Aquaculture 300 (2010) 59–64

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Heritability of resistance to viral nervous necrosis in Atlantic cod (*Gadus morhua* L.)

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ARTICLE INFO

Article history:
Received 1 July 2009
Received in revised form 5 January 2010
Accepted 6 January 2010

Keywords:
VNN
VER
Nodavirus
Atlantic cod
Gadus morhua
Heritability

ABSTRACT

Nodavirus are the causative agents of viral nervous necrosis (VNN), and has been shown to cause mortality in numerous fish species worldwide, among them is the Atlantic cod (*Gadus morhua* L.). In this study, heritability of VNN resistance in Atlantic cod was estimated through challenge testing of 50 large full sib families (~94 fish per family) comprising two genetically distinct groups of Atlantic cod (Norwegian coastal cod and northeast Arctic cod) and their F1 crossbreds. The overall survival (dead/alive) at end of test was 30%, but coastal cod had considerably higher survival (56%) than northeast Arctic cod (10%), while the F_1 cross was intermediate (31%). Furthermore, enormous variation in family survival was observed within each group (17 to 82% for coastal cod, 0 to 42% for northeast Arctic cod and 0 to 92% for the F_1 cross). As a consequence, the estimated within-strain heritability of survival under challenge testing was extremely high (0.75 ± 0.11 on the underlying scale and 0.43 ± 0.07 on the observable scale). Common environmental effects of full-sib families were not significant in addition to additive genetic effects, but the data structure was not optimal for distinguishing these effects.

1. Introduction

Nodavirus has been reported to cause high mortalities in more than 20 marine fish species worldwide, with the same viral strains causing disease across species (Starkey et al., 2001; Munday et al., 2002). Infections with nodavirus target neural tissues and retina and the disease are thus referred to as either viral encephalopathy and retinopathy (VER) or viral nervous necrosis (VNN). In cod, the outbreaks are typically chronic with moderate mortalities (Hellberg, 2007; Patel et al., 2007). Symptoms of VNN infection include loss of appetite, abnormal schooling and swimming behaviour (looping, spiralling) and change of pigmentation. VNN is diagnosed by the characteristic histopathological changes in the target tissues and by verification of the virus by immunohistochemistry, RT-PCR and cell culture. The disease mainly infects fish in the larval or juvenile stages, but has recently been found to attack older fish (Bricknell et al., 2006; Samuelsen et al., 2006; Hellberg, 2007), although a higher disease pressure or weakened condition of the fish is needed to infect adult cod.

In Atlantic cod, disease outbreaks have been observed in Scotland (Starkey et al., 2001), North America (Johnson et al., 2002) and Norway (Patel et al., 2007). In Norway, nodavirus was officially diagnosed first time in 2006, and in 2008 three cod aquaculture farms were diagnosed with VNN. The virus may be transmitted both vertically and horizontally, can persist as infectious for a long time in subclinically infected fish and may remain infectious for a long time even under extreme environmental conditions (Samuelsen et al., 2006). These characteristics, together with the fact that no commercial vaccines against nodavirus are available, make VNN a great threat to cod aquaculture.

In addition to other preventive measures, selective breeding for more resistant fish may be an opportunity. Effectiveness of selective breeding will, however, rely on whether resistance can be assessed through testing methods, e.g., challenge tests, and, if so, whether a significant heritability can be found. Using challenge testing, substantial heritabilities have been found for a number of different diseases in aquaculture species (e.g., Gjøen et al., 1997; Henryon et al., 2005; Kjøglum et al., 2008), but, to our knowledge, no prior estimates of heritability of VNN exist. Furthermore, aquaculture production of Atlantic cod in Norway is based on a mixed population consisting of two genetically distinct groups (coastal cod mainly from southern Norway, north east Arctic cod and their crosses), which is known to differ in resistance against other diseases (vibriosis). The primary aims of this study were therefore to estimate heritability and differences between sub-populations with respect to challenge test survival (defined as dead/alive).

2. Materials and methods

2.1. Fish

A national breeding program for Atlantic cod was started in 2002 by the Norwegian Institute of Fisheries and Aquaculture Research

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doi:10.1016/j.aquaculture.2010.01.006

(presently Nofima Marin, Tromsø). The base population comprises brood-fish from different geographical areas along the coast of Norway. Two distinct genetic groups were present among the original brood-fish in the base population: Coastal cod (CC) and north east Arctic cod (NEAC). Three year classes with progeny of wild-caught cod (comprising both genetic groups), were formed (2003–2005). During this period, the two populations of cod were bred separately.

The families in the current study were produced in March/April 2007, and had parents from the year-classes 2003 and 2004. Thus, the tested fish were second generation of cod bred in captivity. Descriptive statistics of the data set are given in Table 1. In total, 4714 individually tagged fish were tested, comprising 761 fish from eight full-sib families of CC; 1200 fish from 13 full-sib families of NEAC; and 2753 fish from 29 F_1 cross families. Fish were progeny of 40 sires ($N_{NCC}=22$, $N_{NEAC}=18$) and 50 dams ($N_{NCC}=19$, $N_{NEAC}=31$), thus 10 of the sires were mated with more two dams while the remaining 30 were mated with a single dam. All CC sires, and all but one of the CC dams originated from southern Norway, while the last CC dam originated from northern Norway. The sires and dams of the tested fish originated from two year-classes: 2003 ($N=42$) and 2004 ($N=48$), and were selected for bodyweight at 2+ age (after two summers at sea) and vibriosis resistance. However, some of the parents used in this study came from families not tested for vibriosis resistance (38% and 17% of parents from year-classes 2003 and 2004, respectively). All fish in the current study originated from 76 wild-caught grandparents (35 males and 41 females). As a consequence, all tested families had one or more cousin families represented within the data set.

The number of tested individuals per family varied from 76 to 102 (average of 94). At 175–185 d of age fish were individually tagged with transponders (Jojo automasjon AS, Sola, Norway), at an average body weight of 18.4 g (5.7–58.2 g, CV = 0.35). The pure CC and NEAC (17.4 g and 17.7 g, respectively) tended to be somewhat small than the F_1 crosses (19.0 g). Families were kept in separate tanks until tagging. Cod juveniles were from 200 to 209 d of age when evaluated for survival after challenge with nodavirus at the Fish Health Unit of Tromsø Aquaculture Research Station (Tromsø, Norway).

2.2. Nodavirus isolation and preparation

The challenge test was conducted using injection of a nodavirus suspension, which was obtained as follows: A strain H-NV/RI97 was originally isolated from diseased, small juveniles of Atlantic halibut collected during an outbreak of VNN (Sommer et al., 2004). This strain, now called C06-H-NV/RI97, was re-isolated from Atlantic cod (5 g) that had died in a challenge trial performed in 2006. The fish were kept frozen at −70 °C and brain and eyes were collected after slight thawing. The samples from three individuals were pooled and homogenized in PBS (0.1 g/ml) by mincing and then blending in a whirl mixer. The homogenates were centrifuged for 5 min in 12,500×*g* and supernatants were collected. The cloned E-11 cell line (European collection of Cell Cultures, ECACC), derived from the striped snakehead (SSN-1) cell line, was used for propagation of nodavirus (Iwamoto et al., 2000). The E-11 cells were cultured in a 25 cm^2 tissue culture flask (Primaria, Falcon) at 25 °C using Leibivitz L-15 medium (PAA Laboratories GmbH Austria) supplemented with 4 mM L-Glutamine, 10% FBS (fetal bovine serum) and 50 μg ml^{-1} Gentamicin. The virus containing supernatant was diluted to 1:10 in L-15 medium supplemented with 2% FBS and 10 ml of this suspension was inoculated onto almost confluent monolayer of E-11 cells in the flask. For virus propagation the cells were incubated at 20 °C until full cytopathic effect (CPE) was observed, usually after 7–10 days. The flasks were frozen and thawed, and the cell debris was removed by centrifugation at 1465×*g* for 15 min at 4 °C.

2.3. Titration of virus

The culture supernatant was diluted serially 10-fold in L-15. Eight parallels of each dilution were inoculated onto E-11 cells in 96-well plates (200 μl per well). After 10 d of incubation at 20 °C end point dilution showing CPE was registered, and the 50% tissue culture infective dose ($TCID_{50}$ ml^{-1}) was calculated by the method of Reed and Muench (1938).

2.4. Challenge test

To carry out the challenge test, experimental fish were transferred from the breeding station to the Fish Health Unit of Tromsø Aquaculture Research Station. Here, fish were allocated into two 4 m^3 tanks, each having an equal number of representatives from all families, and kept in this environment throughout the entire test period. After arrival, fish were acclimatised gradually over 14 days from the 10 °C at the breeding station to the challenge temperature of 18 °C, not exceeding one degree per day. The estimated average body weight (based on a subsample of fish) at challenge was 25 g, resulting in an approximate biomass of 16 kg/m^3 per experimental tank. Filtered, oxygenated (140% saturation, 80–90% saturation in outflow), UV-treated normal salinity seawater (34‰) was provided. Feeding was stopped one day prior to the challenge with material from a second passage of C06-H-NV/RI97 in E-11 cell culture.

At the day of challenge (17–21 d after tagging), fish were anaesthetized with Metacaine (0.7 g/l) and subjected to an intramuscular injection (i.m.) of 0.1 ml nodavirus suspension with a titre of 3×10^8 $TCID_{50}$ ml^{-1} in the anterior dorsal region. Fish were fed according to appetite (Dana Feed AS, Horsens, Denmark) under continuous light throughout the whole experiment. Mortality was recorded daily, and the experiment was terminated 35 d post-challenge. Moribund and surviving fish were euthanized with an overdose of benzocaine.

The challenge model of VNN was pre-tested under identical conditions. Here, the injected fish showed typical signs of acute VNN involving the CNS, as disoriented swimming movements, with several belly-up due to gas-inflated swim bladder. High level of nodavirus was detected in brain and eyes of dead fish. Furthermore, no individuals died in a saline injected control group (results not shown). In the current study, similar signs of acute VNN were observed and identical criteria were used for removal of dead/moribund fish from the tank.

Table 1
Descriptive statistics of challenge-test data for VNN resistance in Atlantic cod. Three genetic groups were represented: Norwegian coastal cod (CC), northeast Arctic cod (NEAC) and F1 crosses between them (CROSS).

	CC	NEAC	CROSS	Total
No. of tanks				2
No. of fish	761	1200	2753	4714
No. of families	8	13	29	50
Average no. of fish per family (range)	95 (86–100)	92 (76–98)	95 (86–102)	94
No. of sires, year-class 2003	6	13		19
No. of sires, year-class 2004	9	12		21
No. of dams, year-class 2003	11	12		23
No. of dams, year-class 2004	15	12		27
No. of parents across year-classes	41	49		90
Mean end-survival, % (range)	56 (17–82)	10 (0–42)	31 (0–92)	30
Mean tagging weight, g (SD)	17.4 (5.8)	17.7 (5.3)	19.5 (6.6)	18.9 (6.1)
Pearson correlation between individual tagging weights and survival				0.01