Comparative experimental transmission of cardiomyopathy syndrome (CMS) in Atlantic salmon *Salmo salar*

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ABSTRACT: Cardiomyopathy syndrome (CMS) has been recorded in wild and farmed Atlantic salmon *Salmo salar*. Characteristic heart lesions primarily involving the myocardium are reported in natural outbreaks with associated mortality. To date, no experimental trials have reproduced these lesions in the laboratory. The present study reports on the first successful experimental transmission of CMS in Atlantic salmon in Scotland, with full development of the histological lesions that are described for the syndrome. Tissue homogenates of CMS-infected fish indicative of mild and severe lesions from Scottish and Norwegian natural outbreaks, respectively, were injected into naïve fish, and both induced heart lesions consistent with CMS. Lesion development was earlier and progression faster in the fish group receiving the Norwegian homogenate, but equivalent in both groups by the end time point of the experiment. The study demonstrated that the reported condition for both countries is identical, as evaluated through light microscopy, and that tissue homogenates from either mild or severely affected fish contain the transmissible agent.

KEY WORDS: Cardiomyopathy syndrome · Atlantic salmon · Transmission

INTRODUCTION

Cardiomyopathy syndrome (CMS) is an economically important disease of adult Atlantic salmon *Salmo salar* in seawater (Brun et al. 2003). This enigmatic syndrome currently accounts for significant losses through disease in terms of tonnage (S. Murray pers. comm. 2009). The first presumptive reports of CMS occurred in the mid-1980s for farmed salmon at coastal aquaculture sites in western Norway (Amin & Trasti 1988), but CMS has now been reported to occur in most of the salmon farming areas in Norway (Ferguson et al. 1990), as well as in wild salmon (Poppe & Seierstad 2003). Elsewhere, CMS was documented in Atlantic salmon from the Faeroe Islands, Denmark (Poppe & Sande 1994), from Scotland (Wood et al. 1995, Rodger & Turnbull 2000) and also in pre-harvest salmon from Canada (Brocklebank & Raverty 2002). The syndrome has primarily been observed in adult fish after 12 to 18 mo in seawater (Rodger & Turnbull 2000, Brun et al. 2003); consequently, there is a significant impact on market-size

fish that can die suddenly from presumptive heart failure (Brun et al. 2003).

CMS is diagnosed on the basis of histopathology, whereby a characteristic severe, chronic lesion affecting the spongiosum of the atrium and ventricle can be observed. Fish with CMS may cease feeding and swim sluggishly, developing skin haemorrhaging, raised scales and oedema (Rodger & Turnbull 2000, Poppe & Seierstad 2003). Typical findings at necropsy are ascitic fluid, fibrinous peritonitis and blood clots on the dorsocranial surface of the liver and surrounding the heart, or within the heart, with subsequent cardiac tamponade (Ferguson et al. 1990, Rodger & Turnbull 2000, Poppe & Seierstad 2003). Fish affected by the disease are, however, often in good condition, showing few clinical signs before death.

Grotmol et al. (1997) reported the detection of endotheliotropic nodavirus-like particles in heart tissue of salmon diagnosed with CMS. Positive immunolabelling of cells within the endocardium, epicardium and myocardium was observed through the use of a primary antibody against striped jack nervous necrosis virus. No

further studies on possible causative agents have, however, been published, and, currently, the cause or causes of CMS remains unknown, and no model of the infection is available. The salmon farming industry has requested an international effort towards identifying whether an infective agent is the cause of CMS, in order to improve diagnostics, study the epidemiological basis of infection and, ultimately, improve CMS disease control strategies. This study of pathogenesis was designed to determine if CMS could be reproduced in naïve Atlantic salmon by injecting tissue homogenates taken from severely or mildly affected fish.

MATERIALS AND METHODS

Fish. The experimental Atlantic salmon *Salmo salar* were reared from eggs at the Marine Scotland laboratory's experimental unit at Aultbea, Ross-shire, and transported to the laboratory in Aberdeen 2 mo prior to use. Large fish were selected for this experiment (mean weight: 1.1 kg) as reports indicate that CMS primarily occurs in this size group. The fish had been subject to health checks biannually since hatching, testing negative for infectious pancreatic necrosis virus (IPNV), viral haematopoietic necrosis virus (VHSV), furunculosis and bacterial kidney disease (BKD). In addition, 30 fish from the stock for this experiment were examined for salmonid alphavirus (SAV), infectious salmon anaemia virus (ISAV), IPNV and VHSV before the experiment was started, and bacterial isolation was attempted by inoculating samples onto tryptone soya agar (TSA) (Oxoid) with 2% NaCl, at 15°C. Plates were examined for growth daily for 7 d. Kidney and heart tissue were obtained from the same stock and frozen at –80°C until required for injection into the control fish.

Experimental design. The Scottish homogenate was taken from 3 salmon hatched in 2003 and transferred to the sea in June 2004. Histological examination indicated that these fish had mild lesions associated with the spongiosum of the ventricle, which is consistent with CMS infection. The Norwegian tissue homogenate was taken from 3 fish hatched in 2003 and transferred to the sea between May and July 2004. The sample was collected and pooled on 15 November 2005 after histological examination indicated severe heart lesions consistent with CMS. Tissue portions (kidney and heart) from both groups were stored frozen at –80°C until required.

The experimental Atlantic salmon were divided into 6 replicate groups of 30 fish tank^{-1} (a total of 180 fish). For each group (Scottish or Norwegian), 12 g of mixed kidney and heart tissue from the confirmed CMS cases described above, and similarly from control fish that were negative for CMS by light microscopy, were defrosted. Transport medium, L-15 medium 10% newborn calf serum, penicillin-streptomycin (200 U ml^{-1}), gentamicin (50 mg ml^{-1}) and polymixin B sulphate (400 U ml^{-1}) were added to the tissues and homogenised. The supernatant was then diluted further with transport medium to the total volume required for the inoculum. Syringes were pre-loaded with 1 ml fish^{-1} and kept cool until injection (up to 1 h). A sample of each homogenate was kept for virological screening as described below.

The aquarium seawater was maintained at 10°C throughout the experiment and monitored continually. The fish were fed at a rate of 1% body weight of feed d^{-1} fish^{-1} (Skretting Atlantic 500+), and each tank was provided with flow-through water at a rate of 400 l h^{-1}. The procedures and experimental protocols were licensed according to current UK regulations (The Animals [Scientific Procedures] Act 1986).

Light microscopy. Following terminal anaesthesia with tricaine methane sulphonate (MS222) at 0.1 g l^{-1}, fish were sampled at 52, 143 (final date for Norwegian group) and 150 (final date for Scottish group) days post-injection (dpi), at which times the experiment was terminated. Single moribund fish were also sampled at 110 and 130 dpi.

Tissues were fixed in 10% buffered neutral formalin solution for a minimum of 24 h, they were paraffin embedded, and sections were stained routinely with haematoxylin and eosin (H&E). Additional sections of heart, kidney, spleen and liver were also stained with Perl's stain (Bancroft & Stevens 2007) for haemosiderin and iron. Selected heart sections were stained for demonstration of fibrin with modified Masson's trichrome (Culling 1974) by replacing ponceau 2R stain with 1% acid fuchsin. All slides were coded, and microscopy evaluation was carried out 'blindly' when the whole study was completed, i.e. without knowledge of the group to which they belonged. Tissues were confirmed positive for CMS based on heart lesions as described by Ferguson et al. (1990) and Poppe & Seierstad (2003). Lesion development in the atrium and ventricle was scored for severity (mild, moderate and severe) and recorded separately for the CMS-positive fish. Red and white muscle, pancreas, liver and heart were examined by light microscopy to exclude lesions attributed to alphavirus and HSMI (heart and skeletal muscle inflammation).

Virology. Homogenates were screened for SAV, IPNV, ISAV, IHNV and VHSV by tissue culture for virus isolation. To screen for VHSV and IHNV, the homogenates were inoculated onto BF-2 and FHM, respectively, and were incubated at 15°C for 14 d with sub-cultivation on Day 7. To screen for ISAV, the homogenates were inoculated onto TO (leucocyte

line), and incubated at 15°C for up to 30 d with sub-cultivation on Day 14. To screen for SAV and IPNV, the homogenates were inoculated onto Chinook salmon embryo (CHSE 214), and incubated at 15°C for up to 42 d with sub-cultivation on Days 7 and 14.

Quantitative PCR of serum samples for SAV. Blood was collected in heparinised tubes, and serum was stored frozen at −80°C. RNA extraction was performed using the QIAGEN MagAttract Viral M48 RNA Kit on the Q M48 BioRobot according to the manufacturer's protocol. Resultant RNA was converted into cDNA using the TaqMan Reverse Transcription Reagents kit (Applied Biosystems) as follows: 19.25 µl of total RNA was mixed in a final volume of 50 µl containing the following: 1× RT buffer (25 mM Tris-HCl pH 8.3, 37.5 mM KCl), 5.5 mM MgCl$_2$, 2.5 µl of 50 µM random hexamers, 0.5 mM of each dNTP, 0.4 U RNase Inhibitor and 1.25 U Multiscribe Reverse Transcriptase. The mixture was incubated at 25°C for 10 min, 48°C for 30 min, heat inactivated at 95°C for 5 min and then stored at −20°C. The resultant cDNA was then amplified using a UKAS-accredited assay with primers Q-nsP1 F, Q-nsP1 R and the probe Q-nsP1 P (Hodneland & Endresen 2006). Quantitative PCR (qPCR) was performed using an ABI Prism 7000 (Applied Biosystems) under the following cycling conditions: 1 cycle of 37°C for 10 min, 1 cycle of 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

A total of 46 fish across all groups died during the experiment. A pump failure at 28 dpi resulted in the death of 13 fish (8 from 1 Scottish injected tank and the rest from a control tank). In addition, another 10 control, 10 Scottish homogenate-injected and 13 Norwegian homogenate-injected fish died during a period of 56 d, thus reducing the total number of analysed fish to 134.

RESULTS

Bacteriology and virology

Atlantic salmon *Salmo salar* sampled prior to the start of this experiment showed no evidence of bacterial infection and no histological evidence of CMS, HSMI, IPN, ISA, or alphavirus infection. Similarly the injected tissue homogenates checked by virus culture for alphavirus, ISAV and IPNV and by PCR for ISAV and alphavirus all showed negative results. Serum samples analysed by qPCR were also negative for SAV.

Gross findings

Fish sampled at scheduled time points did not show any specific external lesions that could be linked to CMS. Of the 46 fish that died, most occurred during the night and were unsuitable for sampling; however, 2 fish were found moribund and were sampled in full. Externally, they showed mild ventral haemorrhaging of the skin, while, internally, dilatation of the atrium and sinus venosus and a few blood clots near the heart were observed. These lesions were not recorded in the control groups. Mortality started at 67 dpi, and the last dead fish was registered at 123 dpi. For an additional 27d, until the termination of the experiment, no further mortalities were recorded.

Histopathology

Initial results from blind examination were recorded in an Excel file, and then records and sections were re-organised according to treatment group and sampling date, to enable interpretation and analysis of the histopathological changes. Both injected groups recorded heart lesions at 52 dpi, but with different levels of severity and tissue involvement. In the Scottish injected group, 20% of the fish sampled showed small foci of mild endocardial proliferation in the atrium, namely a thickened endocardium due to a combination of hyperplasia and hypertrophy of endothelial cells (Fig. 1A). At this time point, 100% of the fish sampled from the Norwegian group showed widespread changes compatible with early CMS in both atrium and ventricle. Typical histopathological changes included diffuse endocardial proliferation and subendocardial mononuclear infiltration (monocytes, macrophages) and, occasionally, neutrophils. These changes were mild and primarily noted in the atrium of the Scottish group, but widespread and also involving the ventricular spongiosum of the Norwegian group (Fig. 1B). Myocyte disarray was particularly noticeable in the spongiosum and atrium of the Norwegian group, but still largely absent in the Scottish group at 52 dpi. In addition, slight loss of myofibre striation and scattered hypertrophic, pleomorphic nuclei were seen in the atrium and ventricle in both groups (Fig. 1C). The large, wavy and elongated nuclei (up to 40 µm) were similar to the Anitschkow-like nuclei cells described by Ferguson et al. (2005), though lacking the wavy chromatin pattern.

At 110 dpi, a freshly moribund fish sampled from the injected Norwegian group showed extensive lesions consistent with CMS (Fig. 2), and, by 143 dpi (the final sampling for the Norwegian groups), 63% of the remaining fish had severe CMS lesions, primarily involving the ventricle, with extensive myocardial infiltration and endocardial proliferation still evident. Hypertrophic, pleomorphic and centrally located nuclei were prominent at this time point within the ventricle spon-
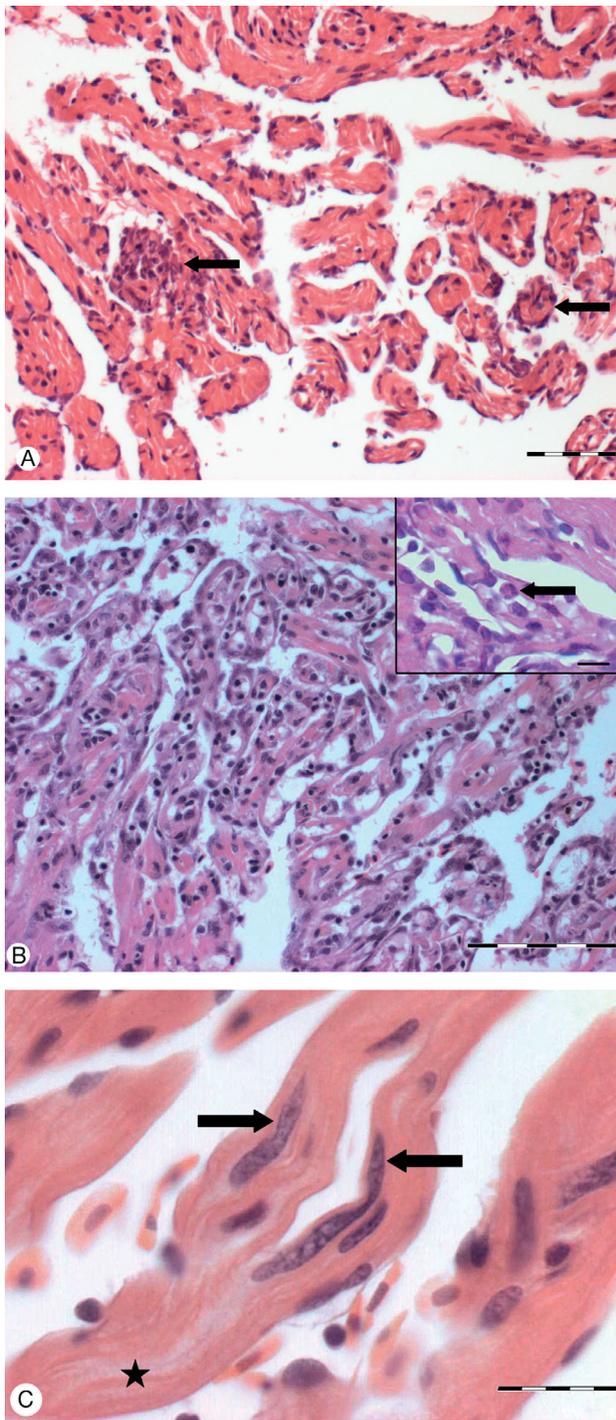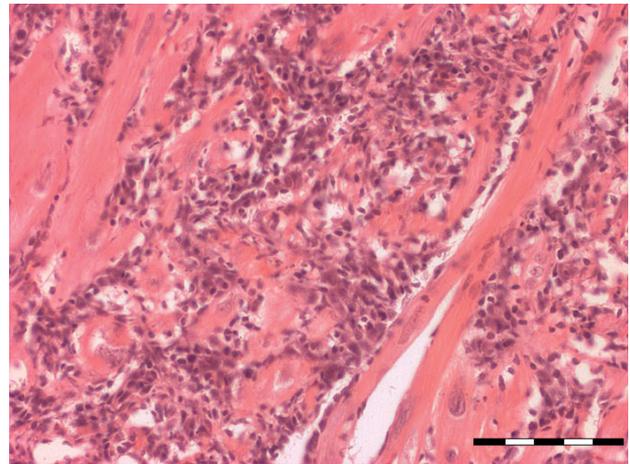
Fig. 2. *Salmo salar.* Cardiomyopathy syndrome in Atlantic salmon at 110 d post-injection. Norwegian group: severe myocardial infiltration. Haematoxylin and eosin staining; scale bar = 100 µm

Fig. 1. *Salmo salar.* Cardiomyopathy syndrome in Atlantic salmon at 52 d post-injection. (A) Scottish group: focal, mild thickening of the endocardium in the atrium (arrows). (B) Norwegian group: endocardial proliferation and sub-endocardial infiltration in the ventricle spongiosum. Occasional neutrophils were observed (see inset). (C) Both groups: slight loss of myofibre striation (★) and scattered hypertrophic, pleomorphic nuclei (arrows). All panels haematoxylin and eosin staining; scale bars = 100 µm (A,B) and 20 µm (B inset, C)

giosum, often adjacent to areas of massive infiltration, degeneration and necrosis of the myocardium (Fig. 3A). Some fish however showed evidence of early regeneration and fibrosis within the atrium (Fig. 3B), with an overall reduction in the inflammatory response. The Scottish group at 143 dpi showed 57% of the sampled fish had moderate to severe lesions consistent with CMS (Fig. 3C), and, at 150 dpi (final sampling for the Scottish group), 75% of the remaining fish were diagnosed with widespread characteristic CMS lesions involving both the atrium and ventricle (Fig. 4). However, fish were still displaying moderate to severe infiltration and muscle degeneration, involving both the atrium and ventricle, in comparison to the 143 dpi terminal sampling of the Norwegian group. Focal to diffuse epicarditis ranging from mild to moderate was recorded in both injected groups throughout the experiment, and, occasionally, although mild, was also observed in control fish. Thrombi in the heart chambers were infrequent, and multinucleated giant cells, melanin accumulation, or melanisation as previously described by Ferguson et al. (2005) were not observed during this experiment.

The control fish did not show lesions consistent with any of those reported for CMS. Additional observations in other tissues from the homogenate-injected fish included mild to moderate congestion within the ellipsoids in some spleen sections and an increase in haemosiderin from the CMS groups at 143 dpi. At the same time, a mild focal but spreading necrosis in the hepatic parenchyma was also noted.

Overall, both groups developed CMS lesions, though on different time scales. At the end of the experiment, heart-specific CMS lesions were recorded in 57 and
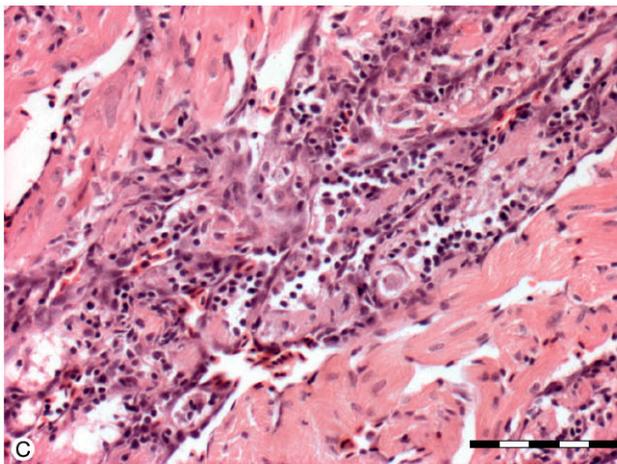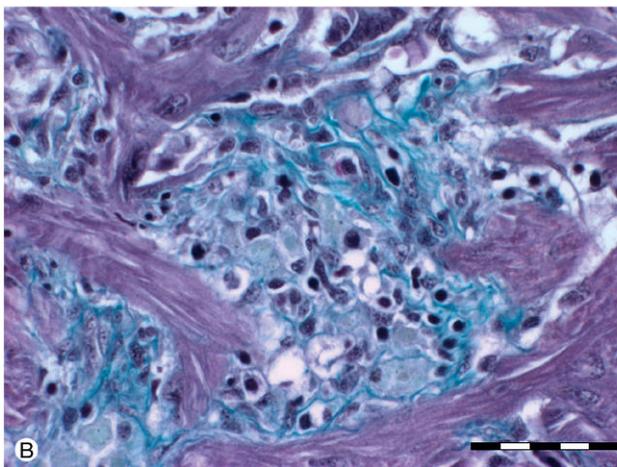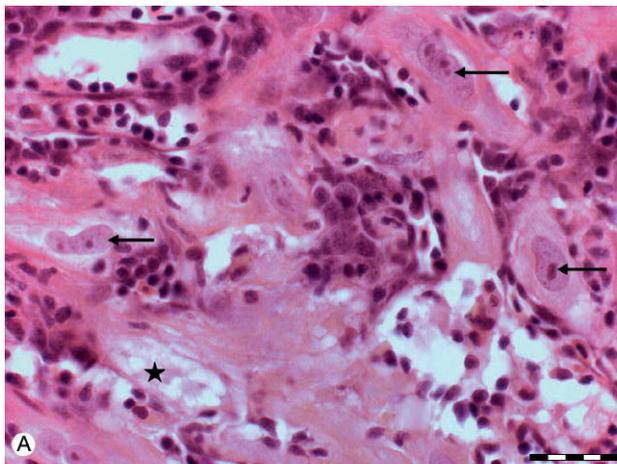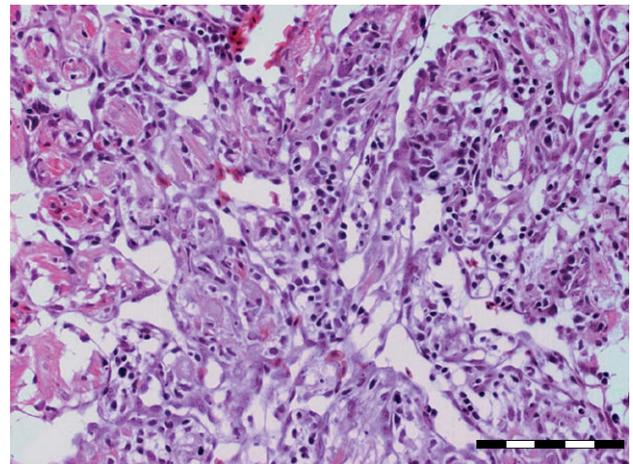
Fig. 4. *Salmo salar.* Cardiomyopathy syndrome in Atlantic salmon at 150 d post-injection. Scottish group: severe, widespread, myocardial infiltration and degeneration involving the ventricle. Haematoxylin and eosin staining; scale bar = 100 µm

60% of the sampled Scottish and Norwegian infected groups, respectively. CMS lesions in the Norwegian group were evident prior to those in the Scottish fish and showed lesions consistent with CMS by 52 dpi. Severe CMS was recorded at 110 dpi, and regenerative processes were evident by 143 dpi. CMS in the Scottish group showed mild changes by 52 dpi, but did not reach maximum severity until 150 dpi.

DISCUSSION

Cardiomyopathies are heart conditions characterised by dysfunction of the myocardium. CMS is one of several diseases affecting the cardiac muscle of Atlantic salmon *Salmo salar* (Ferguson et al. 1990, Poppe & Seierstad 2003, Poppe et al. 2007). In the current study, the condition was fully reproduced by injection of Scottish and Norwegian homogenates; hence, the frozen tissues proved to be infective, indicating that the causative agent is present in both mildly or severely affected fish and is able to survive at least 1 freeze/thaw cycle at −80°C. The CMS-associated lesions described in the current study parallel those published for natural infections of wild and farmed salmon (Ferguson et al. 1990, Poppe & Seierstad 2003), including endocarditis, infiltration, muscle degeneration, necrosis and finally fibrosis. These findings were supported by the removal of bias during the initial reading of the slides, the absence of similar lesions in the control groups and the fact that CMS lesions were reproduced from both the Scottish and Norwegian homogenates. Furthermore, all of the fish tested prior to the experiment were free of specific

Fig. 3. *Salmo salar.* Cardiomyopathy syndrome in Atlantic salmon at 143 d post-injection. (A) Norwegian group: hypertrophic, pleomorphic centrally located nuclei (arrows) within the spongiosum myocardium adjacent to areas of myocardium infiltration, degeneration and necrosis (★). Haematoxylin and eosin (H&E) staining. (B) Norwegian group: evidence of reparative processes (fibrosis) in the ventricle. Masson's trichrome. (C) Scottish group: focal but severe characteristic lesions developing in the ventricle. H&E staining; scale bars = 50 µm (A, B) and 100 µm (C)

pathogens and the testing of the injected tissue homogenate was free of known salmonid viral agents. It is noteworthy that very early changes described in the atrium of challenged fish, including diffuse proliferation and hypertrophy of individual endocardial cells, were not previously attributed directly to CMS. We now believe these to be indicative of early morphological changes due to the syndrome.

The progression of the observed lesions in both infected groups demonstrated that lesions initially occur in the atrium, with subsequent progression to the ventricle. Furthermore, the fish injected with the Norwegian homogenate developed lesions far earlier than the Scottish group. Lesions in the atrium developed at a similar rate in both groups, though obviously displaced in time; hence, throughout the study, at the same point in time lesions were always more widespread in the Norwegian group. However, towards the end of the experiment, heart lesions in both groups reached equivalent levels of severity. It was concluded that the difference in the onset of the disease may reflect different levels of 'infectious agent' present in the original tissue homogenate; if this is true, it would indicate that severely affected fish harbour (and eventually shed) higher amounts of the infectious agent compared to fish with early lesions. However, this result could also reflect different strains of an infectious agent, with different levels of pathogenicity. The specific cause for the destruction of the cardiomyocytes in CMS-infected fish is, at present, unknown, but may be associated with the severe and extensive inflammatory response affecting the myo- and endocardium, which can occur through virus-mediated myocarditis, leading to lysis. Such a progression, for example, has been reported in association with Coxsackievirus B in man, resulting in myocyte damage and cardiovascular disease (MacArthur et al. 1984, Horwitz et al. 2000).

Hypertrophic and pleomorphic cardiac myocyte nuclei were recorded in the ventricle spongiosum and atrium, and are believed to represent a compensatory reaction to a failing heart, as suggested by Ferguson et al. (1990). The long, wavy and elongated nuclei seen in some fish from both injected groups resembled Anitschkow myocytes, which are believed to be pathognomonic of acute rheumatic carditis in man (Guilherme et al. 2004). Ferguson et al. (2005) suggested that the Anitschkow-like nuclei were linked with the myocardial repair or regeneration of the heart and skeletal muscle inflammation-like condition in Atlantic salmon. In the present study, however, this cell type was observed throughout the experiment, but primarily during the early heart lesions associated with CMS. The pathological changes in the heart muscle of CMS-infected fish could result in an increase in cardiac workload, inducing mechanical stress, as reported following continued damage to the stressed heart and haemodynamic overload in humans, which may well induce the critical transition from compensatory hypertrophy to decompensated heart failure (Diwan & Dorn 2007). Only a few thrombi were noted in the heart chamber in the experimental fish, which Ferguson et al. (1990) suggested was common in severely affected salmon, and we expect that, with longer periods of experimental observation, an increase in this lesion type would be found.

CMS-associated mortality is associated with presumptive heart failure, which suggests that the high metabolic activity and increased oxygen extraction required by the subendocardium would be significant for large, fast-growing fish. However, small fish generally consume more oxygen than larger fish, on a unit weight basis (Forsberg 1994); hence, the apparent absence of CMS in post-smolts is unlikely to be explained on the basis of oxygen demand by heart tissue alone. However, as a chronically developing disease, it is not known whether smolts might be infected but do not develop obvious lesions until they are grown, when they are no longer able to cope with the dysfunctional heart. Although there is increasing evidence that intensively reared fish significantly alter some aspects of their cardiac anatomy (Santer et al. 1983, Farrell 2002, Poppe et al. 2007) and physiology (McDonald et al. 1998), the occurrence of CMS in both wild and farmed fish indicates that aquaculture constraints are not the primary factors causing this syndrome.

Fish from the injected groups showed a mild to moderate multifocal hepatic necrosis consistent with reports for natural outbreaks (Grotmol et al. 1997, Rodger & Turnbull 2000). This might be attributed to a circulatory disturbance or reduced cardiac output, leading to hypoxia, and might be consistent with the presence of ascites recorded in natural infections. Anaemia is not reported as a specific feature of CMS, but the hepatocyte necrosis noted in the present study and the presence of haemosiderin within the spleen parenchyma suggest that there is some level of destruction of erythrocytes. An increase in fibrosis was associated with resolving focal lesions within the atrium and ventricle during cardiomyocyte regeneration and similar to that reported in the zebra fish *Danio rerio* following ventricular resection (Ross et al. 2002). In mammals, reparative fibrosis subsequent to myocardial cell necrosis is a well-described process (see Weber 1989), and acute myocarditis can lead to healing with fibrosis and cardiomyocyte hypotrophy (Billingham & Tazelaar 1986, Hasumi et al. 1986, Larsson et al. 1999).

In addition to CMS, since the mid-1980s, other significant heart-associated pathologies have been reported in farmed fish, including subendocardial fibro-

elastosis (Amin & Poppe 1989), haemorrhagic smolt syndrome (HSS) (Nylund et al. 2003), HSMI (Kongtorp et al. 2004a,b), myocardial necrosis (Poppe et al. 2007) and infections attributed to a SAV (McLoughlin & Graham 2007, Murphy et al. 2006). Of these, only CMS has been reported from wild salmonids (Poppe & Seierstad 2003). CMS, SAV and HSMI present overlapping histopathology, and this has led to discussion regarding these conditions as representing separate diseases with distinct aetiologies or new conditions or variants of existing diseases (Kongtorp et al. 2004a). From our present knowledge CMS, HSMI and HSS represent distinct syndromes, although interestingly Ferguson et al. (2005) reported presumptive HSMI from farmed salmon in Scotland, with heart lesions that were similar to those described for CMS, but also showing an increased inflammatory reaction and an increase in multinucleate 'nests' within the myocytes, which are not reported for CMS.

For a differential diagnosis, CMS and HSMI are characterised by mononuclear infiltration affecting the myocardium and endocardium (Ferguson et al. 1990, Kongtorp et al. 2004b), and HSMI and alphavirus infection both show severe myocarditis and myocardial necrosis, but in association with skeletal muscle involvement (McLoughlin et al. 2002, Kongtorp et al. 2004b). However, in CMS, the myocardial fibres are gradually replaced by inflammatory cells, as noted in the present study, and are only rarely recorded for HSMI (Kongtorp et al. 2004a,b). Data from those studies (op. cit.) and those published for CMS, HSMI and alphavirus infections (Rodger & Turnbull 2000, Ferguson et al. 2005, Kongtorp et al. 2004a,b, McLoughlin & Graham 2007) indicate that histological separation is possible for typical cases. However, the chronic nature of CMS, as reported in the present study, and HSMI (Kongtorp et al. 2006) would suggest that fish can experience concurrent or overlapping infections, so that early clinical signs and the cell types involved become important for diagnosis at a stage when not all the lesions characteristic of each disease are present.

Further work is required to establish the infectious agent for CMS. Poppe & Seierstad (2003) postulated a possible viral aetiology due to the occurrence of CMS in wild Atlantic salmon, while the occasional observations of intranuclear inclusion bodies were also postulated as indication of viral infection by Amin & Trasti (1988). However, the only published paper on a proposed viral aetiology of CMS was presented by Grotmol et al. (1997), in which the cardiac atrial and ventricular trabeculae were reported to display positive immunohistochemistry against a nodavirus, although a causal relationship between the viral particles and CMS was not established. The antibiotics present in the tissue culture medium used to homogenise the tis-

sues prior to injection in the current study would have been expected to inhibit bacteria during the estimated contact time of from 1 to 2 h, suggesting that a bacterium is not involved, as reported by Amin & Trasti (1988). Reports to date (Rodger & Turnbull 2000, Brun et al. 2003) note that CMS occurs in fish that are entering or are in their second year of seawater production, and Saunders et al. (1992) noted coronary heart lesions increased with rapid growth at sea. However, as noted above, cardiomyopathy can result from numerous causes, including congenital defects, acute or chronic infections, connective tissue disorders, deformities, nutritional deficiencies and autoimmunity (Amin & Poppe 1989, Poppe & Taksdal 2000, Nylund et al. 2003, Poppe et al. 2003, 2007). Furthermore, there may well be other influencing factors; for example, Flysand et al. (1992) noted that salmon accumulate adrenaline in atrial tissue during periods of stress, and, in high amounts, this is reported to be cardiotoxic (Carlsten et al. 1983).

The current study has established that kidney/heart tissues of natural CMS outbreaks contained infective material, which reproduced CMS lesions in naïve fish. Moreover, similar results to those reported here have been recently obtained in a study using smaller fish in Norway (Fritsvold et al. 2009). The injection of homogenates from Scotland and Norway groups provided clear evidence that a transmissible agent was present in both inoculated homogenates, those taken from fish with mild (i.e. Scotland) as well as severe (i.e. Norway) CMS lesions. Furthermore, the results also confirm the chronic development of CMS. This is important to our understanding of the disease, and to the development of appropriate management practices to hinder its transmission.

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