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NOTE

Concentrations of iron, copper, zinc and selenium in liver of Atlantic salmon *Salmo salar* infected with *Vibrio salmonicida*

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ABSTRACT: Lethal infections with *Vibrio salmonicida* result in a significant reduction in liver concentrations of iron, copper, and selenium expressed as mg kg^{-1} wet wt liver. Infections also increase liver weight as the hepatic somatic index (HSI) is higher in dead and moribund fish than in survivors and non-infected fish. The net result is that total liver content of these minerals is not significantly different between healthy and diseased, experimentally infected fish.

Hitra Disease is a significant disease problem in Norwegian fish farming. While some authors suggest this is a complex multifactorial disease syndrome resembling vitamin E/selenium deficiency in domesticate animals (Fjølstad & Heyeraas 1985, Poppe et al. 1985, 1986), others (Egidius et al. 1981, 1984) emphasize the importance of a *Vibrio* sp. frequently isolated from fish suffering from Hitra Disease. This *Vibrio* sp. can produce the clinical signs of Hitra Disease in challenge tests (Hjeltnes et al. 1987) and has been proposed as a new species, *Vibrio salmonicida* (Egidius et al. 1986).

Hapatic levels of iron, copper, zinc, and selenium have been reported to be much lower in farmed Norwegian salmon than in wild salmon, and fish suffering from Hitra Disease have levels that are even further reduced (Poppe et al. 1986). Several authors have viewed this as support for the hypothesis that nutritional and/or metabolic disorders are important components of Hitra Disease. The data suggest 2 opposing hypotheses: infection of fish with *Vibrio salmonicida* may be secondary to tissue deficiency of these minerals, the deficiency actually enhancing susceptibility to the bacterium; or, reduced tissue levels of trace elements may be a result of infection with the bacterium. This study was undertaken to investigate the latter hypothesis.

Materials and methods. Experimental fish were 150 to 200 g Atlantic salmon *Salmo salar* smolts kept in 400 l aquaria with 9 °C, UV-treated sea water (30 ‰ salinity) and fed commercial dry pellets. One group of 20 fish was challenged with *Vibrio salmonicida* (NCMB 2262) by intraperitoneal (i.p.) injection of 10^6 bacteria in 0.1 ml of 1.5 % NaCl. A control group of 20 fish was injected with 0.1 ml 1.5 % NaCl. Concentrations of iron, copper, zinc, and selenium in the feed were 283, 14, 184, and 2.4 mg kg^{-1} dry wt, respectively. Fish were not fed during the experiment.

Following injection, dead and moribund fish were collected, livers were removed, and samples of kidney tissue were cultured at 15 °C on tryptone soy agar (TSA) supplemented with 5 % human blood and 1.5 % NaCl. The API-50 system (API system s.a., France) with 1.5 % sterile NaCl added to the solvent was used for biochemical characterization of bacterial isolates. After 17 d, survivors in the infected group and fish in the control group were killed and processed in an identical manner.

The total weights of the fish and their livers were recorded. Livers were freeze-dried to constant weight, homogenized, stored in tightly sealed bottles, and then digested in a mixture of concentrated nitric and perchloric acids (Suprapure; 9:1) as described by Julshamn et al. (1982). Iron, copper, and zinc were determined by flame atomic absorption spectrophotometry (AAS), whereas selenium was determined by graphite furnace atomic absorption spectrophotometry (GFAAS). Uncoated graphite tubes with an inserted L'vov platform and nickel matrix modifier (Maage et al. 1991) were used to stabilize the selenium. The accuracy and precision of the element analyses were confirmed with standard reference material (SRM Oyster

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Table 1. *Salmo salar*. Concentration of iron, copper, zinc, and selenium in liver of *Vibrio salmonicida* infected and non-infected fish

Element	Treatment	N	mg mineral kg^{-1} (wet wt) liver	Mineral content in whole liver (µg)
Fe	Noninfected	20	246 ± 44[a]	514 ± 200[a]
	Infected			
	Dead/moribund	13	136 ± 37[b]	498 ± 233[a]
	Survivors	7	270 ± 24[a]	543 ± 130[a]
Cu	Noninfected	20	50 ± 14[a]	94 ± 62[a]
	Infected			
	Dead/moribund	13	20 ± 11[b]	83 ± 65[a]
	Survivors	7	57 ± 17[a]	115 ± 47[a]
Zn	Noninfected	20	23 ± 3.9[a]	44 ± 13[a]
	Infected			
	Dead/moribund	13	23 ± 3.3[a]	85 ± 36[b]
	Survivors	7	24 ± 2.1[a]	49 ± 16[a]
Se	Noninfected	20	3.1 ± 0.6[a]	6.5 ± 2.9[a]
	Infected			
	Dead/moribund	13	1.5 ± 0.3[b]	6.1 ± 3.2[a]
	Survivors	7	3.6 ± 0.9[a]	7.1 ± 1.7[a]

In any given column, values for a particular mineral are significantly different when they do not share the same superscript letter ($p < 0.05$) (*t*-test; Snedecor & Cochran 1967)

Tissue 1566) from National Institute of Standards and Technology (NIST).

Results and discussion. Within 1 wk of challenge with *Vibrio salmonicida*, 13 of the 20 fish in the group had died (6 fish) or were collected in moribund condition (7 fish). Gross pathology was typical of infections with *V. salmonicida* (Hjeltnes et al. 1987) and the bacterium was isolated from all dead and moribund fish. None of the fish in the control group died during the experiment. No bacteria were isolated from survivors in the challenged group or from the control group.

The hepatic levels of iron, copper, and selenium in liver samples (mg kg^{-1} wet wt) were significantly reduced in fish dying from infection (Table 1). Inexplicably, and in contrast to the findings of Poppe et al. (1986), the concentration of zinc was not found to be significantly altered. In addition, there were no differences in hepatic levels of iron, copper, zinc, and selenium between challenged survivors and the control group. Both dead and moribund fish had grossly enlarged livers. Liver weights calculated as a percentage of the body weight and expressed as hepatic somatic index (HSI) were 1.81 ± 0.29 (range 1.57 to 2.26) in the dead and moribund fish. In survivors and in noninfected fish the HSI was 0.89 ± 0.08 (range 0.75 to 0.99) and 0.91 ± 0.11 (range 0.77 to 1.12), respectively. This supports the observations of Waagbø et al. (1988) who reported HSI (LSI) values of 1.83 ± 0.21 in fish suffering from the Hitra Disease and HSI values of 1.14 ± 0.18 in healthy fish. These authors suggested that HSI value differences were the result of higher lipid and water content in diseased fish livers following pathologic disturbances in liver metabolism. The change in HSI should be considered when the fate of trace minerals during an infection is studied. When the total amount of Fe, Cu, Zn, and Se was calculated for whole livers in the 3 groups of fish (controls, survivors, and dead/moribund groups), the only significant difference found between the groups was for zinc, which, in the dead/moribund group, was almost twice that for the control and survivor groups. This finding for zinc was consistent with the fact that the infection appeared not to affect the zinc concentration in the liver.

Poppe et al. (1985, 1986) reported a difference in the levels of Fe, Cu, and Se in wild fish, apparently healthy farmed fish, and farmed fish with Hitra Disease. The concentrations of trace minerals were expressed as mg kg^{-1} wet wt of the liver, and these authors proposed that dietary imbalance explained the observed differences. Our results suggest, however, that the difference between apparently healthy farmed fish and farmed fish with Hitra Disease is actually the result of liver enlargement associated with *Vibrio salmonicida* infection.

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