TISSUE TROPISM OF PISCINE MYOCARDITIS VIRUS (PMCV) IN EXPERIMENTALLY CHALLENGED ATLANTIC SALMON

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INTRODUCTION
Cardiomyopathy syndrome (CMS) is a severe cardiac disease affecting both farmed and wild Atlantic salmon (1). The disease was first described in Norway in 1985, and outbreaks occur along most of the Norwegian coast usually during the spring and autumn months affecting large fish in their second year in seawater. Accumulated mortality can be very high, causing significantly economic losses. Fish suffering from CMS may suddenly die without previous signs of disease and showing very few, if any, symptoms. CMS is diagnosed on the basis of histopathology, and is characterized by severe inflammation and degeneration of the cardiomyocytes in the atrium and the spongious part of the heart ventricle.

Recently Haugland et al. showed that the causative agent of CMS is a double-stranded RNA virus likely belonging to the family Totiviridae (1). As the virus causes a necrotizing myocarditis in salmon the name piscine myocarditis virus (PMCV) was proposed.

Real-time PCR or quantitative PCR (qPCR) has during the last years been established as a reliable and accepted method for diagnostic testing. It is specific and sensitive, has a rapid protocol and is widely used as a diagnostic and quantitative method.

Histopathology is the microscopic examination of tissue in order to study the manifestations of disease. The tissue samples are fixed in 10% buffered formalin, embedded in paraffin, sectioned and finally stained. The most commonly used stain in histopathology is a combination of hematoxylin and eosin (HE).

AIM OF THE STUDY
The aim of this study was to assess tissue tropism of a PMCV isolate following i.p. challenge of unvaccinated post-smolts of Atlantic salmon in sea water. The objectives were threefold;

• To assess histopathological changes in gills, heart, kidney, liver, spleen, pancreas and muscle at different time points between 0 and 12 weeks post injection
• To assess presence and quantity of the virus by qPCR screening of organ tissue and serum at different time points between 0 and 12 weeks post injection
• To assess the ability of the virus to infect cohabitant fish horizontally during challenge

MATERIALS AND METHODS
The experimental challenge was performed at ILAB in Bergen, Norway. Atlantic salmon kept in 12 °C sea water were injected intraperitoneally with a 0.2ml dose of challenge inoculum propagated in cell culture (GF-1 cells). Liver, gill, spleen, kidney, muscle and heart tissue, in addition to serum were sampled from 10 fish each at 0, 1, 2, 4, 6, 8, 10 and 12 weeks post injection.

Preservation. The sampled tissue were stored in RNAlater for qPCR-analysis and in formalin for histopathological analysis. Serum was frozen directly at ~20 °C until analysis.

qPCR. Standard protocol of Qiagen’s RNeasy mini kit was used to extract total RNA from the tissue samples. The RNA-concentration was measured by nanodrop and normalized to 1µg/µl into the cDNA-synthesis. For muscle and heart tissue, the RNA amount was 500µg/µl. The realtime analysis was run in a 2-step procedure, using Invitrogen’s SuperScript III Platinum Two-Step qRT-PCR kit with SYBR Green. Roche’s LightCycler system was used for the final qPCR step.

RESULTS and DISCUSSION

Virus load in tissue
The mean virus load (n=10), is given as Ct-values, of detectable virus load in serum for each sampling date 0-12 weeks post infection. These results show that spleen and kidney tissue carry detectable amounts of PMCV already at 1 week post infection. The virus load roughly peaks at 4 weeks post infection for all tissues. Thereafter, the Ct-values decreases for all tissues up to 10 weeks post infection, after when a slight increase is seen by 12 weeks. The pattern is more or less the same for all tissues indicating a chronic stage of infection.

Virus load 12 weeks post infection
The mean virus load, given as Ct-values, at 12 weeks post infection, injected and cohabitant fish are compared. Cohabitant fish carry higher virus load than the injected fish at this time point. These findings indicate that the two different routes of infection; intraperitoneal injection and horizontally spread, results in equal distribution of virus in the different organs in fish.

Virus load in serum
The number of fish, at each sampling (n=10), with detectable virus load in serum for each sampling date 0-12 weeks post infection. The virus load increases clearly by 2 weeks post infection and peaks at 4 weeks, then declining. These findings are in support of spleen and kidney harboring a PMCV infection 1 week post challenge and is not merely a result of virus circulating in blood.

Histoscores in heart
Histoscores (heart histopathology) from 6 to 12 weeks post vaccination in injected (H-I) and cohabitant (H-C) fish. The scores indicated are the sum of atrium and ventricle (+SEM) at the different time points indicated. No changes were observed before 6 weeks post challenge. Heart lesions are first observed 4 weeks post viral peak (upper figure) and decline thereafter up to 12 weeks. First lesions in cohabitant fish are delayed by 2 weeks post infection. No lesions were observed in any of the other organs examined.