNA2) (Mori et al., 1992; Frerichs et al., 1996). The RNA1 (3.1 Kb) encodes the RNA-dependent RNA polymerase, and the RNA2 (1.4 Kb) encodes the capsid protein (CP, 42 kDa) (Mori et al., 1992; Comps et al., 1994). It has also been reported a subgenomic transcript originated from the RNA1 segment (RNA3, 0.4 Kb), which encodes the non-structural B1 (11 kDa) and B2 (8.4 kDa) proteins (Tan et al., 2001).

Traditionally, betanodaviruses have been clustered into four genotypes: barfin flounder nervous necrosis virus (BFNNV), redspotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV) and tiger puffer nervous necrosis virus (TPNNV) (Nishizawa et al., 1997). This classification is based on the phylogenetic analysis of a variable region within the RNA2 segment. Further studies have evidenced the importance of analysing both RNA segments for VNNV characterization, since the reassortment between genomic segments seems to be a frequent event. As a matter of fact, reassortant isolates combining genomic segments from the SJNNV and RGNNV genotypes have been obtained from farmed European sea bass (*Dicenthrachus labrax*) and Senegalese sole (*Solea senegalensis*) (Toffolo et al., 2007; Olveira et al., 2009; Panzarin et al., 2012).

Although the susceptibility of European sea bass to RGNNV is well-known (Skliris et al., 2001; Toffolo et al., 2007; Panzarin et al., 2012), the knowledge on the susceptibility of this fish species to other genotypes is very poor to date. In fact, to our knowledge, there is only one study analysing the susceptibility of European sea bass to different VNNV genotypes by experimental infection (Vendramin et al., 2014). The studies about the Senegalese sole susceptibility to VNNV are even more scarce, since only a few RGNNV/SJNNV reassortant isolates (composed of RGNNV RNA1 and SJNNV RNA2 segments) have been associated with mortalities in this species so far (Olveira et al., 2009).

In the present study, the susceptibility of European sea bass and Senegalese sole to RGNNV, SJNNV, and RGNNV/SJNNV isolates was evaluated. In addition, viral RNA production and viral infectivity were determined in different experimental infections.

2. Materials and methods

2.1. Virus and cell culture

Several VNNV isolates were used in this study: (i) ERV378/102-5/04 (RGNNV genotype), kindly provided by Dr G. Bovo (Istituto Zooprofilattico Sperimentale delle Venezie, Italy), and previously used in European sea bass experimental infections (Lopez-Jimena et al., 2011, 2012); (ii) SpSs-IAusc160.03, a RGNNV/SJNNV reassortant isolate obtained from diseased Senegalese sole (Olveira et al., 2009), and (iii) SJ93Nag, a reference SJNNV strain.

All viral isolates were propagated on E-11 cells (Iwamoto et al., 2000) grown in Leibovitz L-15 (Gibco) medium supplemented with penicillin (Gibco, 100 units/ml), streptomycin (Gibco, 100 mg/ml), and 2% foetal bovine serum (FBS, Lonza). Inoculated cells were incubated at 25 °C. Viral titration was performed in 96-well plates (Nunc), and expressed as the viral dilution infecting 50% of the cell cultures ($TCID_{50}$), following the methodology described by Reed and Müench (1938).

2.2. Experimental challenges

European sea bass and Senegalese sole were obtained from commercial fish farms and acclimatized for at least 7 days after arrival to the quarantine facilities sited at the IFAPA Centre El Toruño (Cádiz, Spain) (sea bass), and at the University of Santiago de Compostela (Spain) (sole). Fish were maintained at a maximum density of 100 fish in 100 l aquaria with aeration and were fed ad libitum with a commercial diet.

Prior to experimental infections, they were tested for the presence of VNNV, infectious pancreatic necrosis virus (IPNV), viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) genome. VNNV detection was performed using a combination of RT-PCR and dot-blot hybridization, according to Lopez-Jimena et al. (2010a), whereas IPNV, VHSV and IHNV were analyzed by RT-PCR and nested-PCR following the procedures described by Lopez-Jimena et al. (2010b), López-Vázquez et al. (2006) and Dopazo et al. (2002), respectively. Amplified products were run on 2% agarose gels stained with ethidium bromide, using the 100-bp DNA ladder (Lonza).

2.2.1. Bath challenge

Both, European sea bass and Senegalese sole (2 g, average weight), were split into four groups (100 fish per group): (i) ERV_{bath}/bass-sole, challenged with the RGNNV isolate (ii) $Ss160.03_{bath}$/bass-sole, infected with the reassortant isolate, (iii) $SJ93Nag_{bath}$/bass-sole, challenged with the SJNNV isolate and (iv) control group (negative control). Animals were exposed to each virus (10^5 $TCID_{50}$/ml) in 3 l for 3 h (Senegalese sole) or 1 h (European sea bass) and strong aeration was supplied to the water during the challenge. Negative controls were exposed to L-15 containing no viruses. Temperature was maintained between 22 and 25 °C throughout all the experiment. Mortality was daily recorded, and dead fish were stored at −80 °C until virological analyses.

2.2.2. Intramuscular challenge

Fish (5 g, average weight) were intramuscularly (i.m.) injected with 0.1 ml of viral inoculum (5×10^5 or 10^5 $TCID_{50}$/fish, for sea bass and sole challenges, respectively) or L-15 medium (control group), and were distributed in four groups (100 and 60 individuals per group in sea bass and sole trials, respectively) named as follows: (i) $ERV_{i.m.}$/bass-sole, challenged with RGNNV, (ii) $Ss160.03_{i.m.}$/bass-sole, infected with the reassortant, (iii) $SJ93Nag_{i.m.}$/bass-sole, challenged with SJNNV, and (iv) control group, infected with 0.1 ml of L-15 medium. Temperature was maintained between 22 and 25 °C throughout all the experiment. Mortality was daily recorded, and dead fish were stored at −80 °C until virological analyses.