Rapport

Cardiomyopathy syndrome (CMS): a literature review

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Summary

CMS is a severe cardiac disease affecting farmed Atlantic salmon during the second year in seawater. Cardiac lesions similar to CMS have also been observed in wild Atlantic salmon. From 1998 to 2002 there was an increase in the number of diagnosed cases in farmed fish. Outbreaks occur along most of the coast, but are most frequent in Mid-Norway. Disease outbreaks usually occur during the spring and autumn months. Fish in affected cages may die suddenly without prior signs of disease, or show symptoms such as abnormal swimming behaviour and anorexia. Accumulated morality may be very high, causing significant economic losses. There is at present no treatment for CMS, but minimizing stress appears to reduce mortality during outbreaks. CMS is diagnosed on the basis of histopathology, and is characterized by severe inflammation and degeneration of spongy myocardium in the atrium and ventricle. Multifocal liver necrosis is also a common finding, but is considered to be a secondary effect of circulatory disturbances.

The cause of CMS is unknown, and very little research has been done to determine the aetiology. A hypothesis suggesting viral origin has been the most debated. Both intracellular inclusions and virus-like particles have been observed in fish from affected farms, but the reports are not consistent. In one study, cardiac tissue from fish with CMS displayed positive immunohistochemistry with an antiserum against nodavirus. CMS has also been transmitted from diseased to healthy fish by intraperitoneal injection of cardiac, renal and hepatic tissue filtered through a 220 nm filter. These results have not been published scientifically, and the causal relationship between the observed viral particles and CMS has not been firmly established. There are almost no reports on investigations of the presence of bacteria, parasites or other factors that may potentially be associated with CMS. Nutrition, other environmental factors or autoimmunity have also been suggested as possible causes of CMS, but no studies have yet been published on these topics. In conclusion, there is a great need for research on the aetiology, pathogenesis and risk factors for CMS, in order to find measures to prevent or reduce disease occurrence.

Introduction

Cardiomyopathy syndrome (CMS) is a severe cardiac disease. It mainly affects farmed Atlantic salmon during the late seawater phase; typically 14-18 months post sea transfer. CMS was first diagnosed in Norway in 1985, and has since been diagnosed in Scotland and the Faeroe Islands (1-3). Suspected cases have also been reported from Canada (4). Histopathologically, CMS is characterized by inflammation and degeneration of spongy myocardium in the atrium and ventricle (5). Pathological investigations indicate that this is a chronic disease developing over a period of several months. Inflammation and necrosis of myocardial cells may progress to such a state that the wall of the atrium or sinus venosus weakens or breaks. This causes severe circulatory disturbances or death due to haemorrhage or haemopericardium. Affected fish may show symptoms of lethargy and abnormal swimming behaviour prior to death, but sudden deaths may also occur (6). Mortality is usually moderate, but may be elevated for long periods. As CMS generally affects large fish, the economic losses may be extensive (7-9). Very little research has been published on CMS the last few years, in spite of its widespread occurrence and economic importance in Norwegian salmon farming. The cause of CMS is not yet established, but some reports have suggested a viral aetiology (10-12). At present, there is no treatment against CMS (6). The purpose of this review is to investigate the current knowledge on occurrence, aetiology and prevention of CMS. Literature included is scientifically published articles that are easily accessible, as well as Norwegian reports and non-scientifically published articles.

Clinical features, pathology and differential diagnoses

During outbreaks of CMS one may observe two different clinical patterns; one that entails sudden or peracute deaths of apparently healthy fish (5;6), and another featuring moderately elevated mortality over a period of many months (1;7). In outbreaks with prolonged duration, fish may show abnormal swimming behaviour and anorexia prior to death. At autopsy, fish with CMS typically have skin haemorrhages, raised scales and exophthalmos. Ascites is a common finding, and fibrinous casts over the liver capsule have also been observed. The atrium and sinus venosus are usually enlarged, and blood or blood clots often fill the pericardial cavity (13). In some fish, the atrial wall may be ruptured, but this may be difficult to see (3). Ultrasound examination and echocardiography of tranquilized fish show that the atrium is dilated and the cardiac ventricle is compressed in terminal stages of CMS. Pericardial fluid may also be observed by these techniques, as well as the presence of pericardial clots (14).

Histopathological findings include severe inflammation, degeneration and necrosis of spongy myocardium of the atrium and ventricle. Inflammatory infiltrates mainly consist of mononuclear cells, possibly lymphocytes and macrophages. Inflammation may also be extensive in the epi- and endocardium. The epicardium may contain a range of inflammatory cells, including lymphocytes, macrophages, plasma cells, eosinophilic granular cells and neutrophils. Compact myocardium of the ventricle is

usually not affected, however epicardial infiltrates may extend into the compact layer along vessels (5).

Mild, and possibly early lesions are characterized by a multifocal pattern of affected myocardium, clearly separated by normal tissue. Vacuolation is common, especially in the subendocardial space. Multifocal lesions gradually develop into diffuse and extensive lesions. Severely affected fish display extensive inflammation and myocardial damage, leaving almost no intact cells in the atrium and spongy layer of the ventricle. Atrial thrombosis is often seen. Affected myocardial cells show loss of striation, eosinophilia and lysis of contractile fibres. Hypertrophic nuclei and trabecular fibrosis are also observed, and are thought to represent compensatory and regenerative processes. Liver lesions include multifocal to anastomosing necrosis of hepatocytes and fibrinous coating of the capsule (5). Other organs may show changes such as congestion of the spleen and gills, possibly due to circulatory failure (3).

Due to the lack of knowledge on aetiological or other possible characteristic features of the disease, the CMS diagnosis is presently based on observations of clinical features, autopsy and histopathology. Several diseases may, however, cause lesions in the heart, and there is a great need for further development of tools that may support the diagnosis. The most obvious differential diagnoses to CMS are heart and skeletal muscle inflammation (HSMI) and pancreas disease (PD), which both cause severe myocarditis and myocardial necrosis (15;16). PD is caused by salmon pancreas disease virus (SPDV), whereas the aetiology of HSMI has not yet been firmly established (17;18). HSMI and PD affect all layers of the heart, as well as red skeletal muscle, and are in typical cases clearly distinguishable from CMS, due to histopathological differences (16). PD also causes necrosis and loss of exocrine pancreatic tissue. Multifocal liver necrosis has been reported from HSMI cases, but not from PD cases. The liver necrosis seen in CMS may also resemble the lesions occurring in infectious salmon anaemia (ISA) (6). Atypical disease outbreaks or concurrent outbreaks of several diseases may represent diagnostic challenges for all these diseases.

Occurrence in Norway

Outbreaks of CMS occur along most of the Norwegian coast, with a hot spot in Mid-Norway, namely in the counties Møre og Romsdal, Sør- and Nord-Trøndelag. CMS is, however, rarely reported from the northernmost counties, Troms and Finnmark. In a prospective study lasting from September 2000 and 12 months onward, fish on 14.3 % of the farms in Sør-Trøndelag were diagnosed with CMS. In comparison, only 2.2 % of the farms in Troms were affected by the disease during the same period (8). In this study, the number of outbreaks was also linked to age of the fish, or more accurately the recorded time of sea transfer. The results showed that there was a considerable variation across age groups. As CMS probably develops over a long period, and mortalities usually occur late in the production cycle, this result would be expected in a study conducted within a limited time frame.

From 1998 to 2002, there was an increase in the number of diagnosed CMS outbreaks, from 25 to 101 (7). Records from the National Veterinary Institute show

that the number of diagnosed cases has remained high during the recent years. Most outbreaks occur in Atlantic salmon farms, but cardiac lesions similar to those seen in CMS have also been observed in wild Atlantic salmon (19). CMS has been registered throughout the year, but the number of outbreaks is somewhat higher during the spring and autumn months. This may be due to more unstable environmental conditions during these periods. A clinical outbreak of CMS may last from one to six months or more (8).

Typically, fish are affected by CMS about 400 days post sea transfer (from 253 to 595 days) and weighs 2-3 kg. Accumulated mortality during an outbreak is estimated to be 6 % on average, which is significantly higher than in comparable fish groups on farms not affected by CMS (6). Østvik and Kjerstad (8) reported that in 82 % of the cases studied, only a few cages were affected by CMS during the outbreak. Still, when CMS is present on a farm, this disease alone may account for 80 % of the total loss. Observations indicate that mortality increases in association with stressful events (6).

Although few papers have been published on the occurrence of CMS outside Norway, the disease appears to be of concern, especially as an important differential diagnosis to PD (16;20). Observations of focal cardiomyopathy of uncertain aetiology recorded on routine examination of diseased Atlantic salmon in Scotland has also been related to CMS (2;21).

Aetiology

Myocarditis and myocardial necrosis are associated with several diseases in terrestrial species, and the causes are quite diverse. For instance, myocarditis is an important feature of canine parvovirus infection in puppies. Autoimmune responses induced by viral infections may be even more damaging for cardiac tissue than the virus itself, as is the case with coxsackievirus B3 infections in humans (22). Inflammation and necrosis of cardiac tissue may also be caused by infections with bacteria or parasites, e.g. *Erysipelothrix rhusiopathiae* in pigs or *Listeria monocytogenes* and *Toxoplasma gondii* in sheep (23). Toxic plants or chemicals such as coffeeweed and ionophores may change the transport of molecules across membranes, causing myocardial necrosis and subsequent inflammation (24). Nutritional deficiencies are other conditions that may be responsible for damage of cardiac tissue. Selenium-vitamin E deficiency is a common cause of diffuse cardiac necrosis in young animals of many species. Lack of other important substances in the diet, for instance potassium, copper and protein may also cause cardiac injuries (23).

The aetiology of CMS has not yet been established, and there has been very little research on the subject. A number of hypotheses have been put forward, however, mainly based on the comparative knowledge from other species. Possible microbial, nutritional, autoimmune and environmental causes will be considered in this review. The virus hypothesis is the most debated, and will therefore be discussed in detail.

Virus

A possible viral aetiology of CMS was suggested already in the first description of the disease. Amin and Trasti (3) observed intranuclear eosinophilic inclusion bodies in unaffected myocardial cells situated adjacent to degenerated cells; an indication of viral infection. The authors did not explore the possible causal implications further. Rodger and Turnbull (2) also reported eosinophilic inclusions, but these were located to the endocardium and were seen in only one of eight fish. Ferguson et al (5) did not observe inclusion bodies. Neither Rogder and Turnbull (2) nor Ferguson et al (5) found viral particles by transmission electron microscopy (TEM). Attempts to isolate virus from pools of heart, spleen and kidney by inoculation onto cell cultures also failed (2). Viral-like particles in cardiac tissue have, however, been described from other CMS outbreaks (10-12). The authors of these papers did not report observations of inclusions by histology, but virus-like particles were still found in the cytoplasm of epi-, myo- and endocardium by TEM. Grotmol et al (10), Nylund (11) and Watanabe et al (12) all appear to describe elements from the same study. Neither of the papers authored by Nylund (11) and Watanabe et al. (12) are published in journals with a system of peer-review, and both are unfortunately written in such a manner that it may be difficult for other scientists to evaluate the results or to repeat the study.

The only scientifically published paper on the proposed viral aetiology of CMS, is that of Grotmol et al. (10). In this work, 12 fish from four farms located in western Norway were studied. Sampled fish had been diagnosed on the basis of outbreak history, clinical signs and the presence of cardiac and hepatic lesions consistent with CMS. Grotmol et al (10) reported that areas of the cardiac atrium displayed positive immunohistochemistry by using an antiserum against a nodavirus. Subsequent findings of virus-like particles of 25 nm in these areas by TEM thus support the results from the immunohistochemical investigation. The image presented in the paper is, however, not of sufficient quality to determine whether the particles resemble nodavirus. Grotmol et al (10) do not describe any attempts to establish a causal relationship between the described viral particles and CMS. Isolation and further characterization of the virus have not yet been published. Later immunohistochemical studies with antisera against nodaviridae on cardiac tissue from fish with CMS have not given positive results (1;2).

Atrial myocardia of fish from seven farms were examined in the study reported by Watanabe et al (12). The number of samples is not stated, but fish had been collected over a number of years, and were of different geographical origin (from Troms to Hordaland). It is uncertain whether the material investigated came from individuals displaying histopathological changes consistent with CMS. The results are also somewhat unclear. Watanabe et al (12) first state that several viral particles were found, but that the particles discovered were few and not consistently present in the studied material. The authors conclude that there are no indications that these particles represent causal agents for CMS. Later in the same article, Watanabe et al describe the discovery of a large number of icosahedral viral particles measuring 39.8 nm ± 1.5 in fish with CMS. Nylund (11), referring to Watanabe et al (12), informs that most of the discovered particles measured about 40 nm, but that particles identical to nodavirus were found in one case. The author later refers to Grotmol et al (10) when stating that the hypothesis

of nodavirus infection was confirmed by immunohistochemistry. It therefore appears that Grotmol et al (10) have examined a sample of the same material that Watanabe et al (12) studied.

CMS has been transmitted to healthy experimental fish by injection of cardiac, renal and hepatic tissue from diseased fish with virus-like particles in the heart. The inoculation material had been passed through a 220 nm filter before intraperitoneal injection (11;12;25). Unfortunately, this study has not been published scientifically. Material from this challenge study would, however, constitute an excellent basis for an attempt to fulfil Koch's postulates on causal relationships between virus and disease. The authors should therefore be urged to isolate and characterize the virus, as well as to further explore the association with CMS. The findings of cardiac lesions similar to CMS in wild fish suggest that the disease may be transmissible, and thus gives some support to the virus hypothesis (19). Recent investigations on HSMI, however, show that late HSMI lesions may resemble early CMS-lesions (26). HSMI has shown to be transmissible by intraperitoneal injection of cardiac tissue from diseased fish and by cohabitation to injected fish (27). It is thus likely that HSMI may be transmissible from farmed to wild fish. As the lesions observed in the wild fish were in apparently healthy individuals, the diagnosis is therefore somewhat uncertain.

Several known viruses have been suggested as candidates for causing CMS. An epidemiological study of CMS showed a significant association between CMS and previous outbreaks of infectious pancreatic necrosis (IPN) in the same fish group. The risk of experiencing an outbreak of CMS on the farm was four (from 1.6 to10) times as high when IPN had been diagnosed in the early seawater phase (6;7). The prevalence of the causal virus (IPNV) is extremely high in Norwegian fish farms, and there is reason to believe that the virus is ubiquitous in Norway (28;29). Records from the National Veterinary Institute also show that outbreaks of IPN are frequent, with more than 170 outbreaks each year. As both CMS and IPN are common diseases in Norwegian salmon farms, an incidental association may occur. In spite of this precaution, the results are interesting, and should be pursued further.

Rodger and Richards (21) found a significant association between focal cardiomyopathy and intraerythrocytic inclusions resembling erythrocytic inclusion body syndrome (EIBS). The nature of this relationship is not clear, as the cardiac changes did not appear to have clinical significance. Myocarditis was also observed in association with EIBS by Graham et al (30), but this was not a consistent finding. EIBS is caused by a partly characterized virus, but it is uncertain whether the virus causes a specific disease, or if it merely facilitates the introduction of other pathogens (31-33). Investigations show that the EIBS virus is a common finding in farmed Atlantic salmon, and that it is not necessarily associated with disease (21;34;35).

A recent paper reported that Norwegian salmonid alphavirus (NSAV), a close relative to SPDV, has been cultured from fish diagnosed with CMS (36). It is somewhat unclear whether the isolates came from individual fish with CMS lesions, or if fish with PD were also present in the population. Concurrent disease with CMS and PD in the same individuals is also a plausible hypothesis, as CMS is such a

common disease in Norway. Virus was not consistently isolated from fish with CMS in the study. NSAV has also been isolated from other diseases, such as haemorrhagic smolt syndrome (37). The causal relationship between CMS and the isolated virus is yet to be determined.

Bacteria or parasites

No bacteria or parasites have so far been associated with CMS (6). However, hardly any of the published studies have actually included specific testing for these organisms. Nylund (11) mentions that bacteriological investigations have been negative, but does not describe study material and methods. Bacteria or parasites have, however, not been observed histologically (3;5). Amin and Trasti (3) used various staining techniques on their material, but found no indications of bacteria in tissues prepared with Gram's stain. Future studies of CMS should include investigations of bacteria and parasites that may potentially be part of the pathogenesis.

Nutrition

CMS lesions may resemble nutritional myopathies, but no association between CMS and nutritional factors have so far been established (25). Again, this may be due to a lack of research on the topic. Fish with CMS lesions have normal plasma vitamin E and selenium levels (4;5). In a case of CMS in Scotland, moribund fish had low plasma potassium levels, but these results were not significantly abnormal (2).

Autoimmunity

Viral infections may induce an autoimmune reaction causing myocarditis in humans (38;39). CMS has been diagnosed in fish with a history of other diseases of known or suspected viral aetiology, and it may occur secondarily to one or several of these diseases (1). Outbreaks of IPN in the early seawater phase have, for instance, been epidemiologically associated with later outbreaks of CMS (6;7). No studies have been performed to explore this hypothesis further.

Environmental factors

The lesions seen in CMS mainly occur in spongy myocardium (5). This tissue has no arterial blood supply, but is oxygenated by venous blood that is pumped through the heart (40). Environmental factors, such as oxygen levels and temperature, may therefore potentially affect the metabolism of myocardial cells in the spongy layer. Possible associations between CMS and unfavourable environmental conditions have not been investigated (6).

Preventive measures

Stress-related episodes during an outbreak of CMS appear to increase mortality (6). Losses due to CMS may therefore be reduced by minimizing stress during outbreaks. Potentially stressful events, such as grading, moving, delousing or other handling of fish should therefore be avoided when CMS is suspected or diagnosed on the farm.

The use of methods to prevent or minimize losses due to CMS probably varies between farms, and there are almost no reports on this topic. Moreover, the effectiveness of preventive measures has not been evaluated. A survey conducted among fish health services in Norway revealed that in 26.3 % of the disease outbreaks, production routines were not changed on the farm. Most farmers did take steps to reduce the problem, however, as 63.2 % changed their feeding routines. Forced slaughter is another damage-reducing action, which in this survey had been used in 42.1 % of the cases (6).

If CMS is caused by an infectious agent, general biosecurity measures would probably be effective to prevent disease outbreaks. The time of potential agent introduction into the population is, however, unclear. Furthermore, there are no reports on risk factors for the introduction of CMS to a farm. More research is therefore required in order to establish satisfactory preventive measures.

Topics for future research

There are many unresolved questions about the pathogenesis, risk factors, occurrence and possible prevention of CMS. Some of the issues that are important to address are listed below:

1. Increase the knowledge of disease development by a longitudinal study of selected farms from early seawater phase and through an outbreak of CMS. Investigations should include autopsies, histopathology, mortality patterns, microbiological investigations and disease history of contact fish.
2. Investigate a possible causal relationship between IPN and CMS. It may also be appropriate to study possible associations between CMS and other diseases.
3. Determine the cause(s) of CMS:
    a. Virus:
        i. Cell culture trials should be performed with material from early seawater phase, some weeks prior to the outbreak and from the outbreak itself.
        ii. Challenge studies are essential to establish the transmissibility of CMS. A pilot study is being run in Scotland (autumn 2005), as an attempt to develop a challenge model for CMS (1). It is important that this study is followed up by a larger challenge study.
        iii. Electron microscopical examinations of CMS-material may be a useful tool in order to discover viruses.
        iv. In the event of virus isolation and establishment of a causal relationship with CMS, the virus should be characterized further.
    b. Environmental factors: Epidemiological investigations of possible causal relationships between CMS and environmental factors, such as water exchange, oxygen levels, sea floor topography, water quality, fish density, etc.
    c. Post viral myocarditis/ autoimmunity: Investigate possible associations between autoimmunity and CMS.

4. Epidemiology: Map risk factors for CMS in order to establish measures to prevent or reduce losses due to CMS.
5. When the aetiology of CMS is established, more accurate diagnostic tools should be developed, in order to efficiently obtain an accurate CMS diagnosis.

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