MSD
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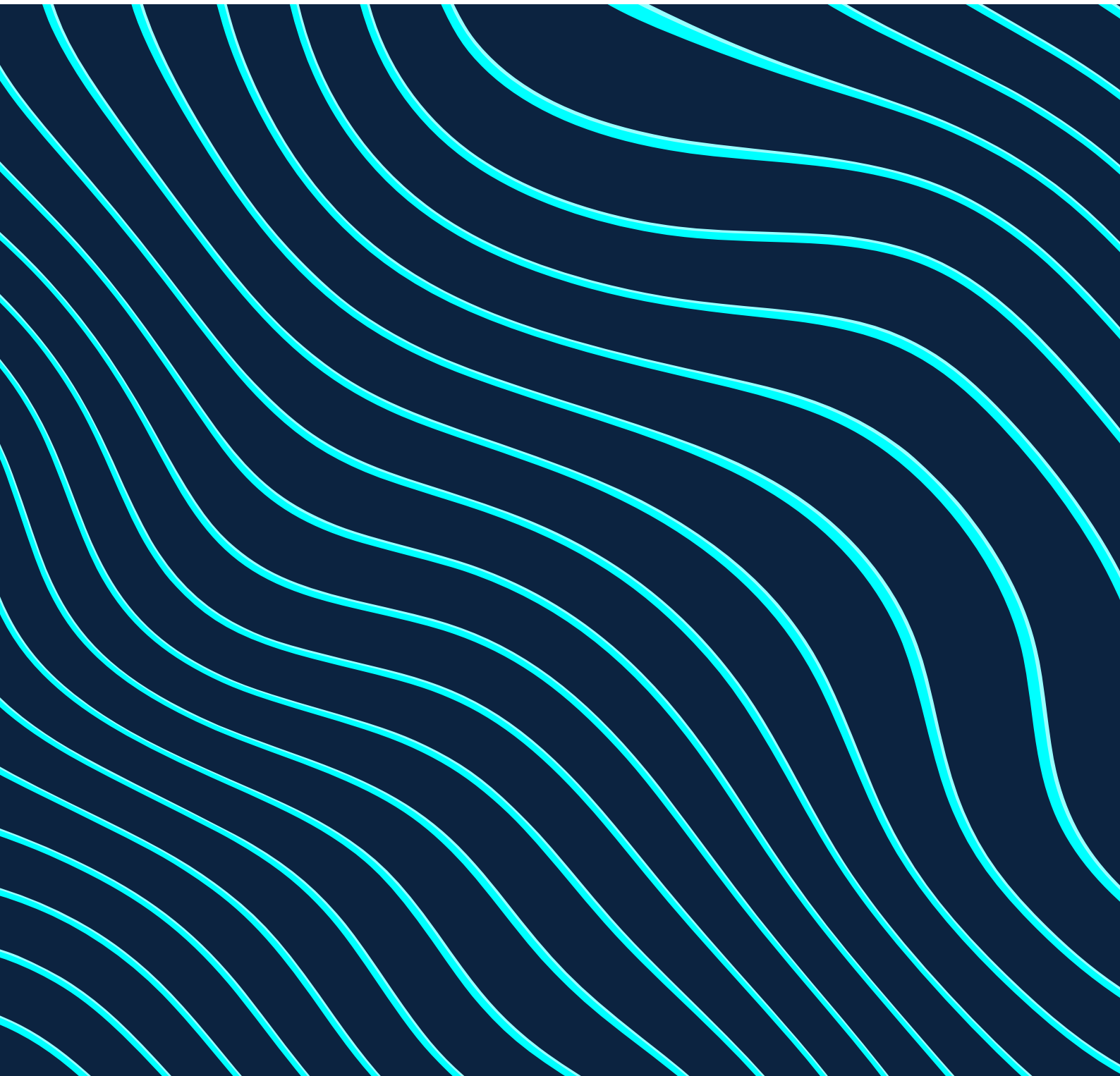
TECHNICAL REPORT

AN OVERVIEW OF EMERGING DISEASES IN THE SALMONID FARMING INDUSTRY



TECHNICAL REPORT 2025

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DEAR READER,

As an animal health company, present in the main salmonid production regions, MSD Animal Health has been in a privileged position to work closely with farmers. We know the health challenges and understand the true impact of emerging diseases globally.

The World Organization for Animal Health (WOAH) defines an emerging disease as “a disease, other than listed diseases, which has a significant impact on aquatic animal or public health resulting from a change of known pathogenic agent or its spread to a new geographic area or species; or a newly recognized or suspected pathogenic agent”*.

Over the years, we have experienced emerging diseases. Ones occurring frequently in the various aquaculture sectors. Some of these diseases have shown devastating effects locally, nationally, as well as internationally by rapidly spreading through transboundary trade and other activities.

In 2019, we launched the first edition of this report where we described six important diseases affecting farmed salmon globally. The document has been widely used by producers, scientists, and academia. New knowledge has been discovered and gathered over the years, and we are proudly releasing the current edition for use by the entire industry and its stakeholders. The report contains a wide review of six aquatic diseases that are having an impact today.

We have identified them as emerging for these reasons: there is new knowledge on agent dynamics, they are re-occurring, or they are well-known in one region and may become a threat in another.

Knowledge-sharing and awareness of salmonid production, fish health, and emerging diseases have become a key focus of the industry with dedicated resources. Being proactive in preventing the introduction of disease and having early detection systems in place to restrain an unwanted event is necessary. It will help everyone meet the ultimate goal of controlling emerging diseases.

This report aims to help veterinarians, fish health specialists, and the global salmon farming industry fulfill the important work of keeping salmon healthy globally in order to feed the world, ensuring a better future for all.

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Jose F Rodríguez
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*https://www.woah.org/fileadmin/Home/eng/Health_standards/aahc/current/glossaire.pdf
Last accessed on December 2024

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CARDIOMYOPATHY SYNDROME IN FARMED ATLANTIC SALMON

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INTRODUCTION

Cardiomyopathy syndrome (CMS) is a viral disease in farmed Atlantic salmon, causing severe inflammation in the spongious tissues of atrium and ventricles of the heart. CMS usually affects larger salmon in the late seawater phase including broodstock, causing moderate to severe mortality, reduced fish welfare, significant management-related challenges, and substantial economic losses (Sommerset, Wiik-Nielsen *et al*., 2023; Brun, Poppe *et al*., 2003). The disease was first described in mid-Norway in 1985 (Amin and Trasti, 1988). Today, CMS is widespread along the Norwegian coast, but still with a "hot spot" with regards to the number of cases in mid- and southern Norway (Sommerset, Wiik-Nielsen *et al*., 2023). Since first being described, CMS has been detected in the Faroe Islands, Scotland, and Ireland (Ferguson, Poppe *et al*., 1990; Rodger and Turnbull, 2000; Rodger, McCleary *et al*., 2014). A similar condition has been observed in Canada (Brocklebank and Raverty, 2002). CMS-like lesions have also been described in wild salmon, but without clinical disease (Poppe and Seierstad, 2003).

In the 1980s and 1990s, the cause of CMS was still unknown. Several hypotheses were proposed, including breeding-related causes affecting cardiac development, resulting in less robust hearts, effects of environmental factors like temperature, oxygen levels in seawater, and nutritional factors on the salmonid heart, geographical location of sites relative to sea currents and oxygen levels, and autoimmunity secondary to other infections. A viral etiology was suggested in the first published description (Amin and Trasti, 1988), and nodavirus-like particles were observed in cardiac tissue of CMS affected fish in the mid-1990s (Grotmol, Totland *et al*., 1997; Watanabe, Økland *et al*., 1995). Attempts to cultivate a possible viral agent from tissue samples of CMS diseased fish, however, failed, and have up until now not been published. Experimental transmissibility by intraperitoneal (i.p.) injection was demonstrated in 2009 by researchers at both the Marine Institute, Aberdeen, Scotland (Bruno and Noguera, 2009) and the Norwegian Veterinary Institute (Fritsvold, Kongtorp *et al*., 2009), both groups utilizing tissue homogenate from hearts and kidneys of CMS diseased fish. Transmissibility by cohabitation has also been demonstrated (Haugland, Mikalsen *et al*., 2011). Using next generation sequencing, piscine myocarditis virus (PMCV) was identified as the associated pathogen by two independent research groups in 2010 (Lovoll, Wiik-Nielsen *et al*., 2010) and 2011 (Haugland, Mikalsen *et al*., 2011), respectively.

PISCINE MYOCARDITIS VIRUS (PMCV)

Piscine myocarditis virus (PMCV) was first described as the etiological agent of CMS in 2010-2011 (Lovoll, Wiik-Nielsen *et al.*, 2010; Haugland, Mikalsen *et al.*, 2011), and this non-enveloped RNA virus has a single, non-segmented, double-stranded (ds) genome of 6688 bp in a spherical capsid.

Three open reading frames, ORF1, ORF2 and ORF3 have been identified on the positive sense (+) strand of the genome. Based on knowledge of genomic characteristics and organization of Totiviridae, and the homology of the ORF2 and encoded protein shown to Totiviridae, ORF1 is expected to encode the capsid protein and ORF2 a RNA-dependent polymerase (RdRp). A -1 ribosomal frameshift (Haugland, Mikalsen *et al.* 2011; Lovoll, Wiik-Nielsen *et al.*, 2010) enables these two ORFs to be translated to a capsid –RdRp fusion protein. Extensive research is focused on ORF3, as the function of its protein has not yet been described, but the main hypotheses are either a non-structural protein involved in infection/viral entry and/or release of cells, and/or a protein with immune modulating properties affecting the host (Sandlund, Mor *et al.*, 2021).

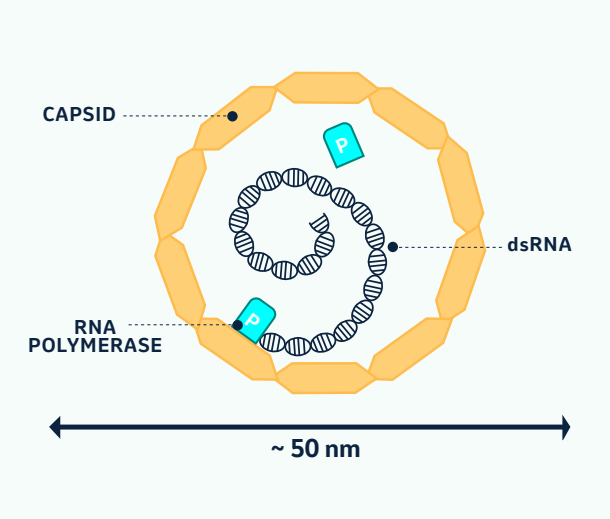


Figure 1: Simplified figure of a PMCV virion based on comparison to viruses of Totiviridae (Fritsvold, 2021).

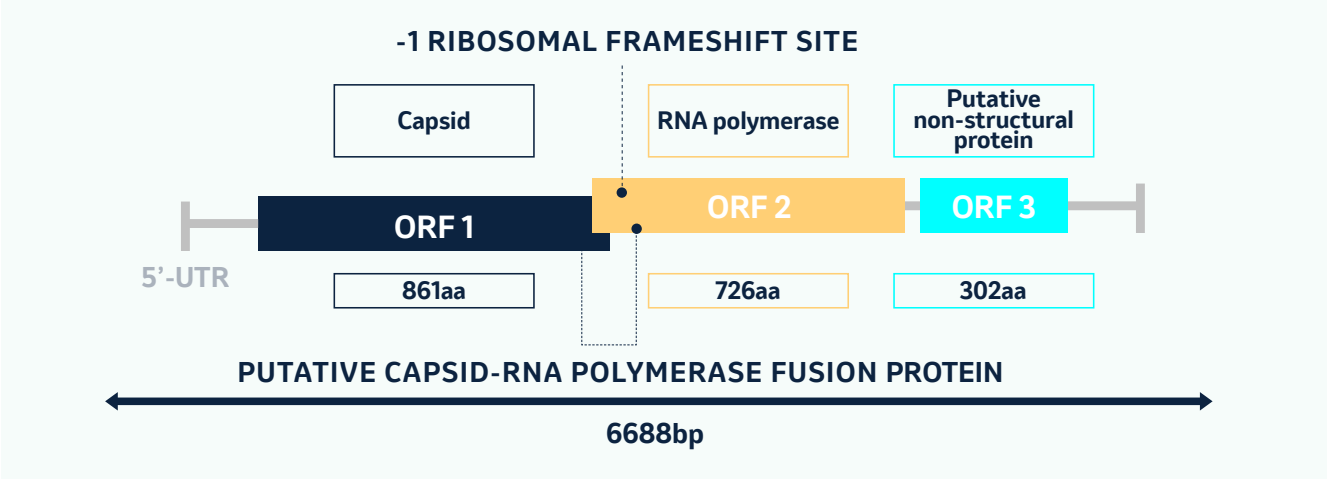


Figure 2: Overview of the proposed organization of the PMCV genome (adapted (Fritsvold, 2021) from A.B. Mikalsen (Sandlund, Mor *et al.*, 2021).

PMCV is not officially assigned to the Totiviridae family, but is described as a toti-like virus together with five other recently discovered toti-like viruses of fish: golden shiner toti-like virus 1 and 2 (GSTLV-1 and -2), blue gill toti-like virus 1 (BGTLV-1), common carp toti-like virus 1 (CCTLV-1) and *Cyclopterus lumpus* toti-like virus (CLuTLV), respectively (Sandlund, Mor *et al.*, 2021).

Typically, Totiviridae cause latent persistent infections in unicellular organisms like fungi and protozoan parasites (*i.e., Trichomonas sp., Leishmania sp. and Giardia lamblia*), where they are transmitted intracellularly to new host cells through cell division, cell fusion, or sporogenesis (Jansen, Jensen *et al.*, 2015), (Sandlund, Mor *et al.*, 2021). However, several toti-like viruses with a more advanced host-spectrum have been discovered in arthropods (crab, shrimp, fruit fly, mosquito, and ants) (Koyama, Urayama *et al.*, 2015; Poulos, Tang *et al.*, 2006; Wu, Luo *et al.*, 2010; Zhai, Attoui *et al.*, 2010; Shi, Lin *et al.*, 2016; Prasad, Shyam *et al.*, 2017), planarians (flatworms) (Burrows, Depierreux *et al.*, 2020) and, as PMCV, in some fish species. Toti-like viruses infecting these more advanced hosts show, or are expected to have, an extra-cellular transmission route, and have, compared to the Totiviridae, extra genomic material suggested to facilitate cell entry and infection of their multicellular hosts. PMCV is phylogenetically closely related to GSTLV-1, CCTLV-1 and CLuTLV in a distal branch of the Totiviridae. As this group are the only toti-like viruses with an extra ORF with Protein-coding sequences, it is suggested to define them as a separate genus, *Pistolvirus* (Piscine Toti-like virus) (Sandlund, Mor *et al.*, 2021).

According to genome analysis of isolated sequences of PMCV, the Norwegian PMCV strains constitute a genetically homogenous group, with a few geographical clusters. Interestingly, different isolates sampled at some farms have shown a relatively high variability on site (Wiik-Nielsen, Alarcon *et al.*, 2013). This was also observed in a smaller study of Irish isolates, and when comparing Irish and Norwegian strains (Rodger, McCleary *et al.*, 2014; Tighe, Carlsson *et al.*, 2019). Isolates of Norwegian wild Atlantic salmon were closely related to the isolates of Norwegian farmed Atlantic salmon (Garseth, Biering *et al.*, 2012; Garseth, Sindre *et al.*, 2016). Both Irish and Norwegian isolates had a higher amino acid diversity in ORF3 compared to ORF1 (capsid) (Rodger, McCleary *et al.*, 2014; Wiik-Nielsen, Alarcon *et al.*, 2013).

PMCV AS A PATHOGEN OF FARMED ATLANTIC SALMON

Typical cases of cardiomyopathy syndrome affect larger Atlantic salmon in good condition in their second year post-seawater transfer, often close to slaughter. However, experimental studies have demonstrated that also younger salmon can both be infected and develop CMS lesions (Fritsvold, Kongtorp *et al.*, 2009; Hansen, Haugland *et al.*, 2011; Hillestad and Moghadam, 2019; Martinez-Rubio, Morais *et al.*, 2012; Timmerhaus, Krasnov *et al.*, 2011; Timmerhaus, 2011). The incidence of CMS in atypical, smaller fish has increased in the last decade (Fritsvold, Mikalsen *et al.*, 2021; Sommerset, Wiik-Nielsen *et al.*, 2023). CMS can present with low to moderate increased mortality for a longer period of time, or as sudden outbreaks with high mortality, usually related to stressful handling events such as transport, delousing, or other treatments. As most fish with CMS are large and close to harvest, and very sensitive to stress, the economic impact is significant and aggravated by frequent delousing.

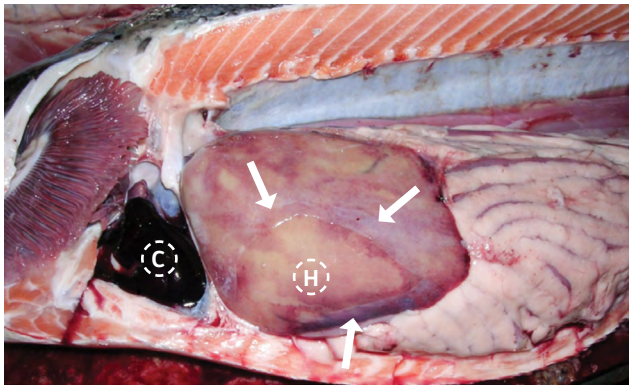


Figure 3: Cardiomyopathy syndrome (CMS). a) Necropsy findings in a CMS fish: Ruptured heart (C), almost completely covered by a blood clot filling the entire pericardial cavity, liver (H) with discoloration, multifocal hemorrhages, and fibrinous casts on the surface (arrows). I = pyloric caeca with adipose and pancreas tissue. The swim bladder can be seen as a grey-white area above the liver and adipose tissue. Photo by Brit Tørud, Norwegian Veterinary Institute (Sommerset, Walde *et al.*, 2022).

> ***Experimental studies have demonstrated that also younger salmon can both be infected and develop CMS lesions.***
>
> *(Fritsvold, Kongtorp et al., 2009; Hansen, Haugland et al., 2011; Hillestad and Moghadam, 2019; Martinez-Rubio, Morais et al., 2012; Timmerhaus, Krasnov et al., 2011; Timmerhaus, 2011.)*

Microscopically, CMS is characterized by a severe inflammation of the inner, spongious tissues of both atrium and ventricle of the heart, which in typical and severe cases lead to impaired cardiac function, causing secondary lesions of circulatory disturbances and congestion in other organs like the liver, kidneys, and spleen.

Initially, multifocal endo- and myocardial inflammation can be observed in the atrium and in these early phases, usually less severe, in the inner spongious layers of the ventricle. As the disease progresses, the multifocal pattern develops into a more confluent one, and finally, all spongious tissues of both atrium and ventricle are included in the inflammation. In most cases, the outer, compact layers of the cardiac ventricle are not affected, but in moderate to severe cases a cellular epicarditis can be present, and may progress as perivascular inflammation around coronary vessels of the compact layers of the ventricle (Bruno, Noguera *et al*. 2013; Ferguson, Bjerkas *et al*. 2006). Also, degenerative changes and necrosis occur in CMS, and in moderate to severe phases, the thrombosis is relatively commonly observed as blood clots in the cardiac lumen.

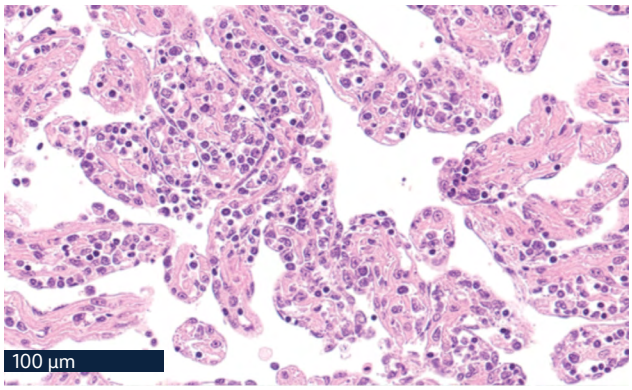


Figure 4: Severe atrial CMS lesions, infiltrated by various inflammatory cells, both subendocardial and in the myocardium (200x magnification, bar = 100 µm, H&E stain). Photo by Camilla Fritsvold, Norwegian Veterinary Institute (Fritsvold, 2021).

> ***Stressful events, like net cleaning, transports, stocking, and delousing are considered potential triggers of CMS outbreaks and mortality .***
>
> *(Sommerset, Wiik-Nielsen Et Al., 2023)*

The highly cellular inflammation of CMS is dominated by mononuclear cells, in addition to lymphocytes, macrophages, and occasional granulocytes (Bruno, Noguera *et al.*, 2013; Wiik-Nielsen, Lovoll *et al.*, 2012). From sparse subendocardial leukocyte infiltration in early stages, the disease progresses to also involve the spongious myocardium, and initially hypertrophic endocardial cells become hyperplastic as the severity and extent of the spongious inflammation increase. In severe cases of CMS, the intense leukocyte infiltration and endocardial lesions weaken the naturally thin walls of atrium and sinus venosus, initially causing heart failure and severe congestion, and eventually resulting in rupture, hemopericardium and sudden death (Ferguson, Poppe *et al.*, 1990; Ferguson, Bjerkas *et al.*, 2006).

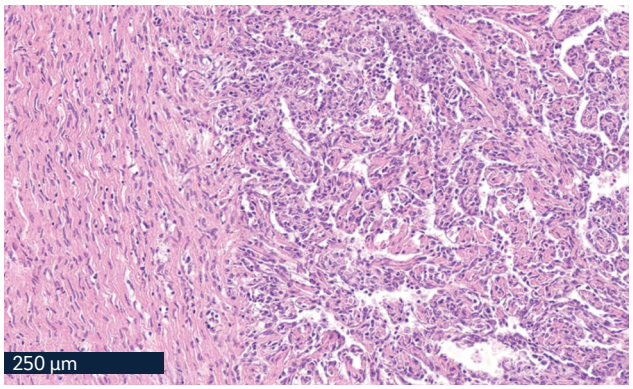


Figure 5: Severe to very severe CMS lesions of the ventricle. Ventricular compact layer to the left, with no or very sparse inflammation, ventricular spongious layer to the right, with intense infiltration of inflammatory cells (100x magnification, bar = 250 µm, H&E stain). Photo by Camilla Fritsvold, Norwegian Veterinary Institute.

In an experimental study with CMS, a decreased load of PMCV specific RNA was observed in all blood samples at 52 dpc (Fritsvold, Mikalsen *et al.*, 2022), possibly indicating an immune response limiting the infection in the organs targeted in the first phases of the PMCV infection. Some early phase immune responses have been described in previous CMS challenges, together with a possible trends of healing of cardiac lesions (Timmerhaus, Krasnov *et al.*, 2011), in contrast to a very long 42 weeks challenge (Fritsvold, Kongtorp *et al.*, 2009) where no indications of recovery were observed. Most experimental trials of CMS have a too short duration and/or too few individuals to evaluate if healing occurs, but there are still no publications of field cases of CMS where the fish group recovers. This is also contrary to what is seen in the most important differential diagnoses to CMS, pancreas disease (PD), and heart and skeletal muscle inflammation (HSMI), where the surviving fish seems to be able to recover and heal both cardiac and, in the case of PD and HSMI, skeletal muscle lesions with time.

Salmonid hearts have an impressive extra functional capacity, and sometimes CMS affected fish with severe cardiac inflammatory lesions can present without, or with only minor, clinical signs. The fish keep their appetite until they suddenly die, due to a stress-induced circulatory failure resulting in a ruptured atrium. However, even though some levels of CMS-lesions are not necessarily fatal *per se*, they do reduce the cardiovascular capacity and make affected fish more susceptible to stress or physical strain. In this perspective, CMS represents an animal welfare problem, especially considering the increasing handling of the fish due to intensive mechanical salmon lice treatments. Stressful events, like net cleaning, transports, stocking, and delousing are considered potential triggers of CMS outbreaks and mortality (Sommerset, Wiik-Nielsen *et al.*, 2023).

Concurrent health problems such as impaired gill health may potentially have an additional negative impact on the outcome for CMS-fish after stressful events.

DIAGNOSTICS

CMS is not a notifiable or listed fish disease by the World Organization for Animal Health (WOAH), the EU, or the Norwegian Food Safety Authority. Hence, no officially approved diagnostic criteria are available.

Today, CMS is primarily a histopathological diagnosis, based on a histopathological evaluation of the cardiac atrium and ventricle and identification of typical pathological lesions. To eliminate other or concurrent diseases as the cause of the cardiac lesions, a selection of other organs, i.e., gill, pyloric ceca with pancreatic tissue, mid-kidney, spleen, and skin/skeletal muscle, should also be included in the histopathological evaluation. Real-time RT-PCR to detect PMCV-specific RNA in heart and kidney samples is in common use in screening of fish groups without clinical signs of CMS, and the use of real-time RT-PCR in support of histopathological evaluations is increasing in CMS diagnostics (Sommerset, Wiik-Nielsen *et al*., 2023). The heart is the target organ of CMS with the most specific pathological lesions and is the organ of choice for sampling in the active phases of PMCV infection, when typical CMS cardiac pathology and disease is present (Fritsvold, Mikalsen *et al*., 2021; Fritsvold, Mikalsen *et al*., 2022; Sommerset, Wiik-Nielsen *et al*., 2023; Timmerhaus, Krasnov *et al*., 2011). The heart is also the organ presenting the highest load of PMCV-specific RNA at that phase, when Ct-values below 10 are not unusual. However, detected RNA may not all represent infective or complete virions. In early phases of PMCV infection, before cardiac lesions manifest, results indicate that samples of kidney should be included to increase the sensitivity of virus detection by PCR (Fritsvold, Mikalsen *et al*., 2022).

Successful attempts to propagate PMCV in cell culture for more than a few passages have not yet been published, indicating that standard cell cultures available for fish viruses are not optimal for PMCV cultivation. A suitable cell culture would make it possible to evaluate the biophysical properties of the virus, namely resistance to disinfectants and to evaluate if PMCV-detected RNA derives from infective viral particles or not.

An immunohistochemistry (IHC) method with polyclonal antibodies directed towards a truncated part of the ORF3-expressed protein has been established at the Norwegian Veterinary Institute (Fritsvold, Mikalsen *et al*., 2021), but the method is not very robust, and is not recommended for diagnostic use. However, a much more robust and highly specific *in situ* hybridization method, RNAscope® (Wang, Flanagan *et al*., 2012), is available and has been used successfully in research (Fritsvold, Mikalsen *et al*., 2022) and has the potential to become a very useful tool both for diagnostic and research purposes. This method uses sets of PMCV target probes consisting of 18-25 bases, linked to a 14-base unique "tail" sequence. The "tail" sequences of each probe pair join and form a 28-base site for hybridization when a probe binds immediately next to the other probe of the pair, hence only target-specific signals are amplified.

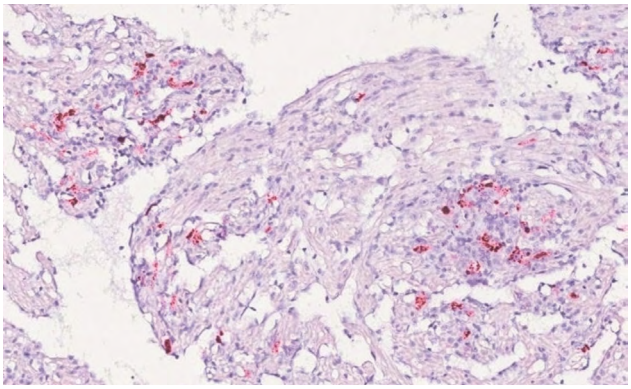


Figure 6: Detection of PMCV (ORF-1) by RNAscope® *in situ* hybridization in a histological section of the atrium of an Atlantic salmon with CMS (experimental infection). PMCV-specific RNA is marked with a dark red staining. Conventional light microscopy, 200x. Photo by Camilla Fritsvold, Norwegian Veterinary Institute (Sommerset, Walde *et al*., 2022).

DIFFERENTIAL DIAGNOSES

Indications of a viremia (10 and 20 days post challenge, dpc) preceding the first cardiac lesions in a CMS challenge trial (Fritsvold, Mikalsen *et al*., 2022), makes non-lethal sampling of blood a promising method to detect PMCV at a very early stage in field cases. The same study shows that samples of mucus can be used for detection of PMCV by PCR, but mucus samples did not reach a similar detection level as plasma samples before the late phases of CMS.

Serum proteins as biomarkers to indicate cardiac disease have been in common use in human medicine for decades, and are also used for some animal species, but for salmon, only a limited number of biomarkers have been described (Costa, Del Pozo *et al*., 2021). Comparing the proteins expressed in samples from fish experiencing a CMS outbreak and clinically healthy fish, showed a different protein profile between the two fish groups. Similar proteins detected in sera from salmon with CMS are known to be associated with cardiac diseases in humans. Further clinical studies have to be performed to evaluate these proteins as biomarkers in Atlantic salmon, but the results are promising for identifying serum biomarkers as an early diagnostic tool for CMS and/or cardiac diseases in general.

Pancreas disease (PD) (Taksdal, Olsen *et al*., 2007; McLoughlin and Graham, 2007), heart and skeletal muscle inflammation (HSMI) (Kongtorp, Kjerstad *et al*., 2004) and, in some cases, infectious salmonid anemia (ISA) (Thorud and Djupvik, 1988; Evensen, Thorud *et al*., 1991) are the most important differential diagnoses of CMS. But, typical cardiac CMS lesions are in most cases easily distinguishable from typical cardiac lesions of PD or HSMI by histopathology. However, co-infections, both at individual and at site level, of two or more of these diseases has become a relatively common finding, and combinations of concurrent, early, sparse, or untypical cardiac lesions of these diseases can complicate the histopathology in diagnostic work.

Histopathological examination of a selected set of organs, in addition to the heart, is essential and also useful in these mixed or complicated cases, as the distribution of other lesions in other organs can help to differentiate between the diseases. Real-time PCR (for all three disease causing agents, or even better, in situ methods, like IHC (for PD, ISA and HSMI) or RNAscope® *in situ* hybridization (Wang, Flanagan *et al*., 2012) (i.e. CMS), can be of great value to determine the correct diagnosis in these cases.

RESERVOIRS AND TRANSMISSION

The only confirmed source of PMCV in the marine environment is the Atlantic salmon itself. PMCV has not been detected in a wide range of environmental samples of sediments, plankton, biofilm, and smaller organisms collected at a salmon farm with an ongoing CMS outbreak (Hellebø, Stene *et al*., 2014). In a survey of 32 marine fish species, a separate PMCV strain was detected in nine individuals of Atlantic Argentine *(Argentina silus)*. Although CMS-like cardiac lesions have been described in wild Atlantic salmon (Poppe and Seierstad, 2003), examined wild salmon spawners had a low prevalence of PMCV (0.25 %) (Garseth, Biering *et al*., 2012; Garseth, Sindre *et al*., 2016), indicating they are of minor importance as a reservoir of PMCV for the farmed salmon. In an Irish Atlantic salmon farm, PMCV has been reported in two cleaner fish species with unspecific cardiac pathology: corkwing wrasse *(Symphodus melops* L.*)* and ballan wrasse (*Labrus bergylta* (Ascanius)) (Scholz, Ruane *et al*., 2018), most likely infected by cohabitation with CMS-diseased Atlantic salmon at the site. In Norway, CMS has never been described in rainbow trout (*Oncho- rhynchus mykiss* (Walbaum)), despite being farmed alongside Atlantic salmon since before the first description of CMS in 1985.

Challenge experiments have demonstrated horizontal transmission of CMS between i.p. injected Atlantic salmon and cohabitants that developed typical CMS lesions in the heart (Haugland, Mikalsen *et al*., 2011). Detection of PMCV in young Atlantic salmon (Fritsvold, Mikalsen *et al*., 2021) and in hatcheries led to a discussion of a possible vertical transmission (Wiik-Nielsen, Ski *et al*., 2012; Bang Jensen, Nylund *et al*., 2019; Mikalsen, Lund *et al*., 2020), but this has so far not been proven and true vertical transmission does not seem to be of major importance in the transmission of PMCV (Mikalsen, Lund *et al*., 2020).

Route of entry of PMCV is not yet identified, but the highest viral load can be found in the heart, spleen, and kidneys in both early infection phases and when pathologic cardiac lesions peak (Hansen, Haugland *et al*., 2011; Timmerhaus, Krasnov *et al*., 2011; Fritsvold, Mikalsen *et al*., 2021).

Compared to other viruses with horizontal transmission, i.e., PD, the pattern of how CMS spreads within a fish farm seems to be more unpredictable, regarding both geographical direction (to which of the other pens), and speed of the spread. The initial PMCV infection in a cohort study of 12 sites from sea transfer to slaughter was estimated to be between 1 to 7 months after sea transfer, with a median time from first detection to a CMS outbreak (in 6 sites) of 6.5 months (Svendsen, Nylund *et al*., 2019). In another study, including all Norwegian salmon cohorts from 2004 to 2012, the median time from sea transfer to CMS disease was 16 months (Bang Jensen, Brun *et al*., 2013). In a case study where CMS was diagnosed in 1 of 6 pens, 6 months post sea transfer, none of the other pens were diagnosed with CMS until 49 weeks after the index pen and 10 weeks after slaughtering of this pen (Fritsvold, Mikalsen *et al*., 2021).

> ***In a farm with CMS diseased fish, the level of stress, handling and treatments should therefore be kept at a minimum, and this should be implemented in the management plans of the site.***

TREATMENT AND PROPHYLAXIS

There is no treatment for PMCV-infection. As CMS is a transmissible disease, the principal preventive measure is to block introduction of PMCV to aquaculture facilities and implementation of good biosecurity measures, including the "all-in-all-out" principle combined with fallowing. Introduction to a sea site by infected smolts is a possibility. Keeping the number of introductions and sources of origin of the fish low will reduce the risk of introducing PMCV to a farm (Jarp, Gjevre *et al*., 1995; Jarp and Karlsen, 1997).

To prevent spread of PMCV between seawater pens and sites, pre-slaughter of diseased fish or stamping-out of infected sites could be used. Use of seawater in hatcheries constitutes a risk of PMCV introduction. As PMCV is a naked virus and probably fairly robust, there is a likelihood for transmission through water and for spread of PMCV by personnel and fomites, especially if shared between sites. The strategic location of farms relative to seawater currents and well boat routes and an "adequate" distance between farms may also have a preventive effect. But, as the ability of PMCV to travel in seawater has not been studied, it is difficult to know which distances to recommend. To reduce and keep the overall infection pressure low, the most important measure is to prevent the spread of PMCV within and between farms.

Salmon with CMS have a reduced cardiovascular capacity, proportional with severity of the cardiac lesions, which make the fish less robust to stress and physical strain (Brun, Poppe *et al*., 2003; Sommerset, Wiik-Nielsen *et al*., 2023; Skrudland, Poppe *et al*., 2002). In a farm with CMS diseased fish, the level of stress, handling and treatments should therefore be kept at a minimum, and this should be implemented in the management plans of the site.

There is a significant inter-family variation in CMS morbidity and subsequently mortality. Breeding companies have included cardiovascular health and capacity in their breeding programs. QTL-selected disease-resistant strains, claiming less severe cardiac lesions, are available in Norway.

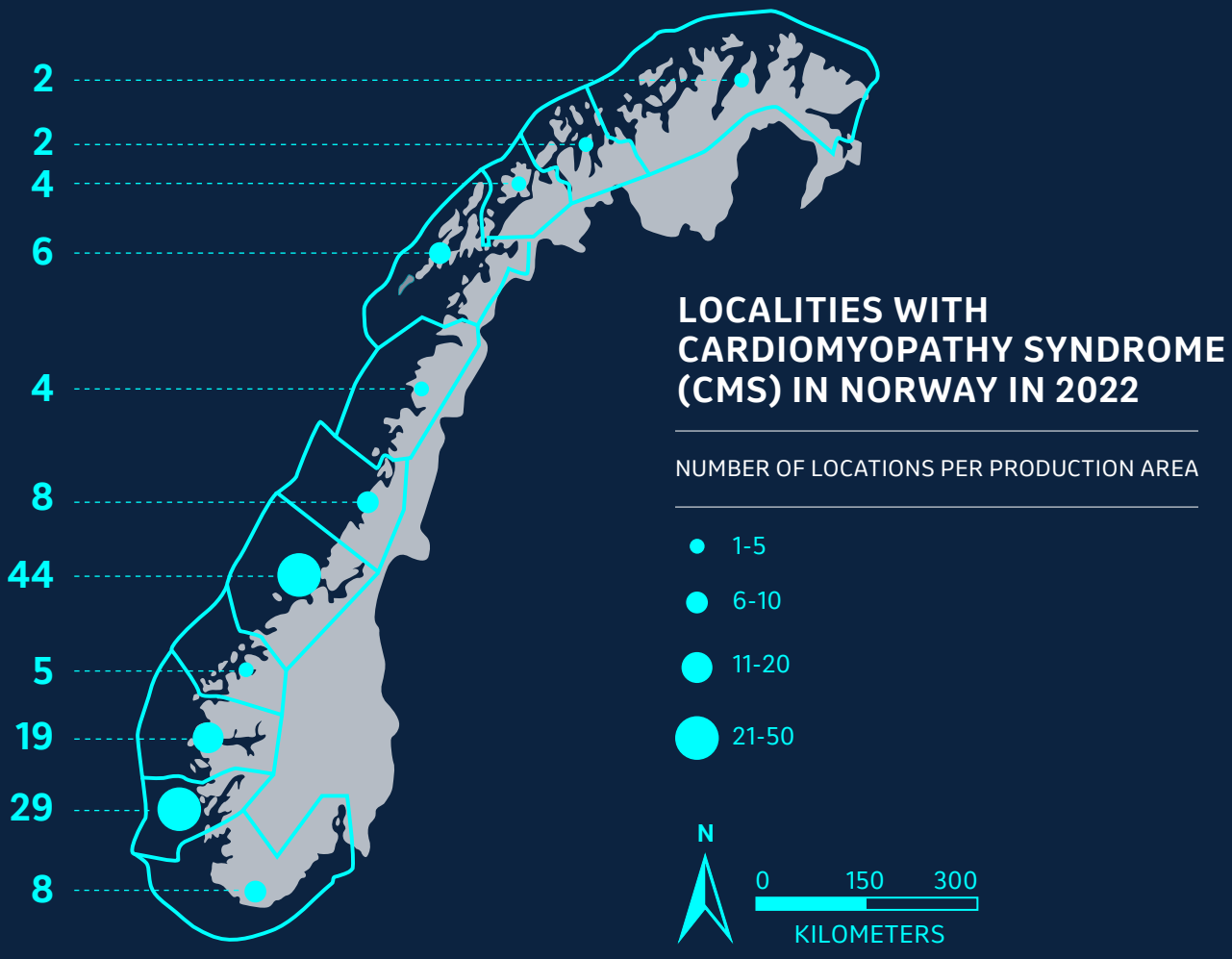
Screening for detection of PMCV by PCR in salmon without clinical lesions is relatively common in Norwegian Atlantic salmon farming. Early detection of PMCV infection can be useful when planning the management of the site to avoid triggering CMS mortality in later phases of the production, and perhaps also to limit the spread of the virus.

Functional feed (diets with extra health promoting qualities designed to facilitate feed uptake, promote healing of pathological lesions, limit or reduce inflammatory responses, increase stress tolerance and/or strengthening cardiovascular health in general) are available from several commercial feed companies. Most of these feeds are intended for use during outbreaks of CMS, PD and HSMI, but are also recommended to be used as early as possible in the course of disease and before expected risk periods.

There are no available vaccines against CMS, but the low genetic variation observed for the Norwegian PMCV isolates is promising for a potential efficient vaccine.

THE NORWEGIAN SITUATION

From 2006 to 2011, the annual number of Norwegian sites with a CMS diagnosis was about 70. An increase began around 2013, with 100 sites with CMS, reaching a maximum of 154 and 155 sites in 2020 and 2021 respectively (Sommerset, Wiik-Nielsen *et al*., 2023). In 2022, 131 of almost 1000 active Norwegian sites were registered with a CMS diagnosis, and the disease continues to represent a significant challenge for the Norwegian aquaculture industry. The increased number of sites with CMS for the last 6-7 years coincides with increasing salmon lice problems, introduction and increased use of non-medical delousing methods, in addition to an increasing number of delousing treatments per year per fish, and it is natural to assume some degree of association.



CMS IN SCOTLAND, IRELAND, AND THE FAROE ISLANDS

CMS contributes significantly to the economic losses experienced in Scotland (Soares, 2022), Ireland and the Faroe Island's salmon farming industries. As in Norway, this is not a notifiable disease and larger market size fish are typically affected. The first case of CMS in Scotland was reported in 2000 (Marine Scotland 2023). The most reliable source of incidence of CMS in Scotland is based on the industry's Code of Good Practice (CoGP). The number of CMS cases/sites totaled 35, 40 and 44 for 2019, 2020 and 2021 respectively. During this time, CMS represented about two-thirds of all the viral cardiac disease cases (CMS, PD and HSMI) reported while affecting approximately 20% of the industry's sea-cage sites. The CMS outbreaks lasted 2.5 months on average. These are reported to occur in all the production regions, suggesting that PMCV is enzootic throughout Scotland.

The first outbreak of CMS in Ireland occurred in 2012. It later appeared on one site in 2014. From 2016 to 2021, Ireland has experienced CMS outbreaks at between 4 and 6 sites annually out of 13 sites total in use. (Mitchell, 2023).

According to Debes Hammershaimb Christiansen, Head of the National Reference Laboratory for Fish Diseases, Faroese Food and Veterinary Authority, the first CMS diagnosis in the Faroe Islands occurred in the 1990s, prior to the ISA epizootic during the period before the entire industry was fallowed in 2005. PMCV and CMS were thereafter not confirmed until 2013. CMS was confirmed only sporadically until 2017. Since 2018, CMS has had a greater impact on the Faroe Islands industry. In recent years, presence of PMCV and subsequent CMS are in some cases found earlier post seawater transfer in smaller fish than before, resembling the development of this epizootic in Norway.

According to Bernhard Laxdal, a practicing fish veterinarian with long experience in the Icelandic salmon farming, no PMCV or CMS has been confirmed in Iceland to date.

> ***From 2016 to 2021, Ireland has experienced CMS outbreaks at between 4 and 6 sites annually out of 13 sites total in use.***
>
> *(Mitchell, 2023)*

DISCUSSION

CMS has, for the last few years, been rated in the Fish Health Report as the most important infectious disease in farmed Atlantic salmon in Norway (Sommerset, Wiik-Nielsen *et al*., 2023). Because most cases of CMS occur late in the production cycle, the impact leads to significant economic losses as well as reduced animal welfare (Garseth, Fritsvold *et al*., 2018).

Although the demonstration of transmissibility and the discovery of PMCV led to increased insight into CMS and the behavior of PMCV, implementation of effective control measures is hampered by the lack of essential knowledge of both the virus, infection dynamics, and the disease.

The knowledge regarding the pathogenesis of CMS is still relatively limited. Characterization of the structure of PMCV, the functions of its encoded proteins, and more details on virulence, antigenicity, shedding and resistance to environmental factors are needed. Other important questions are, what role does the immune system play in developing cardiac lesions and why does it fail to limit or eliminate the infection? Can Atlantic salmon survive CMS, do the cardiac lesions heal in some fish, and is it possible for infected fish to eliminate PMCV?

The mechanisms determining the large variation in time from PMCV detection to clinical signs are recognized and CMS diagnosis should also be investigated.

A functional *in vivo* model for propagation of PMCV, more sensitive and specific antibodies towards viral proteins and methods for non-lethal sampling of blood and mucus would all be very useful tools for both CMS diagnostics and further research on the virus and the disease.

> ***CMS has, for the last few years, been rated in the Fish Health Report as the most important infectious disease in farmed Atlantic salmon in Norway.***
>
> *(Sommerset, Wiik-Nielsen et al., 2023)*

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PASTEURELLOSIS IN FISH

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INTRODUCTION

The term pasteurellosis is used to describe disease caused by variants/species within the bacterial genus *Pasteurella*. Previously, the term has also been used in relation to systemic infections in fish caused by the bacterium *Photobacterium damselae* subsp. *piscicida* of the Family *Vibrionaceae* (Gauthier *et al*., 1995), formerly known as *Pasteurella piscicida* (Jan-ssen and Surgalla, 1968). Some earlier reports also exist of *Pasteurella*–like organisms isolated from diseased fish, which were subsequently confirmed as not belonging to the *Pasteurellaceae* (Ajmal and Hobbs, 1967; Håstein and Bullock, 1976, Jones and Cox, 1999).

Pasteurellosis in fish caused by true members of the Family *Pasteurellaceae* appears to be limited to the North-East Atlantic area. Only one aetiological agent has been validly according to the International Code of Nomenclature of Prokaryotes (ICNB), i.e., *P. skyensis* (Birkbeck *et al*., 2002). Another related bacterial species, for which the name *'Pasteurella atlantica'* has been proposed, but not validly published, is associated with disease in Norwegian farmed Atlantic salmon *(Salmo salar)* (Valheim *et al*. 2000) and lumpfish *(Cyclopterus lumpus)* (Alarcon *et al*. 2015).

One variant known as *"Pasteurella atlantica genomovar (gv.) salmonicida"* has been isolated from individual disease outbreaks in sea-farmed salmon several years apart along the Norwegian coast. The first registered outbreak of the disease, then known under the name "Varracalbmi" – the Lappish term for "bloody eye", occurred in Northern Norway at the end of the 1980s, before later reoccurring in single outbreaks in 2000 and 2012 in Western Norway. In 2018, the disease again re-emerged in this region, but contrary to previous instances, has become established and spread northward along the south-western coast, with the number of affected sites ~50 since 2020 (Figure 1) (Sommerset *et al*. 2021, 2022; Valheim *et al*., 2000; Gulla *et al*., 2020; Legård and Strøm, 2020; Sandlund *et al*., 2021).

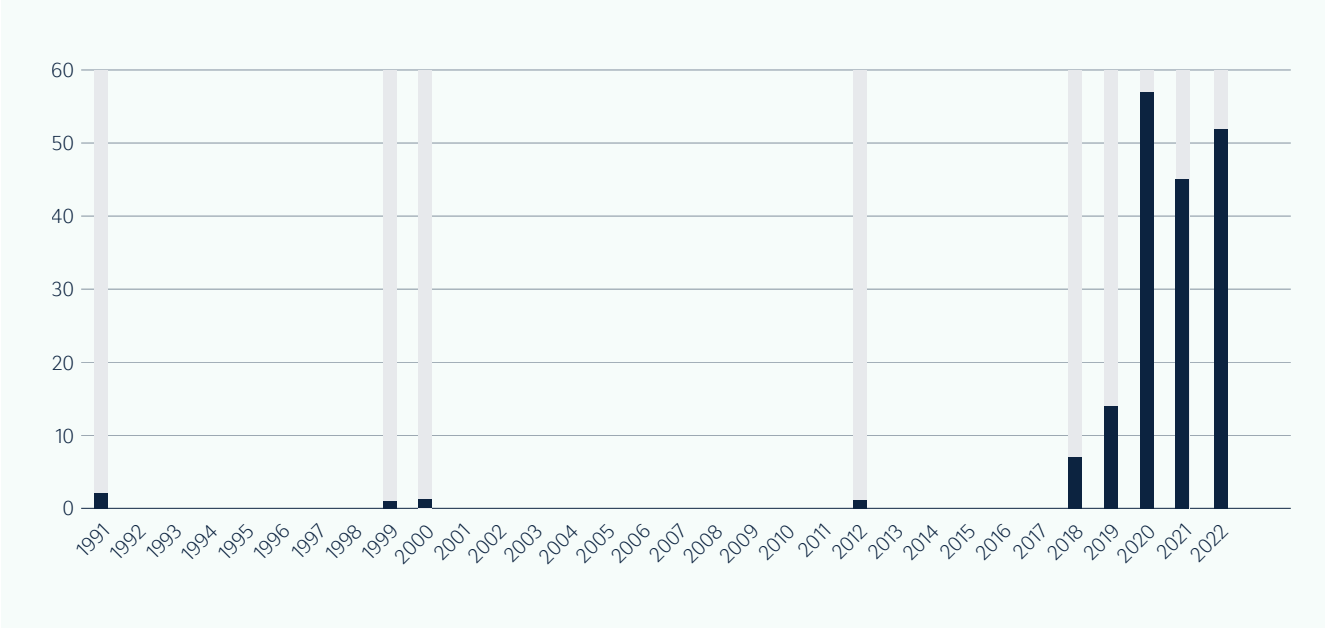

Figure 1: Atlantic salmon farming localities diagnosed with pasteurellosis in Norway 1991-2022.

Another genetic variant', *"P. atlantica gv. cyclopteri"*, is associated with disease in lumpfish used as "cleaner fish" for biological delousing in marine fish farms (Alarcón *et al*., 2015) and has been regularly detected in this fish species in both Norway and Scotland for several years. This variant has never been detected in salmon.

P. skyensis has, since the mid-1990s, caused sporadic problems in sea-based salmon farming in Scotland. However, frequency of outbreaks in salmon also increased in the Scottish aquaculture industry during the same period as the ongoing epizootic of *"P. atlantica gv. salmonicida"* in Norway, frequency of outbreaks in salmon also increased in the Scottish aquaculture industry (Soares *et al*., 2019; ICES, 2020; Marine Scotland Directorate, 2022). *P. skyensis* was detected for the first (and currently last) time in Norway in 2020, from two neighboring farms on the south-western coast (Strøm and Nilsen, 2021).

TAXONOMY

To date, very little has been published regarding the taxonomic position, genetic architecture, and population structure of *"P. atlantica"*, which has not yet received an official standing in prokaryotic nomenclature. However, unpublished work conducted at the Norwegian Veterinary Institute verifies that *P. skyensis* and *"P. atlantica"* constitute two distinct bacterial species within the Family *Pasteurellaceae*, although the association of both with genus *Pasteurella* is less clear, and a renaming at the genus-level may be forthcoming (Gulla *et al*., unpublished; Nilsen *et al*. unpublished). As mentioned, two different variants (genomovars) of *"P. atlantica"* affect lumpfish and salmon respectively, while further stratification of *"gv. salmonicida"* reveals discrete lineages linked to the various outbreaks occurring in Norwegian farmed salmon since the late 1980s, and the epizootic ongoing since 2018 is associated with one such lineage (Gulla *et al*., unpublished). A similar situation, involving various lineages, seems to have unfolded for *P. skyensis* in Scottish salmon.

~50

NUMBER OF AFFECTED SITES SINCE 2020 (SOMMERSET *ET AL.* 2021, 2022; VALHEIM *ET AL.*, 2000; GULLA *ET AL.*, 2020; LEGÅRD AND STRØM, 2020; SANDLUND *ET AL.*, 2021) .

PASTEURELLA SPP. AS PATHOGENS OF FARMED SALMONIDS

Pasteurellosis typically occurs in large sea-farmed Atlantic salmon (2-5+ kg), and occasionally, the disease has been diagnosed in brood fish. The disease has not, so far, been diagnosed in fish during the freshwater stage of culture.

Analyses of pasteurellosis cases diagnosed by the NVI suggests that outbreak risk is indeed higher following thermal delousing operations, but the statistical significance and possible causal basis of the association remains to be assessed (Stige *et al*., unpublished). The highest number of cases are identified from August to January, which coincides with the period of the year with the highest salmon lice infestation pressure and the most frequent lice treatments.

Point of entry of the bacterium is not known, but introduction via the gastrointestinal tract or gills seem likely. Colonization via mechanically induced microlesions deteriorating the skin barrier cannot, however, be ruled out.

In salmon with pasteurellosis caused by "*P. atlantica gv. salmonicida*", typical macroscopic findings are greyish fluid in the pericardial cavity and grey material covering the heart, abdominal wall, and pseudobranch. Also, haemorrhaged and necrotic foci in skeletal muscles and at the base of the pectoral fins are often present. Protruding, sometimes bloody and inflamed eyes are common, but do not occur in all fish.

Histopathological investigation reveals varying presence and extent of haemorrhage, tissue necrosis, leucocyte infiltration (neutrophils and macrophage-like cells) and aggregates of Gram-negative bacterial rods in affected organs including pseudobranch, gill, eye, internal organs and serous membranes like the epicardium and peritoneum. The clinical findings reported from one of the two Norwegian farms diagnosed with *P. skyensis* infection differed slightly from those observed during *"P. atlantica* gv. *salmonicida"* infections, including haemorrhages in the swim bladder wall and in fatty tissues, in addition to pericarditis and exophthalmia. Histopathological investigation revealed granulomatous inflammation in connective tissues of the swim bladder wall, consistent with earlier reports of *P. skyensis*-associated pathological changes (Jones and Cox, 1999). There was also inflammatory cell infiltration in various tissues, but this finding was not consistent. Perivascular inflammation involving neutrophils could be seen and special staining (MGG) showed suspected bacterial rods dispersed in the inflamed tissues.

"P. atlantica gv. salmonicida" has been shown to have low virulence in salmon challenge trials, while lumpfish have been confirmed as susceptible to both *"P. atlantica. gv. salmonicida"* and "gv. *cyclopteri"* (Sandlund *et. a*l., 2021). Recent infection trials (Colquhoun unpublished) suggest that *P. skyensis*, whilst not particularly virulent, may be slightly more virulent than *"P. atlantica gv. salmonicida"*.

DIAGNOSIS

Positive culture and identification from diseased fish confirms the infection. PCR (Sandlund *et a*l., 2021) has been used for detection of *"P. atlantica. gv. salmonicida"* eDNA in seawater and on swabs from gill and skin surfaces, and tissues from diseased fish.

CULTURE AND CLASSIFICATION

Both *P. skyensis* and *"P. atlantica"* are gram-negative, non-motile, pleomorphic, cocco-bacillar rods. They are demanding to cultivate and are dependent on blood products in culture media. Colonies develop as grey, low, convex, friable, non-adherent, smooth and circular with an entire margin ≤ 1 mm in diameter, following 2-4 days incubation at 22°C. The two species are not particularly biochemically active and while they can be separated biochemically, species identification has been based on sequencing of the 16S *rRNA* or *rpoB* genes (Reid and Birkbeck 2015; Alarcon *et a*l., 2016).

RESERVOIRS AND TRANSMISSION

Very little is currently known regarding the marine reservoirs and infection routes of fish-pathogenic *Pasteurella*, and the underlying causes of the current enzootic situations in Norway and Scotland remain unidentified. A possible yet unverified link towards marine mammals, as previously suggested by Reid and Birkbeck (2015), has been substantiated by the identification of closely related bacteria from various marine cetacean species (Gulla *et al*., unpublished). Concurrent outbreaks in neighbouring salmon farms, combined with a high degree of genetic similarity between isolates, point to a likely common source for the ongoing Norwegian salmon epizootic.

Preliminary results from investigations carried out at NVI indicate poor survival in seawater over time. During thermal delousing procedures where fish are bathed briefly in heated water, investigations conducted by NVI have shown an increase in bacterial load using qPCR testing of water samples (Holmeset 2022). Disease outbreaks have been reported in salmon brood fish, but vertical transmission is not documented. *"P. atlantica"* has, however, been detected in lumpfish eggs and milt (Kui S, 2017). As the disease affects salmon late in the production cycle, vertical transmission appears unlikely.

TREATMENT AND PROPHYLAXIS

The aim of prudent use of antibiotics in aquaculture makes it clear that this is not a recommended option for treatment. Jones and Cox (1999) reported treatment failure with trimethoprim-sulphadiazine in Atlantic salmon suffering from infection with a bacterium subsequently identified as *Pasteurella skyensis*. As the disease occurs late in the production cycle, early harvesting may be the best option. General hygiene measures, such as frequent changes of delousing water during treatment to prevent possible build-up of infectious agents shed from infected fish, as well as disinfection of equipment and personnel between treatments, will likely serve a preventive purpose. Minimizing the number and extent of potentially stressful handling procedures is recommended to avoid compromising the general health and disease resistance of the fish. Autogenous vaccines against *"Pasteurella atlantica"* are used, but the degree of protection awarded in the field has not yet been documented.

Frequent removal of dead and moribund fish will reduce infection pressure. Obligatory cleaning and disinfection of service boats visiting the farms has been indicated as a possible effective biosecurity measure (Apablaza, P. 2022)

> ***Very little is currently known regarding the marine reservoirs and infection routes of fish-pathogenic Pasteurella, and the underlying causes of the current enzootic situations in Norway and Scotland remain unidentified.***

THE NORWEGIAN SITUATION

Pasteurellosis in fish is not notifiable in Norway, therefore a full overview of its prevalence in Norwegian aquaculture is not available. Nevertheless, since 2018, pasteurellosis has been one of the major disease and welfare challenges in salmon aquaculture in Western Norway, primarily affecting large fish at the end of the production cycle. In 2022, the disease was documented on 52 marine salmon farms, confined to production areas 1 to 5 on the south-western coast. As in previous years, the highest number of affected sites were in production areas 3 and 4 (Sommerset *et al*., 2023). With a few exceptions, the bacterium *("P. atlantica gv. salmonicida")* has been detected primarily in diseased fish, and fish health services report increased mortality during outbreaks. The bacterium has also been recovered from apparently healthy fish, but the duration and prevalence of subclinical infections remains unknown. The clinical picture seen in moribund fish and revealed by autopsy has commonly involved pericarditis, peritonitis, and necrotizing inflammation in muscles, internal organs, and near the pectoral fins. The disease primarily occurs in large fish (2-5+ kg), but the bacterium has also been detected in <1 kg fish. Handling procedures (e.g., delousing) commonly precede outbreaks (Apablaza, 2022). A complex clinical picture involving various pathogenic agents is often identified (Sommerset *et. al* 2023).

To the best of our knowledge, there are no reports available describing detection of *"P. atlantica"* or *P. skyensis* from Ireland, Canada, or Chile.

DISCUSSION

The number of pasteurellosis outbreaks in farmed Norwegian salmon through 2022 remained high with 52 affected sea farms, a slight increase compared to 45 farms in 2021. Clinical pictures including involvement of several other infectious agents, and potentially stressful handling events commonly preceding outbreaks, complicate diagnostic interpretations. Pasteurellosis in salmon is now an established bacterial disease in Norwegian sea-based aquaculture and threatens fish welfare and sustainability. Since 2018, the disease has spread along, but remains limited to, the southwestern coast of Norway. There is a considerable risk of transmission to new areas. The causative agent is a specific lineage of *"P. atlantica* gv. *salmonicida".*

Pasteurellosis has also been regularly diagnosed in lumpfish used as cleaner fish over the last ten years or so. However, as such cases involve a distinct genetic variant of the bacterium *("P. atlantica gv. cyclopteri")*, which has never been detected in salmon, the risk for transmission from lumpfish to salmon with subsequent disease development is considered minimal.

Currently, very little is known regarding the marine reservoirs and infection routes for fish-pathogenic *Pasteurella*. The degree to which individual fish and/or fish stocks may recover from an outbreak and eliminate the infection remains unclear. Furthermore, the potential role of subclinically infected carriers is also uncertain, but such carrier fish may conceivably play a part during stress-triggered outbreaks e.g., following non-medical delousing.

Despite the fact that previous outbreaks have been short-lived, local, and occurring years apart, the current epidemic seems to be firmly established and represents a serious future threat to fish health and welfare. Vaccination to control bacterial salmon diseases has historically been a success, and this could be a possible tool if problems with pasteurellosis continue to grow, although this will require improved challenge models. Currently, implementation of knowledge-based biosecurity measures aimed at decreasing infection pressures and spread of infection, and maintaining a robust fish stock are perhaps the most pertinent approach towards mitigating pasteurellosis problems in sea-farmed salmon.

Despite the fact that previous outbreaks have been short-lived, local, and occurring years apart, the current epidemic seems to be firmly established and represents a serious future threat to fish health and welfare.

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A REVIEW OF *PISCIRICKETTSIA SALMONIS*, AN AGENT CAUSING PISCIRICKETTSIOSIS IN SALMONIDS

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INTRODUCTION

Rickettsia-like organisms (RLO) have been reported in both terrestrial and aquatic organisms such as mollusks and crustaceans (Olsen, *et al*., 1997; Fryer & Lannan, 1994).

Salmonid Rickettsial Septicaemia (SRS), also known as Piscirickettsiosis, was first reported in 1989 in Chilean coho salmon *(Oncorhynchus kisutch)* (Bravo and Campos, 1988). Piscirickettsiosis was reported in 4 farms in 1989 (Cvitanich *et al*., 1991) whereas 32 farms were infected in 1990 (Fryer *et al*., 1992). Piscirickettsiosis external features include lethargy, anorexia, darkening, multiple white spots and petechiae on the skin. Lesions are related to septicemia symptoms including granulomatous nodules in the liver, ascites, enlarged spleen and gray enlarged kidney (Rozas and Enríquez, 2014).

In Chile, piscirickettsiosis is a major cause of infectious mortalities in net pen salmonids including Atlantic salmon *(Salmo salar)*, coho and rainbow trout *(Oncorhynchus mykiss)* (Rozas and Enríquez, 2014). In fact, piscirickettsiosis is a major disease in commercial salmon production causing significant economic losses. For example, in 2006, the economic losses caused by piscirickettsiosis were estimated at US$200 million, which represented 25% of total revenue of salmon exports in Chile (Henríquez *et al*., 2016).

Although piscirickettsiosis has been reported in several countries producing Atlantic salmon such as Norway, Scotland, Ireland, and Canada, it has lower mortality rates in these countries in comparison to Chile. For example, in Canada, the mortality rate is lower than 1% (Cusack, Groman and Jones, 2002). However, more frequent piscirickettsiosis outbreaks have been reported during these last five years in Western Canada. In Norway, piscirickettsiosis outbreaks seem to be related to environmental changes such as high temperatures and algae blooms (Olsen *et al*., 1997).

The causative agent of piscirickettsiosis is referred to as rickettsia-like organism (RLO). The bacteria responsible for piscirickettsiosis in Chile was characterized as *Piscirickettsia salmonis (P. salmonis)* (isolate LF89) (Fryer *et al*., 1992). Based on the 16S ribosomal DNA (rDNA) sequence of LF-89, *P. salmonis* was grouped within the Proteobacteria group rather than Proteobacteria known as true rickettsiae (Fryer *et al*., 1992). *Piscirickettsia salmonis* is a gram-negative (-) intracellular bacteria (0.5 to 1.5 µm) that has been identified as the etiological agent for piscirickettsiosis. *P. salmonis* can be found as pairs or aggregates of basophilic coccoid organisms. *P. salmonis* can replicate in several antibiotic-free cell culture media including Chinook salmon embryo cell line (CHSE-214) and Epithelioma Papulosum Cyprini (EPC). The bacteria can also grow on cell-free agar growth media supporting the hypothesis that *P. salmonis* is a facultative intracellular bacterium (Mikalsen *et al*., 2008). Several solid and broth media, have been tested and a differential growth has been observed depending on the isolate and the media. For example, Canadian isolates grew preferentially in cysteine-enriched piscirickettsiosis blood agar media (Otterlei *et al*., 2016).

Phylogenetic analysis has revealed that there is a genetic variation between different strains of *P. salmonis*. This has been investigated through the analysis of 16S ribosomal DNA, internal transcribed spacer, and 23S ribosomal DNA sequences. The analysis has led to the identification of two main groups - “LF-89” and “EM-90” - that are genetically distinct (Mauel *et al*.,1999). Recently, a third group has also been reported in *P. salmonis* isolated from Norway and Canada (Shrober, 2022). Several studies have confirmed this genetic diversity, including one by Otterlei *et al*. (2016) who used both 16S rDNA and housekeeping genes to cluster *P. salmonis* into the LF-89 and EM-90 genotypes. Saavedra *et al*. (2018) also confirmed this diversity and highlighted differences in susceptibility to antibiotics, growth rate, and bactericidal action of serum between the two genotypes. Additionally, virulence differences between isolates have been shown by House *et al*. (1999). Furthermore, recent research reviewed by Rozas and Enríquez (2014) suggested that the EM-90 strain is more virulent than the prototype LF-89 based on gene expression mechanisms. This highlights the need for further research into the virulence of *P. salmonis* and the genetic factors that contribute to this virulence.

EPIDEMIOLOGY

The epidemiological complexity of piscirickettsiosis in salmonids is influenced by various factors such as the presence of the bacterium in the environment, host susceptibility to the pathogen, and management practices. The transmission of *P. salmonis* can occur horizontally through fish contact, shedding or water contamination (Long and Jones, 2021). Additionally, environmental temperature, salinity, and water quality are also contributing factors in the development of this disease in salmonids. Warmer temperatures may increase host susceptibility, leading to the growth of bacteria. Studies showed that piscirickettsiosis outbreaks are typically prevalent in water temperatures between 9 and 16°C (Rozas and Enríquez, 2014; Jones, 2019).

Piscirickettsia salmonis could persist in saltwater, and piscirickettsiosis is primarily reported in seawater and estuaries, with few cases found in lake-farmed rainbow trout (Bravo, 1994). The epidemiology of piscirickettsiosis is significantly influenced by management practices in the farmed environment. Improper nutrition, high-density in net pens and stressful conditions are all contributing factors in the risk of disease outbreaks (Almendras *et al*., 1997).

Research into experimental infections has shown that the preferred route of *P. salmonis* entry in coho is through the skin, followed by intestinal intubation, and finally, gill infection (Smith *et al*., 2004).

The complexity of piscirickettsiosis highlights the need for effective management practices to reduce the risk of disease outbreaks in farmed salmonids. Studies highlighted the importance of monitoring mortality during the first 4 weeks post-saltwater entry (Jakob *et al*., 2014). It was suggested that early monitoring can help producers to take preventive measures. A subject matter expert survey identified additional risk factors for piscirickettsiosis outbreaks including fouling of cages, density of farms in close proximity, presence of sea lions and early maturity of salmonids (Estévez *et al*., 2019). Outcomes from this survey suggested that other factors could be used to mitigate outbreaks such as synchronising farm fallowing period, frequent surveillance, and necropsy training (Estévez *et al*., 2019).

The complexity of piscirickettsiosis highlights the need for effective management practices to reduce the risk of disease outbreaks in farmed salmonids.

DIAGNOSIS OF *PISCIRICKETTSIA SALMONIS*

HISTOPATHOLOGY

The histopathology of piscirickettsiosis is characterized by the presence of inflammatory cells, primarily macrophages, within cytoplasmic or free vacuoles. These dark-colored basophilic cells typically measure 0.5-1.5 µm in size and exhibit a characteristic morphology with a large nucleus and a small cytoplasm (Branson and Diaz-Munoz, 1991).

The infection can cause necrosis or death of infected cells, resulting in the formation of granulomas or nodules. Renal pathology in *P. salmonis*-infected fish is characterized by multifocal necrosis of haematopoietic cells, resulting in anemia, and glomerulonephritis followed by vacuolization and edema of the renal capsule (Branson & Nieto Díaz-Muñoz, 1991; Rodger & Drinan, 1993).

In the liver, infected cells may contain vacuoles, as well as the formation of fibrin thrombi. Infected livers also exhibit multifocal necrosis of hepatocytes and infiltration of inflammatory cells. Within the necrotic regions, aggregates of *P. salmonis* can be observed (Branson & Nieto Díaz-Muñoz, 1991; Rodger & Drinan, 1993).

The skeletal muscle of infected fish shows degeneration and necrosis of myocytes, leading to the loss of striation. Inflammatory responses and infiltration of polymorphonuclear cells, associated with the immune response, are also observed. The brain and spinal cord also exhibit moderate granulomatous inflammation and thrombosis (Rodger & Drinan, 1993).

Gill pathology may manifest as epithelial multifocal hyperplasia resulting in lamellae fusion, focal necrosis, and the occurrence of fibrin thrombi within lamellar capillaries (Branson & Nieto Díaz-Muñoz, 1991).

ISOLATION AND CULTURE

The diagnosis of piscirickettsiosis is typically based on the detection of the bacteria in fish tissues. The bacterium *P. salmonis* was initially isolated using a Chinook salmon embryo cell line (CHSE-214) (Fryer *et al*., 1990). This bacterium can replicate in cell-free media, which classifies it as a facultative intracellular organism (Rozas and Enríquez, 2014; Gomez *et al*., 2009).

Subsequent investigations have confirmed the successful cultivation of *P. salmonis* on solid media, although most of these cultures have been established using CHSE-214 cell cultures (Yanez *et al*., 2012; Henriquez *et al*., 2013; Makrinos and Browden, 2016). Nonetheless, a few cases have reported the direct isolation of *P. salmonis* from infected fish using cell-free solid and liquid media (Gomez *et al*., 2009, 2017; Yañez *et al*., 2012). Various types of agar-based media have been explored for the growth of *P. salmonis*, including Cystine Heart Agar (CHAB) (Mikalsen *et al*., 2008), TSHem Agar (Yanez *et al*., 2013), and SRS-BA Agar (Otterlei *et al*., 2016), which have demonstrated successful colony formation (Makrinos and Browden, 2017).

However, further research is still needed to identify the most suitable cell lines and media for the primary isolation of this bacterium from infected fish.

MICROSCOPY, IMMUNOASSAYS, AND MOLECULAR TECHNIQUES

Several techniques have been used to screen for *P. salmonis* (Fryer *et al*., 1992; Jamett *et al*., 2001). The diagnostic procedures primarily involve the utilization of microscopy and immunoassay methods to observe the presence of *P. salmonis* within host cells. Staining techniques like Gram, Giemsa, or methylene blue were used to identify signs of the disease in fish (Rozas and Enríquez, 2014). The identity of *P. salmonis* can be confirmed through serological techniques such as immunofluorescence (IFAT) utilizing fluorescent antibodies (Olsen *et al*., 1997), enzyme-linked immunosorbent assay (ELISA) (Aguayo *et al*., 2002), or immunohistochemistry. These methods are applied to detect the bacterium in tissue imprints, histological sections, or cell cultures (Lannan, Ewing and Fryer, 1991). While the kidney, liver, and blood are the preferred tissues for isolating the bacterium during active infection, the brain is also important for detecting *P. salmonis* (Rozas and Enríquez, 2014).

In recent years, PCR-based techniques have gained popularity for *P. salmonis* testing due to their high throughput workflow and rapid turnaround of results. These techniques commonly target housekeeping genes such as 16S rRNA, internal transcribed spacer (ITS), and 23S rRNA, using PCR, including real-time PCR (Jones, 2019). PCR assays, specifically targeting the more variable ITS region, which allows for accurate screening of *P. salmonis* isolates compared to the 16S region are used in nested PCR. Real-time PCR assays have been developed to offer higher throughput and sensitivity compared to nested PCR, with the opportunity to quantify number of bacteria in samples (Karatas *et al*., 2008). For localization of bacteria in cells, *in situ* hybridization assays have also been implemented, using specific DNA probes designed against the bacterium (Venegas *et al*., 2004).

GENETIC DIVERSITY AND GENOMIC FEATURES OF *P. SALMONIS*

Research has found genetic and phenotypic heterogeneity of *P. salmonis* isolates. Using multiple markers such as 16S ribosomal DNA, internal transcribed spacer and 23S ribosomal DNA sequences, it was discovered that type strain LF-89, Chile; SLGO-94, Chile; C1-95, Chile; ATL-4-91, Western Canada; NOR-92, Norway were similar but different from strain EM-90 (Mauel *et al*., 1999). Another study has found similar heterogeneity among isolates from Northern Europe and Canada isolates (Reid *et al*., 2004). A genetic analysis using ribosomal DNA and housekeeping genes unraveled 2 distinct genotypes (LF-89 and EM-90) associated with piscirickettsiosis in Chilean aquaculture farms (Otterlei *et al*., 2016). On the other hand, the Western Canadian isolates harbor genetic dissimilarity in comparison to the Chilean genotypes (Otterlei *et al*., 2016).

Saavedra *et al*. (2018) suggested that the dissimilarities in Chilean isolates of *Piscirickettsia sp.* are related to their host preferences, *in vitro* growth, and sensitivity to antibiotics. However, a recent phylogenetic analysis of a larger number of *Piscirickettsia sp.* isolated worldwide by Shober (2022) found no evidence of geographic distribution or host-specific infection. For example, although the EM genogroup was previously believed to be restricted to Atlantic salmon, it has also been found in coho *(Oncorhynchus kisutch)*, rainbow trout *(O. mykiss)*, and Chinook salmon *(O. tshawytscha)*. Thus, it appears that neither geographic location nor host specificity are significant evolutionary drivers of *Piscirickettsia sp.* (Shober, 2022).

The emergence of the next generation of sequencing has paved the way for a comprehensive examination of the genomic sequence variations among different isolates. Currently, there are 87 drafted and completed genome sequences of *P. salmonis* that are available publicly (GenBank, May 15, 2023). Pan-genome analysis of 19 *P. salmonis* genomic sequences confirmed the two marked genogroups LF-89 (2,924 genes in pan-genomes, 2,170 genes in core genomes) and EM-90 (2,778 genes in pan-genomes, 2,228 genes in core genomes) (Nourdin-Galindo *et al*., 2017).

Although several genes related to pathogenesis were shared between the two genogroups, 148 and 273 genes encoding for protein related to colonization, invasion factors and endotoxins were exclusive to each LF and EM genogroup respectively (Nourdin-Galindo *et al*., 2017).

The availability of these sequences has enabled researchers to refine the genetic diversity among the isolates and unravel the genomic architecture that encodes for virulence and antibiotic factors (Eppinger *et al*., 2013; Bohle *et al*., 2017). Through genomic analysis, it has been discovered that *P. salmonis* harbors genes that are involved in virulence, including protein system types I, II, and IV, as well as two toxin-antitoxin systems and four protein-encoding proteases (Yañez *et al*., 2014).

Additionally, genes involved in intracellular survival, such as the protein system dot/icm (defect in organelle trafficking/intracellular multiplication), have been identified (Bravo and Martinez, 2016). Genetic manipulation of icmB, one component of the type four secretion systems (T4SS) mutant, resulted in attenuation of infectivity of *P. salmonis* (Mancilla *et al*., 2018).

Other studies have focused on the plasmids harboring multidrug-resistant genes against tetracyclines, aminoglycosides, and sulfonamides (Bohle *et al*., 2017). Susceptibility studies of *P. salmonis* to florfenicol and oxytetracycline antibiotics revealed the involvement of 140 genes encoding for resistance including tet-1 Transporter, Tet-C, Tet-2 Transporter, Bcr/Cfla, Bcr2/Cfla and Bcr3/Cfla (Cartes *et al*., 2017). Analysis of oxytetracycline resistance genes including gene coding for porin (OmpF) showed an association between their gene expression and the presence of single nucleotide polymorphisms (SNP) (Figueroa *et al*., 2019).

Recently, a third genogroup, known as non-Chilean genogroup, including Canadian and Norwegian genome sequences has been characterized and described in Shober's thesis where 73 *P. salmonis* genomes have been sequenced (Schober, 2022). The NOR/CAN genogroups have larger genomes, more genes, and plasmids compared to the Chilean genogroups (Table 1).

	LF	EM	Norway	Canada
Genome Size (Mb)	3.42-3.6	3.25-3.75	3.77	4.07-4.15
Chromosome length	3.17-3.22	3.04-3.34	3.32	3.54-3.58
Extrachromosal elements	3-5	1-5	7	5-6

Figure 1: Comparison of genomes features from the 3 genogroups (LF, EM and Norway/Canada). Information provided from Schober, 2022.

MITIGATION STRATEGIES

USE OF ANTIBIOTICS

Florfenicol and oxytetracyclines are the two main antibiotics used in salmon farms to combat piscirickettsiosis. Between 82 to 90% of all antibiotics used in Chilean farms are prescribed for the treatment of piscirickettsiosis (Price *et al.*, 2018). The low efficacy of the antibiotic treatment could be related to the intracellular pathogenesis of *P. salmonis* (Fryer *et al.*, 1992) but also associated with the produced biomass of fish (Rozas and Enríquez, 2014). Price *et al.* (2016) suggested that antibiotics should be administered during the early phase of a piscirickettsiosis outbreak when the mortality rate is at the lowest peak.

> ***In Chile, there are currently 34 vaccines available in injection, oral, and immersion forms with the best vaccine reducing the mortality by 22%. Studies have shown that vaccines have a good protection for a short period of time (5 days post vaccination) however are less efficient in long-term protection.***
>
> *(Vargas et al., 2021).*

DEVELOPMENT OF VACCINES

In Chile, there are currently 34 vaccines available in injection, oral, and immersion forms with the best vaccine reducing the mortality by 22%. Studies have shown that vaccines have a good protection for a short period of time (5 days post vaccination) however are less efficient in long-term protection (Vargas *et al*., 2021).

The effectiveness of the vaccines can be influenced by several intrinsic and extrinsic factors such as environmental stressors (low oxygen, high temperature, high fish density, chemical treatment) as well as bacterial genetic variability, sex, and age of the fish. Stressors like sea lice infestation, algae blooms, and *P. salmonis* diversity contribute significantly to the vaccine efficacy (Valenzuela-Aviles *et al*., 2022). It was suggested that the mode of vaccination by co-habitation or immersion, understanding the pathogen diversity prevalent in the sites, decreasing the stress on the fish, and improving host resistant through breeding programs may improve the efficacy of the vaccines (Figueroa *et al*., 2022; Valenzuela-Aviles *et al*., 2022).

GENETIC SELECTION

Over the past decade, the identification of disease-resistant genes has emerged as a promising avenue in salmon breeding programs. Extensive research has been dedicated to unraveling the molecular mechanisms underlying resistance to piscirickettsiosis (Moraleda *et al*., 2021). These investigations have revealed that resistance to piscirickettsiosis is a polygenic trait involving factors related to apoptosis, cytoskeletal organization, and the inflammasome (Correa *et al*., 2015; Barría *et al*., 2017; Barria *et al*., 2019; Moraleda *et al*., 2021). A single nucleotide polymorphism (SNP) located in the genomic region Omy27 has been found to account for the highest level of resistance in rainbow trout (Barria *et al*., 2019). Consequently, multiple studies suggested that genetic selection may help in controlling and mitigating piscirickettsiosis in various salmon species such as coho (Barría *et al*., 2017), rainbow trout (Barria *et al*., 2019) and Atlantic salmon (Bangera *et al*., 2017). Practically, it has been demonstrated that utilizing a low-density SNP chip (even as low as 500 SNPs) in conjunction with an imputation strategy offers an alternative to traditional pedigree-based Best Linear Unbiased Prediction (PBLUP) for genetic selection purposes (Bangera *et al*., 2017). This finding highlights the potential for routine utilization of this approach in salmon breeding programs.

PISCIRICKETTSIOSIS IN CHILE

In Chile, piscirickettsiosis is the primary cause of infectious mortalities in net pen salmonids, accounting for a significant proportion of disease-related outbreaks, ranging from 50 to 90% (Rozas and Enríquez, 2014). To combat piscirickettsiosis, a national surveillance and control plan was implemented in Chilean farms in 2012, aiming to minimize the spread and impact of the disease through early detection and control measures within salmon farms (Rozas-Serri *et al*., 2023).

Through expert surveys, ten risk factors and seven protective factors associated with piscirickettsiosis have been identified epidemiologically (Estévez *et al*., 2019). High -risk factors include bath treatment, high farm density, and fouling of cages, while protective factors include biosurveillance, staff training in necropsy procedures, and the implementation of farm fallowing (Estévez *et al*., 2019).

The conventional treatment for piscirickettsiosis is the use of antibiotics such as florfenicol and oxytetracycline. However, due to the intracellular mode of action of *P. salmonis*, antibiotic treatment has shown limited success. Moreover, excessive antibiotic use may lead to disturbances in mucosal microbial dysbiosis and the emergence of antibiotic-resistant bacteria (Yañez *et al*., 2012; Miranda *et al*., 2018). Studies showed that overuse of antibiotics may promote biofilm formation of *P. salmonis*, which can increase the risk of antibiotic resistance and re-infection in cultured fish (Oliver, Céspedes, *et al*., 2023). Recently, there has been a growing emphasis on the discovery of novel antibiotic compounds through a combination of *in silico* analysis and *in vitro* assays (Beltrán *et al*., 2023). Preliminary investigations showed the potential of orbifloxacin, sarafloxacin, and sparfloxacin as therapeutant candidates against *P. salmonis* (Beltrán *et al*., 2023).

In Chilean waters, two main genogroups of *P. salmonis*, LF89 and EF90, are prevalent. These genogroups exhibit distinct infection tropisms, with the EM-90-like genotype associated with a hemorrhagic type of disease and higher mortality, while the LF-89-like genotype is associated with more classic liver and kidney lesions (Rozas-Serri *et al*., 2017a). Co-infection with both genotypes has been observed in Atlantic salmon, with both present in the same tissue of individual fish (Saavedra *et al*., 2017). The prevalence of these genotypes can vary over time, but recent reports indicate a higher prevalence of the LF-89-like genotype (Saavedra *et al*., 2017; Rozas-Serri *et al*., 2023).

Immunological responses to both genotypes are similar, characterized by the activation of innate immunity and the inhibition of T cell-mediated adaptive responses (Rozas-Serri *et al*., 2017b). Fish infected with the EM-90-like genotype exhibit a more pronounced immune response, which may explain the more severe tissue lesions observed in these cases (Rozas-Serri *et al*., 2017b). Additionally, there is a differentiation in susceptibility to antibiotics between *P. salmonis* genotypes, with EM-90-like genotypes being susceptible to commonly used antibiotics such as florfenicol and oxytetracyclines, while LF-89 genotypes show resistance to at least one of these antibiotics (Saavedra *et al*., 2017). The use of the EM-90-like genotype as a live attenuated and bacterin vaccine has been suggested to contribute to the high prevalence of the LF-89-like genotype in recent years (Rozas-Serri *et al*., 2023).

To explore alternative strategies, the Blue Genomics Consortium is investigating the potential of membrane vesicles as a promising avenue for vaccine development. The small structures, Outer Membrane Vesicles (OMVs), released by gram-negative bacteria, harbor virulence factors and toxins. Recent studies conducted with zebrafish have

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demonstrated the protective features of these vesicles, especially when used in combination with chitosan as an adjuvant (Tandberg *et al*., 2017). When OMVs were injected into juvenile Atlantic salmon, they induced the production of IgM antibodies against proteins of *P. salmonis* (Oliver, Coronado, *et al*., 2023). This finding highlights the potential of OMVs as a new approach for stimulating an immune response against *P. salmonis* in salmonids.

Furthermore, ongoing research is focusing on the development of a universal vaccine that can target common epitopes shared among several gram-negative bacteria. Chukwu-Osazuwa *et al*., (2022) have characterized five common antigens present in *P. salmonis*, *A. salmonicida*, *Y. ruckeri*, *V. anguillarum*, and *M. viscosa*, which contain 13 exposed epitopes capable of interacting with T and B cell receptors. This discovery opens new avenues for the development of a more comprehensive and universal vaccine approach to combat co-infections caused by multiple bacteria.

Overall, to enhance our understanding on controlling *P. salmonis* affecting farmed salmon in Chile, Mardones *et al*., (2018) highlight the importance of focusing on eight key research areas including epidemiology, ecology and environmental science, microbiology, immunology, pharmacology, "Omics", human dimensions, and vaccine development. The authors suggested that research studies should be developed through collaborative workforce such as salmon farming industry productivity, the physical and ecological environment, socio-economic sustainability, and policy and management implications. This collaboration between researchers and the industry will foster the development of a sustainable industry, ensuring a holistic approach to disease management (Mardones *et al*., 2018).

PISCIRICKETTSIOSIS IN COLUMBIA

A study conducted by the Department of Fisheries and Oceans (DFO) aimed to evaluate the potential risk of piscirickettsiosis spreading from Atlantic salmon farms in the Discovery Islands to Sockeye salmon populations in the Fraser River (Mimeault *et al*., 2019). Between 2002 and 2016, a total of 1,229 audits were performed on Atlantic salmon farms across all management regions in British Columbia (BC), averaging seven audits per month. DFO veterinarians conducted these audits, focusing on various factors, including farm history, environmental conditions, mortality records, treatment history, clinical presentation, and screening of individual fish or fish pools for infection using histopathological examination or PCR testing (Jones, 2019; Mimeault *et al*., 2019).

A total of 36 farm-level diagnoses of piscirickettsiosis were recorded, with approximately 56% of these diagnoses occurring in Fish Health Zone 2.3, (West Coast of Vancouver Island). The presence of the disease was also identified in other zones between 2004 and 2009, but no diagnoses were made in 2010, 2011, and 2012 (Mimeault *et al*., 2019; Jones, 2019).

Furthermore, investigations into the occurrence and risk factors of piscirickettsiosis have been carried out in net pen and tank-reared British Columbian Atlantic salmon populations using raw seawater (Jones *et al*., 2019). Implementing proper management practices can contribute to the prevention of *P. salmonis* spread, thereby reducing the incidence of piscirickettsiosis in salmonids. For instance, the use of UV treatment on infected raw water in experimental setups has demonstrated a reduction in *P. salmonis* infection and the development of piscirickettsiosis (Jones, 2019). In addition to using antibiotics like oxytetracycline and florfenicol for treatment, BC producers also utilize a vaccine primarily

designed to prevent *Renibacterium salmoninarum* causing bacterial kidney disease, has shown potential efficacy against piscirickettsiosis (Jones, 2019). Fish handling protocols, including the storage of deceased fish and disinfection procedures, have been established to prevent further transmission.

The epidemiology of piscirickettsiosis is complex due to the presence of multiple strains of P. salmonis in net pen outbreaks. Identifying and tracking different strains of the bacterium, which can vary in their virulence and geographic distribution, is crucial for disease surveillance and control.

CONCLUSION

Piscirickettsia salmonis is an intracellular gram-negative bacterium (0.5 to 1.5 µm) that serves as the causative agent for piscirickettsiosis. In Chile, piscirickettsiosis is a leading cause of infectious mortalities among net pen salmonids, including Atlantic salmon *(Salmo salar)*, coho *(Oncorhynchus kisutch)*, and rainbow trout *(Oncorhynchus mykiss)* (Rozas and Enríquez, 2014). While piscirickettsiosis has been reported in other countries producing Atlantic salmon, such as Norway, Scotland, Ireland, and Canada, its mortality rates are comparatively lower in these regions than in Chile.

Clinical signs of piscirickettsiosis in fish include anorexia, lethargy, and abnormal swimming behavior. Gross post-mortem examinations of affected fish often reveal hemorrhaging in various organs, gills, and skin.

The epidemiology of piscirickettsiosis is complex due to the presence of multiple strains of *P. salmonis* in net pen outbreaks. Identifying and tracking different strains of the bacterium, which can vary in their virulence and geographic distribution, is crucial for disease surveillance and control. Improved understanding of the interactions between the bacterium, the environment and fish host susceptibility is essential for developing effective prevention and control strategies.

In a comprehensive review by Rozas and Enríquez (2014), the severity of piscirickettsiosis and its impact on infected fish were examined. Histopathological changes observed in piscirickettsiosis are similar to other intracellular bacterial infections, characterized by the presence of inflammatory cells and tissue necrosis. Understanding these alterations is pivotal for unraveling the pathogenesis of piscirickettsiosis.

Florfenicol and oxytetracycline are the two main antibiotics used to combat piscirickettsiosis. However, the efficacy of antibiotic treatment is limited, possibly due to the intracellular pathogenesis of *P. salmonis*. Price *et a*l. (2016) suggest administering antibiotics during the early phase of piscirickettsiosis outbreaks when the mortality rate is at its lowest. Early treatment may help prevent the spread of the disease and reduce overall mortality.

Despite extensive development and usage over a span of 20 years, more than 30 different types of vaccines tested and administered to salmon have had limited success in providing long-term protection against piscirickettsiosis. Studies have demonstrated that while these vaccines offer good protection for a short period they showed low efficacy for long-term protection. To enhance vaccine efficacy, suggestions include considering alternative modes of vaccination such as co-habitation or immersion, understanding the prevalent pathogen diversity at the sites, reducing stress on the fish, and improving host resistance through breeding programs.

Genomic analysis of *P. salmonis* plays a crucial role in deciphering the pathogenicity molecular mechanisms, offering valuable opportunities for the development of novel therapeutic interventions. Furthermore, the identification of disease-resistant genes in salmon breeding programs has emerged as a promising avenue in the past decade. This finding highlights the potential for routine utilization of this approach in salmon breeding.

Genomic analysis of P. salmonis plays a crucial role in deciphering the pathogenicity molecular mechanisms, offering valuable opportunities for the development of novel therapeutic interventions.

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TENACIBACULOSIS IN FARMED SALMON IN NORWAY

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INTRODUCTION

The genus *Tenacibaculum* (Family *Flavobacteriaceae*, Phylum *Bacteroidetes*) represents a group of closely related Gram-negative, yellow-pigmented, strictly aerobic, slender, filamentous, rod-shaped bacteria, which lack flagella and display characteristic gliding movement. *Tenacibaculum spp.* are common and widespread members of the marine microbiota where they may exist as planktonic cells or in close association with marine organisms (Suzuki *et al*., 2001; Ferguson *et al*., 2010) or organic detritus (Kirchman, 2002). The *Tenacibaculum* genus is closely related to the largely freshwater-associated genus *Flavobacterium*, and both genera perform a vital role in environmental carbon cycling (Kirchman, 2002). Representatives of both genera cause serious disease in farmed fish around the world.

Infections associated with *Tenacibaculum spp.* are commonly referred to as “tenacibaculosis”, a term originally coined to describe the ulcerative disease produced in marine fish by *Tenacibaculum maritimum* (Avendaño-Herrera *et al*., 2006). The term has replaced various names based on the clinical signs observed e.g., eroded mouth syndrome and black patch necrosis (Santos *et al*. 1999). Both the awareness and impact of tenacibaculosis as a problem in salmon farming appear to be increasing. A number of new *Tenacibaculum* species and strains associated with this disease have been described in recent years, several of which appear to have a circum-global distribution. Although a great deal of evidence exists linking, in particular *T. maritimum* (Wakabayashi *et al*., 1986) to disease in farmed marine fish species, the pathogenic role of various other *Tenacibaculum* taxa, isolated during diagnostic investigations in salmon farming around the world, is not always clear. The clinical picture identified in the field may be difficult to recreate in the laboratory and development of clinical disease may be dependent on a complex balance of host, agent, and environmental parameters (Avendaño-Herrera *et al*. 2006).

TAXONOMY/DIVERSITY

More than 30 *Tenacibaculum* species have been described (http://www.bacterio.net/*tenacibaculum*.html) and many more as yet undescribed taxa undoubtedly exist. The validly described *Tenacibaculum spp*. associated with fish disease include *T. maritimum* (formerly *Flexibacter maritimus*) originally described from red seabream *(Pagrus major)* (Wakabayashi *et a*l., 1986), *T. piscium* and *T. finnmarkense genomovariants* (gv.) *finnmarkense* and *ulcerous* isolated from Atlantic salmon *(Salmo salar)* (Olsen *et a*l. 2020), *T. dicentrarchi*, originally isolated from farmed sea-bass *(Dicentrarchus labrax)* (Piñeiro-Vidal *et a*l., 2012), *T. discolor* and *T. soleae* isolated from Senegalese sole *(Solea senegalensis)* (Piñeiro-Vidal *et a*l., 2008a; 2008b) and *T. singaporense* isolated from farmed Asian seabass *(Lates calcifer)* (Miyake *et a*l. 2020). *T. ovolyticum* was originally isolated from Atlantic halibut *(Hippoglossus hippoglossus)* eggs (Hansen *et a*l., 1992).

T. maritimum is widely distributed throughout the world and causes disease in many fish species (for reviews see Avandeño-Herrera *et a*l. 2006 and Mabrok *et a*l. 2023), mostly relatively warm-water species but also in cold-water species including lumpfish *(Cyclopterus lumpus)* (Småge *et a*l. 2016). The recognized ranges and host distributions of other fish-associated *Tenacibaculum* species are also being increasingly extended. *T. ovolyticum* was recently identified from the gills of a Chilean farmed Atlantic salmon displaying clinical signs of tenacibaculosis (Avendaño-Herrerra 2022a). *T. piscium,* recently described and associated with Atlantic salmon skin ulcers in Norway (Olsen *et a*l. 2020), has also been subsequently described as the aetiological agent of skin ulcer development in Atlantic salmon in Chile (Avendaño-Herrera *et a*l. 2022b). Both genomovars of *T. finnmarkense* have been identified in British Columbia, Canada (Nowlan *et a*l. 2023) and isolates closely related to *T. finnmarkense* have been described from diseased rainbow trout *(Oncorhynchus mykiss)* and coho salmon *(Oncorhynchus kisutch)* in Chile (Avendaño-Herrera *et a*l. 2020). *T. dicentrarchi* has been associated with skin ulcers in wrasse *(Labridae)*, lumpsucker *(Cyclopterus lumpus)*, Atlantic cod (Gadus morhua) and Atlantic salmon *(Salmo salar)* in Norway (Olsen *et a*l. 2017). *T. dicentrarchi* has also been associated with mortality in Atlantic salmon in Chile (Avendaño-Herrera *et a*l., 2016) and Norway (Klakegg *et a*l. 2019), in rainbow trout and red conger eel *(Genypterus chilensis)* farmed in Chile (Avendaño-Herrera *et a*l., 2016; Irgang *et a*l., 2017), as well as in Chinook salmon *(Oncorhynchus tshawytscha)* in New Zealand (Kumanan *et a*l. 2022).

Apablaza *et a*l. (2017) reported the first isolation of *T. maritimum* from Chilean Atlantic salmon mortalities during a harmful algal bloom caused by *Pseudochattonella spp*.

TENACIBACULUM SPP. AS PATHOGENS OF FARMED SALMONIDS

Tenacibaculosis in salmonids has been reported from all salmon-producing areas of the globe, and can be roughly split into two main types i.e., outbreaks associated with *T. maritimum* and those associated with non-*T. maritimum* species or strains.

Most studies involving *T. maritimum*-associated disease have focused on marine fish species (Avendaño-Herrera *et al*., 2006). This bacterium has, however, for many years also been associated with disease in salmon farming countries with 'milder' climates and moderate sea water temperatures including Tasmania (Schmidtke and Carson, 1995), Spain (Pazos *et al*., 1996), Ireland (Fringuelli *et al*., 2012), USA (Chen *et al*., 1995), and Western Canada (Kent, 1988; Frisch *et al*., 2017). *T. maritimum* has also recently been described for the first time in diseased Atlantic salmon and rainbow trout farmed in Chile (Apablaza *et al*., 2017; Valdes *et al*. 2021) and amongst the various *Tenacibaculum spp*. associated with gill necrosis in Atlantic salmon in Norway.

Non-*T. maritimum* species isolated from Atlantic salmon include *T. dicentrarchi* in Chile and Norway (Avendaño-Herrera *et al*., 2016; Olsen *et al*., 2017), *T. finnmarkense* in Norway, Chile, and Canada (Olsen *et al*. 2020; Avendaño-Hererra *et al*. 2020; Nowlan *et al*. 2023) and *T. piscium* in Norway and Chile (Olsen *et al*. 2020; Avendaño-Herrera *et al* 2022).

DIAGNOSIS OF TENACIBACULOSIS

While tenacibaculosis, particularly when caused by *T. maritimum*, may present as a systemic infection, the majority of cases in Norway and Chile involve topical infections of the integument, mainly affecting head, abdomen, fins, and gills as skin ulceration and fin or gill 'rot'. Tenacibaculosis in Atlantic salmon raised on the Pacific coast of Canada is normally restricted to infection of the jaws and gills, whereas ulceration is also common in Atlantic salmon in Eastern Canada.

Fish of all ages farmed in seawater may be susceptible to tenacibaculosis and different types/species of *Tenacibaculum* may present similar clinical pictures in salmon. Mouth rot (Ostland *et al*., 1999; Frisch *et al*., 2017) is one of the most common manifestations of tenacibaculosis in salmonids caused by both *T. maritimum* (Ostland *et al*., 1999) and non-*T. maritimum*. Corneal infections, rupture of the eye (Handlinger *et al*., 1997; Olsen *et al*., 2011) and necrotic gills are also reportedly associated with different *Tenacibaculum* bacteria (Chen *et al*., 1995; Handlinger *et al*., 1997; Mitchell & Rodger, 2011). The presence of large numbers of *Tenacibaculum* cells may also give affected areas of skin or gills a yellowish hue and macroscopically visible yellow plaques are also reported (Ostland *et al*., 1999). Histopathological findings common in external lesions of general tenacibaculosis include necrosis in collagen-rich tissues like the dermis and the histologically obvious presence of large numbers of long bacterial cells. Inflammatory reactions are usually absent or sparse.

Identification of long, non-motile rods by direct microscopy of smears from ulcers is indicative of *Tenacibaculum* infection. Histopathology is an excellent tool to visualize the infection and increase the detection rate of tenacibaculosis. Immunohistochemistry techniques have been developed (Olsen *et al*., 2011; Faílde *et al*., 2013).

While typical clinical signs may provide grounds for presumptive diagnosis of tenacibaculosis, they do not constitute grounds for definitive diagnosis, as similar clinical signs may result from other causes and ulcerous lesions favor the entry of many different types of bacteria, including *Tenacibaculum spp*.

Several PCR methods have been described for detection of *T. maritimum* (Cepeda *et al*., 2003; Avendaño-Herrera *et al*., 2004; Fringuelli *et al*., 2012), *T. soleae* (López *et al*., 2010; García-González *et al*., 2011) and more recently, Avendaño-Herrera *et al*. (2019) reported a PCR procedure for detection of *T. dicentrarchi* in fish samples. Wilson *et al*. (2019) described a multiplex PCR, which they used to identify the presence of *T. dicentrarchi* and *T. soleae* in Tasmanian aquaculture for the first time and allowed identification and differentiation between these two species and *T. maritimum*. Nowlan *et al*. (2021) concluded that 16S rDNA-based qPCR assays designed to detect *T. dicentrarchi* and *T. finnmarkense* although, satisfactorily sensitive, showed only moderate specificity.

Kumanan and co-workers (Kumanan *et al*. 2022) recently published a study comparing the efficiency of a selective agar type (Marine Shieh's Selective Medium, MSSM), standard marine agar and digital droplet PCR for surveillance of *Tenacibaculum maritimum* infections. They found culture on Shieh's medium to be much more effective for identification of *T. maritimum* than culture on general marine agar or ddPCR. These authors also found Shieh's medium suitable for culture of *T. dicentrarchi* and *T. solea*, identifying these bacteria from Chinook salmon in New Zealand for the first time. Saldarriaga-Córdoba *et al*. (2023) described a standard PCR specific for *T. piscium* sufficiently sensitive to identify acute infections in Atlantic salmon.

As tenacibaculosis is not a notifiable disease in Norway, Chile, or Canada, the infection is often diagnosed at a local level, based on a few indications such as direct microscopy and/or culture of yellow-pigmented colonies of typical morphology on suitable agar. As *Tenacibaculum spp*. are generally unreactive to most metabolic tests, molecular studies (see culture and classification below) are generally required to differentiate and confirm the identity of isolates to species and genomovariant/biotype. Such investigations are not commonly performed at the diagnostic level and local or private diagnosis also means that the incidence of tenacibaculosis is most probably under-reported.

> ***Non-T. maritimum species isolated from Atlantic salmon include T. dicentrarchi in Chile and Norway, T. finnmarkense in Norway, Chile, and Canada, and T. piscium in Norway and Chile.***

CULTURE AND CLASSIFICATION

Tenacibaculum spp. grow with pale to bright yellow colonies consisting of rod-shaped, long hair-like bacterial cells, which may become rounded in older cultures. *T. maritimum* grows with colonies that are particularly adherent to the culture agar. Most *Tenacibaculum* species grow poorly or not at all on general-purpose agars, even when supplemented with NaCl. They may be cultured by inoculation of ulcer material on low nutrient media containing sea salts e.g., FMM *(Flexibacter maritimus-medium)* and Marine Agar 2216 (Pazos *et al*., 1996). The addition of 50 mg/ml kanamycin to culture media has been reported to aid recovery through depression of 'contaminating' bacterial growth (Frisch *et al*., 2017). Further addition of blood to marine agar with- (KABAMA) or without- kanamycin (BAMA) has also been reported as enabling differentiation of hemolytic and non-hemolytic *Tenacibaculum spp*. (Lagadec *et al*. 2021). *Tenacibaculum* are relatively slow growing and cultivation directly from ulcers may be difficult. To increase the likelihood of successful culture, tissue scrapings should be obtained by scalpel and inoculated onto the plate prior to careful spread.

The various species of *Tenacibaculum* display a wide range of optimal culture temperatures, which vary from 15°C to 30°C. It may appear that *T. maritimum* isolated from salmon and other cold-water species have lower optimal temperatures than *T. maritimum* isolates originating from warmer water fish species (Frisch *et al*., 2017). Incubation temperatures between 15 to 18°C are probably suitable for most salmonid-related isolates.

As mentioned above, *Tenacibaculum spp*. are biochemically relatively unreactive and identification to the species level using traditional phenotypic methods may be challenging, particularly given the phenotypical differences reported between Chilean and European isolates of the same species (Piñeiro-Vidal *et al*., 2012; Avendaño-Herrera *et al*., 2016; Irgang *et al*., 2017). With the increasing availability of advanced analytical methods such as PCR, gene sequencing and proteomics, phenotype-based studies are becoming less frequently used.

Given the diversity of undescribed *Tenacibaculum* taxa within the environmental flora, development of specific molecular detection analyses may also be challenging. However, the ever-increasing availability of whole genome sequence data is easing development of molecular tools for identification of particular taxa including the various PCR-based assays mentioned previously.

Genotyping by multilocus sequence analysis (MLSA) and multilocus sequence typing (MLST) has been developed for *Tenacibaculum spp*. **http://pubmlst.org/***tenacibaculum* (Habib *et al*., 2014). Steinum *et al*. (2021) found whole genome-based *in silico* CRISPRcas spoligotyping provided a higher degree of discrimination than MLST when used to study *T. maritimum* isolated from European seabass *(D. labrus)* in Turkey.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), which differentiates bacterial species and strains on the basis of mass-charge of (mainly) ribosomal proteins, has also recently been demonstrated as a useful and extremely rapid tool for the identification and differentiation of *Tenacibaculum* species (Fernández-Álvarez *et al*., 2017). Bridel *et al*. (2020) used MALDI-TOF to identify 20 MALDI-Types and 4 MALDI-groups amongst 131 *T. maritimum* isolates and proposed MALDI-TOF-MS Multi Peak Shift Typing as a cheap, fast and accurate method for large scale epidemiological screening of this bacterium. Spilsberg *et al*. (2022) found MALDI-TOF analysis to represent a rapid and reliable method for identification and differentiation of and between *Tenacibaculum* species, even at the sub-species genomovariant level.

RESERVOIRS AND TRANSMISSION

Tenacibaculum spp. are common and widespread in marine waters, suggesting that the main sources of infection are environmental. While *Tenacibaculum spp*., as rich producers of extracellular proteases (van Gelderen *et al*., 2009), are almost certainly responsible for the majority of the tissue losses observed during outbreaks of tenacibaculosis, the epidemiology may be complex and outbreaks are often associated with compromised skin barriers due to environmental challenges, suboptimal management and/or poor resistance in affected fish. Following establishment of an infection, shedding of bacteria from ulcerated fish will undoubtedly to some extent increase the risk of other fish also becoming infected, but the degree of infectivity is unclear. Intra-outbreak strain diversity within several Norwegian tenacibaculosis cases in farmed salmon suggests that direct fish-to-fish transmission is not the major mode of transmission (Olsen *et al*. 2017; Spilsberg *et al*. 2022).

T. maritimum may be associated with marine organisms and blooms of jellyfish could conceivably provide both a colonization site (via initial nematocyst-related injuries) and a source of *Tenacibaculum* infection (Ferguson *et al*., 2010; Delannoy *et al*., 2011). Barker *et al*. (2009) also suggested that the sea louse *(Lepeophtheirus salmonis)* might serve as an organic substrate capable of extending the persistence of *Tenacibaculum* cells in seawater. The modes of transmission and routes of infection for tenacibaculosis in salmon in general remain unclear. Consistent reproduction of disease under controlled laboratory conditions is often difficult, although clinical signs consistent with *T. dicentrarchi* infection and mortality have been demonstrated in Atlantic salmon and rainbow trout exposed to 3.78 x 10^5 CFU/ml *T. dicentrarchi* for 60 minutes (Avendaño-Herrera *et al*., 2016; Nowlan *et al*., 2021). Despite the association between *T. dicentrarchi* isolation and observed clinical changes in salmon in Chile, there is no conclusive evidence that this bacterium is a primary or opportunist pathogen.

The ability of particular genotypes of *T. maritimum* (Habib *et al*., 2014) and other *Tenacibaculum spp*. (Olsen *et al*., 2017) to colonize multiple fish species within restricted geographic areas may indicate that the pathogen/host relationship is regulated by geographical limitations rather than host specificity within certain strains. A moderately positive correlation between the presence of *T. dicentrarchi* and *Paramoeba perurans*, the causative agent of amoebic gill disease in Atlantic salmon, has been identified (Slinger *et al*. 2020), but the underlying nature of the relationship and role of *T. dicentrarchi* was not elucidated. Levipan *et al*. (2019) found that *T. dicentrarchi* can rapidly form biofilm and postulated that such biofilm development may represent a transient reservoir of infection. Very little is known of the role wild fish may have, if any, in transmission of tenacibaculosis, or the impact *Tenacibaculum* infections in commercial fish-farming may have upon wild fish populations. Bateman *et al*. (2022) did, however, find a positive association between *T. maritimum* infection in migrating juvenile sockeye salmon *(Oncorhynchus nerka)* and increased infection pressure surrounding British Columbian salmon farms.

> ***The modes of transmission and routes of infection for tenacibaculosis in salmon in general remain unclear.***

TREATMENT AND PROPHYLAXIS

Tenacibaculum spp. infections are most often external. Antibiotic treatment may not be successful and is not generally recommended. However, treatments are at times considered necessary and the response to treatment seems to be variable. One measure to reduce mortalities once the infection is detected is rapid removal of fish with macroscopic lesions, thereby attenuating further transmission of infection.

Currently, vaccination is the only measure to prevent the appearance of diseases of bacterial origin in fish farming, although products using the bacteriophage principle are under development for bacterial control. Some success has been achieved in developing vaccines against *T. maritimum* infections in marine fish species (Romalde *et al*., 2005). Despite the favorable results generated following intraperitoneal application of an adjuvanted vaccine against *T. maritimum*-related tenacibaculosis in Atlantic salmon (van Gelderen *et al*., 2010), no commercial vaccine against tenacibaculosis in salmonids is currently available. The genetic and possibly antigenic variety amongst the bacterial isolates associated with tenacibaculosis, particularly in non-*T. maritimum* associated cases, may complicate the choice of candidate strains for vaccine development (Irgang *et al*., 2017, Olsen *et al*., 2017). Handlinger *et al*. (1997) highlighted the improvement of fish management in general as an important reason for the decline in tenacibaculosis outbreaks in Australia. Handling and other management routines such as mechanical anti-lice treatments should therefore be kept to a minimum. Great care should be taken to avoid compromising the skin barrier of the fish.

THE NORWEGIAN SITUATION

Tenacibaculum spp. have been associated with ulcers of farmed Norwegian salmonids since the late 1980's, either alone or as co-infections with *Moritella viscosa* (Olsen *et al*., 2011). During the last decade however, tenacibaculosis (sometimes referred to as 'atypical' winter ulcer) typified by extreme necrosis and tissue loss involving the head/jaw has become increasingly considered a serious threat to the farming industry. While the northernmost areas of the country, i.e. Finnmark and Troms, are most severely affected, outbreaks occur along the whole coastline. Outbreaks involving head ulcerations occur most commonly in smolts, newly transferred to very cold seawater, although larger fish are also affected. The prevalence within affected cages may be extremely high (>80%) and associated with severe, acute mortality. In some cases, the infection has apparently transmitted from cage-to-cage, although most cases only involve single cages without indications of further spread.

The reasons for the continuing emergence of this extreme type of tenacibaculosis in Norway are unclear, but it is thought that current farming practices are contributing to the situation. Stocking practices have changed in relatively recent years from annual transfers of smolts to sea in spring and autumn, to continual sea-transfer of smolts. Even in Finnmark, the northernmost (and coldest) region of Norway, smolts are transferred to sea in the colder months, although this practice is now becoming less frequent. The introduction of physical, non-medicinal delousing methodologies including treatments based on warm-water, physical brushing and/or waterjets, as well as broad use of H_2O_2 in chemical treatments against amoebic gill disease (AGD), may also have played an important facilitating role. Such procedures are commonly associated with both acute mortality events and mechanical injuries that may subsequently become infected.

Despite remaining uncertainties, the etiology of tenacibaculosis in Norway is becoming clearer. *T. maritimum* has recently been isolated from farmed lumpsucker (Småge *et al*., 2016), turbot (Olsen, 2017), ballan wrasse (Olsen, unpublished) and more recently from the gills of Atlantic salmon farmed in Norway. Characterization and valid publication of *T. piscium* and *T. finnmarkense* gv. *finnmarkense* and gv. *ulcerans* (Olsen *et al*., 2020) has to a significant degree clarified the aetiological situation of tenacibaculosis in Atlantic salmon in Norway. Previous studies (Olsen *et al*., 2011; Habib *et al*., 2014; Småge *et al*., 2016; Olsen *et al*., 2017) all indicated that several species and strains were involved, and four main clades (I-IV) were identified (Olsen *et al*., 2017). These clades have now been confirmed (Olsen *et al*., 2020) to represent

the following taxa: Clade I: *T. finnmarkense* g.v. *ulcerans*, Clade II: *T. dicentrarchi*, Clade III: *T. finnmarkense* gv. *finnmarkense*, and Clade IV: *T. piscium*. *'T. finnmarkense'* described by Småge *et al*. (2016) has also been confirmed as *T. finnmarkense* gv. *finnmarkense* (Olsen *et al*. 2021). A recent study (Spilsberg *et al*., 2022) of *Tenacibaculum* isolates from 15 geographically disparate tenacibaculosis outbreaks in relatively newly sea-transferred Atlantic salmon in 2018 and 2019 identified *T. finnmarkense* as the dominating species with a genetically conserved cluster of *T. finnmarkense* gv. *finnmarkense* being isolated from most cases, against a background of relatively diverse gv. *finnmarkense* and gv. *ulcerans* isolates. *T. dicentrarchi* and *T. piscium* were isolated in very few cases as part of a mixed flora. The overall extensive genetic diversity identified between isolates from individual outbreaks, i.e. lack of within-outbreak clonality, suggests however that fish-to-fish transmission may not represent the primary transmission route.

Combined data from the Norwegian Veterinary Institute and private diagnostic laboratories revealed that winter ulcer, irrespective of underlying causes, was diagnosed in 433 Norwegian salmon farms through 2022 (Sommerset *et al*., 2023). *Tenacibaculum spp*., either in pure or mixed culture (e.g., together with *M. viscosa*), was isolated from 205 salmon farms. Approximately 60% of the isolates were identified to species/genomovariant level, with ~50% and ~33% being respectively identified as *T. finnmarkense* gv. *finnmarkense* and gv. *ulcerans*. While both were primarily isolated during the first months of the year, they showed an uneven distribution across production areas (Figure 1). The remaining isolates belonged to either *T. dicentrarchi* (~4%), *T. piscium* (~2%) or *T. maritimum* (~3%). In the annual survey issued to fish health personnel in Norway, *Tenacibaculum*-related disease was ranked sixth among the most important health challenges in sea-farmed Atlantic salmon (Sommerset *et al*., 2023). In this regard, high overall rankings were also awarded to the closely connected problems with delousing-related injuries (2nd), *M. viscosa* (3rd) and non-specific skin lesions (7th).

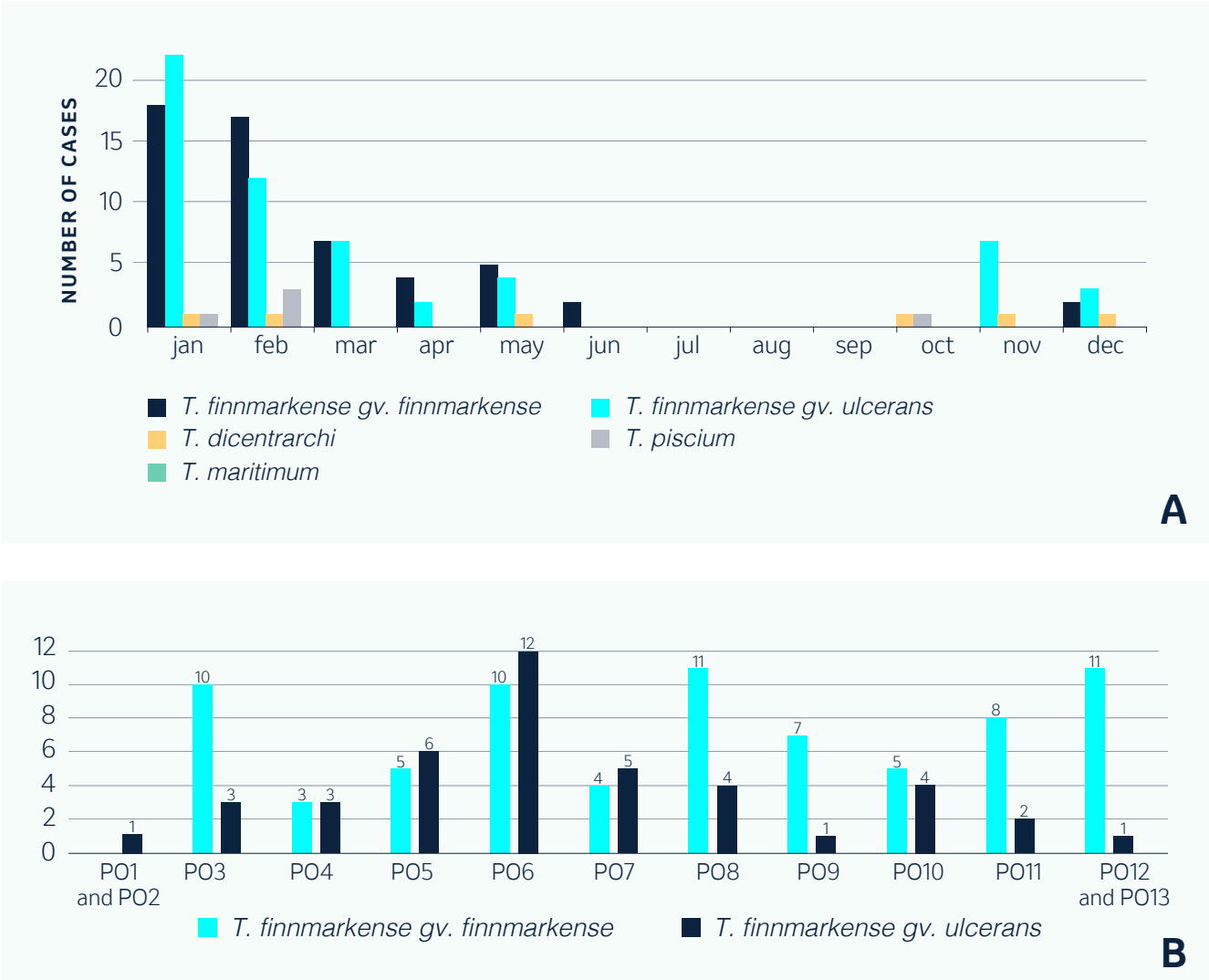

Figure 1: Number of Norwegian salmon farms with *Tenacibaculum spp*. diagnoses made through 2022 (Norwegian Veterinary and private laboratories), per month (A) and per production area/PO (B). Diagrams sourced from Sommerset *et al*., 2023.

DISCUSSION

It is clear that tenacibaculosis in farmed Atlantic salmon, despite many commonalities, is not a uniform disease/infection. The situation in Australia and on Canada's West Coast appears to be entirely dominated by *T. maritimum*-associated infections (Frisch *et al*., 2017), while the Chilean situation is dominated by *T. dicentrarchi* (Avandaño-Herrera *et al*., 2016), *T. maritimum* (Apablaza *et al*., 2017) and *T. piscium* (Avandaño-Herrera *et al*., 2022). In Norway, while *T. finnmarkense* gv. *finnmarkense* and gv. *ulcerans* apparently dominate, *T. dicentrarchi* and *T. piscium* are also occasionally identified (Spilsberg *et al*., 2022). *T. maritimum* is undoubtedly present in Norwegian waters and is for the most related to disease in farmed lumpfish, but it has not yet been identified as the cause of serious pathologies in Atlantic salmon in Norway.

It is evident, therefore, that tenacibaculosis may be associated with several different *Tenacibaculum* species and genotypes. Although we have an increasing understanding of the population structures of *Tenacibaculum* species isolated from diseased fish, we remain relatively in the dark as far as the dynamics of infection and pathogenesis are concerned. Do the fish-related *Tenacibaculum* flora merely represent the *Tenacibaculum* flora of the surrounding environment or are there specific mechanisms involved? Several whole genome sequencing (https://www.ncbi.nlm.nih.gov/genome/?term=*tenacibaculum*) projects have been completed, which have allowed high-resolution population studies and development of highly specific molecular diagnostic tools, as well as identification of various putative virulence factors (Nowlan *et al*., 2023, Pérez-Pascual *et al*., 2017). However, there is no doubt that much work remains to be done in regard to analysis of available and future genome sequences, which undoubtedly contain valuable information relating to pathogenesis, virulence etc. that has not yet been 'mined' and scrutinized.

Successful vaccine strategies have yet to be developed. Little is known of the antigenic and genetic heterogeneity within *Tenacibaculum* populations, particularly non-*T. maritimum* isolates. Such knowledge will be essential for the development of effective vaccines.

Although available evidence indicates environmental factors to be of crucial importance for the pathogenic potential of various *Tenacibaculum* species, manifestation of tenacibaculosis could also be deeply influenced by other factors. Therefore, thorough epidemiological studies are required to precisely determine risk factors such that this knowledge may be used to control and prevent future outbreaks.

Finally, while antibiotic treatment should be considered a last resort, research is needed to evaluate the efficacy of various antibiotic treatments. Likewise, alternatives to chemotherapeutics, such as probiotics and antimicrobial peptides, should be investigated.

As salmon farms become ever larger and farming more technically demanding, there are few reasons to believe *Tenacibaculum* problems will disappear in the near future, unless management routines now known to damage skin health are improved upon or discontinued.

Climate change and the expansion of salmon farming into new geographical areas may also lead to new *Tenacibaculum*-related disease problems. The recent isolation of *T. maritimum* from lumpsucker and salmon in Norway and salmon in Chile shows that this bacterium is already present in areas of still relatively cold water. Even slight increases in water temperature may result in the establishment of *T. maritimum*-associated disease in salmon farming in more northern areas such as Norway. There are constant expansion pressures in salmon farming and increasing sea temperatures may allow farming of salmon in polar areas. This will very likely result in problems associated with the colder water types of *Tenacibaculum*. Therefore, there are many good reasons to maintain awareness and a research focus on *Tenacibaculum.*

Climate change and the expansion of salmon farming into new geographical areas may also lead to new Tenacibaculum-related disease problems.

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TENACIBACULUM SPP IN CHILE

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INTRODUCTION

Although the term tenacibaculosis was originally coined to refer to infections caused by *T. maritimum*, the term is now used to refer to a bacterial disease caused by any of the species of the genus *Tenacibaculum*.

Even though *Tenacibaculum* does not cause outbreaks, it is important to control its occurrence in fish farms. Recent publications indicate that this occurrence may be associated with environmental challenges, inadequate handling and low resistance of fish to the presence of bacteria of this genus.

In Chile, for years it was discussed whether the cases of *Tenacibaculum* were really a primary cause of disease or if they corresponded to secondary infections of cases caused by *Piscicketssia salmonis*. While this debate was happening, cases associated with *Tenacibaculum* infections increased significantly (mainly due to *Tenacibaclum dicentrarchi* infections in Atlantic salmon). According to the information provided by the Chilean national fishing and aquaculture authorities, there has been an important and permanent increase of tenacibaculosis cases in the country. In 2018, 4.3% of cases associated with this pathology were reported, in 2019 these cases grew to 14%, in 2020 the reports increased to 25.3% and in 2021 the mortality due to this pathogen had already increased to 31.3% (SERNAPESCA's annual health reports).

This significant increase in mortality led SERNAPESCA (the office responsible for the regulation and health control of aquaculture in Chile) to issue its Exempt Resolution N° DN - 01606/2021 in which it added a new mortality classification, tenacibaculosis, into the General Health Program for Mortality Management and Classification. This disease was included in List 3 of High-Risk Diseases in passive surveillance, i.e., it is associated with reports delivered monthly by the diagnostic laboratories and to reports of mortalities that the company sends to the authorities. At the same time, it is classified as a re-emerging disease (infectious disease that has already been diagnosed and whose incidence has increased recently, after a period in which there has been a decrease in its incidence or changes in its geographical distribution).

CLINICAL SIGNS

The clinical signs of Tenacibaculosis may vary depending on the stage of the disease and the species of fish affected. However, some of the most important clinical signs to be aware of in cases of tenacibaculosis include:

Skin injuries: tenacibaculosis often produces skin injuries, which may appear as ulcers, erosions, or raised areas (bumps) on the skin. Injuries may be reddish or necrotic in appearance and are commonly found on the fins (the disease can cause severe rotting, leading to frayed or eroded fins, and in advanced cases, fins may even be partially or completely destroyed), on the mouth (mouth erosion with reddening of the skin in the mandibular area, yellowish plaques on the jaw and tooth bone), and on the tail (necrosis of the caudal fin).

Changes in behavior: Fish affected by tenacibaculosis may show behavioral changes such as decreased activity, lethargy and reduced swimming ability.

Loss of appetite: Infected fish may show reduced appetite and decreased feeding activity.

Weight loss: Tenacibaculosis can lead to weight loss in affected fish.

Skin discoloration: the skin around the injuries may become discolored and show reddish or dark areas.

It is important to keep in mind that the clinical signs of tenacibaculosis may overlap with other diseases, making the final diagnosis difficult. Therefore, appropriate diagnostic techniques, such as bacterial culture, PCR or histopathological examination are essential for accurate identification of *Tenacibaculum* species and confirmation of the disease.

It is important to emphasize that early detection and prompt treatment are crucial to effectively manage tenacibaculosis and minimize its impact on aquaculture operations.

> ***The distribution of tenacibaculosis in Chile has extended to all regions where salmonids are farmed in seawater, that is, from Región de Los Lagos to Región de Magallanes and not only affecting Atlantic salmon.***

EPIDEMIOLOGY

All species of *Tenacibaculum* are found exclusively in aquatic environments, attached to or associated with the surface of marine organisms such as macroalgae, various invertebrates and fish. Besides *T. maritimum*, seven other *Tenacibaculum* have been identified as fish pathogens: *T. ovolyticum* in Atlantic halibut *(Hippoglossus hippoglossus)* (Hansen *et al*., 1992; Avendaño-Herrera *et al*., 2022a), *T. discolor* in European and Asian seabass (*Dicentrarchus labrax* and *Lates calcarifer*) (Piñeiro-Vidal *et al*., 2008a), *T. soleae* in Senegalese sole *(Solea senegalensis)* (Piñeiro-Vidal *et al*., 2008), *T. dicentrarchi* in Atlantic salmon *(Salmo salar)*, rainbow trout *(Oncorhynchus mykiss)*, coho salmon *(Oncorhynchus kisutch)*, turbot, sea bass and red conger eel (Piñeiro-Vidal *et al*., 2012; Avendaño-Herrera *et al*., 2016; Avendaño-Herrera *et al*., 2020), *T. finnmarkense* in rainbow trout *(Oncorhynchus mykiss)* and Atlantic salmon *(Salmo salar)* (Olsen *et al*., 2020, Bridel *et al*., 2018), and *T. singaporense* (Miyake *et al*., 2020) and *T. piscium* in corkwing wrasse *(Symphodus melops)* and Atlantic salmon *(Salmo salar)* (Olsen *et al*., 2020; Avendaño-Herrera *et al*., 2022b).

In Chile, of the eight pathogenic species mentioned above, through publications or direct diagnosis in fish, the presence of six of them have been reported: *T. dicentrarchi*, *T. maritimum*, *T. finnmarkense* and *T. piscium* (to this we must add that recent publications refer to an isolation of *T. ovolyticum* and another that mentions the finding of *T. soleae*).

The distribution of tenacibaculosis in Chile has extended to all regions where salmonids are farmed in seawater, that is, from Los Lagos to Magallanes region and not only affecting Atlantic salmon, the cases caused by bacteria of this genus have also spread to species such as *Oncorhynchus kisutch* (coho salmon) and *Oncorhynchus mykiss* (Rainbow trout), (Avendaño Herrera, R., & Co, 2020).

TREATMENT

In Chile, tenacibaculosis has been a major problem in the aquaculture industry, especially in salmon farming. This disease can cause significant losses in salmonid production, due to the high morbidity and mortality associated.

One of the best-known pathogens, and to date the main problematic agent of the genus *Tenacibaculum* in Chile, is *Tenacibaculum dicentrarchi.*

Until now, there is no vaccine against tenacibaculosis so the control and treatment of this disease is done by using antimicrobials, mainly Florfenicol and Oxytetracycline. In 2019 alone, 6.2 tons of Florfenicol and 3.2 tons of Oxytetracycline were used to control this disease. However, between the years 2018 and 2020, some sea fish farms used Tiamulin (extra-label use) to control outbreaks of this disease; these treatments were shown to have high effectiveness, managing to reduce mortalities after 25 to 30 days of the use of this drug (Irgang R., & Avedaño Herrera R., 2022).

Sernapesca has established surveillance, prevention, and control protocols for tenacibaculosis in the Chilean aquaculture industry. This includes biosecurity measures in fish farms, early detection of the disease, application of appropriate treatments and implementation of management strategies to minimize the impact of the disease on fish production.

The control of tenacibaculosis in Chile is based on an integrated approach that combines sanitary measures, such as regular monitoring of fish farms, the use of good management practices and the application of antibiotic treatments when necessary. In addition, scientific research is promoted to improve understanding of the epidemiology of the disease in order to develop more effective control and prevention strategies.

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TENACIBACULUM PREVALENCE IN BRITISH COLUMBIA (CANADA)

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BACKGROUND

Tenacibaculosis, an emerging disease in Canada, causes significant outbreak events among post-seawater entry smolts, often resulting in mortalities of up to 60% in affected cages (Personal Communication). In British Columbia (BC), the disease is clinically identified by the presence of yellow plaques on the fish's mouth, commonly known as "yellow mouth" or "mouth rot". This bacterium was initially documented in BC and Washington State in the late 1980s and continues to affect BC's aquatic population annually (Kent *et al*., 1988; Frelier *et al*., 1994). Local aquaculture companies have reported two distinct peaks in mortalities within BC waters.

The initial peak occurs one to two weeks after the smolts enter seawater, followed by a second peak when the fish reach approximately 500 grams, after around 3-4 months at sea (Hewison, 2019). Reported mortalities attributed to tenacibaculosis seem to occur during periods of elevated seawater temperatures (> 10°C) (Frisch *et al*., 2018a). In terms of economic impact, the annual cost of managing tenacibaculosis outbreaks in BC is estimated to be $1.8 million, based on the last recorded generation in 2016-2017. This translates to a substantial revenue loss of approximately $3.8 million per year for the local aquaculture industry.

EPIDEMIOLOGY

In British Columbia (BC), diagnostic information for farmed salmon is sourced from three channels: Department of Fisheries and Oceans (DFO) audit records, DFO Fish Health Event (FHE) records, and industry-provided data. The identification of mouth rot is conducted through gross pathology, with particular attention to the presence of yellow mouth plaques observed on the farm. Tissue samples are then sent to a diagnostic laboratory for microscopic examination, focusing on filamentous bacteria, and for assessment of inflammation in the jaw (Wade and Weber, 2020). Mouth rot was diagnosed in 7.3% audits (106 out of 1446) between 2002 and 2018. Data showed that bet-ween 2002 and 2013, as well as between 2015 and 2018, a total of 537 FHEs were attributed to mouth rot on Atlantic salmon farms in BC (Wade and Weber, 2020).

Both in the field outbreaks and controlled bath challenges, infected smolts predominantly exhibit mouth lesions. These lesions are focal, severe and progress rapidly, often accompanied by limited associated inflammation. The onset of yellow mouth varies, manifesting between a few weeks to 2 months post-transfer to saltwater, with mortality rates fluctuating from 0.15% to 5% among the regions. Factors such as salinity levels, smoltification and stressors like transportation and harmful algae presence exert significant influence on disease prevalence. It is noteworthy that the disease tends to disappear as the fish reach a certain size, although whether this phenomenon is attributed to fish immunology or disease mechanisms remains an unanswered question (Hewison, 2019). All the Canadian isolates were derived from Atlantic salmon reared at temperatures ranging from 8.7-14.7°C. In contrast, according to Ostland *et al*. (1999), all mouth rot isolates exhibited growth across a temperature range of 12-30°C.None of the isolates demonstrated growth at 5°C or 37°C.

The etiological agent, *Tenacibaculum spp*., has been observed to spread through direct fish contact and the spread of bacteria from infected fish into the water (Frisch *et al*. 2018b; Nowlan *et al*. 2021). It was hypothesized that fish-to-fish contact in densely populated net pens may represent a way of transmission, but the shedding of bacteria into the environment could be another potential avenue for short-term dissemination (Frisch *et al*. 2018a). However, *Tenacibaculum* is ubiquitously found in marine environments and forms biofilm on infrastructure (Nowlan *et al*., 2021). The study identifies a potential relationship between seasonality and the occurrence of tenacibaculosis, with more bacteria recorded in the summer months, along with increased mortality events and antibiotic treatments (Nowlan *et al*., 2021). Nevertheless, further investigation on bacteria transmission origin and water dynamics in BC net pen infrastructure is necessary to understand the epidemiology of the disease.

GENETIC DIVERSITY

Examination of tissue tropism through real-time RT-PCR assays has shown the internal presence of *T. maritimum* in infected smolts, with isolation of the bacteria from the kidney, suggesting systemic dissemination of the bacterium within the host.

Although variations in virulence have been reported between *T. maritimum* isolates, genetic analysis of bacteria isolated from field outbreaks has revealed a homogeneous population. In the region surrounding Vancouver Island, the presence of both CAN1 and CAN2 strains of *T. maritimum* has been genetically characterized.

Frisch *et al*. (2018a) findings revealed that *T. maritimum* isolates obtained from BC Atlantic salmon exhibiting clinical mouth rot grouped into two distinct sequence types (STCan1, STCan2). These BC types were closer genetically to a Norwegian lumpfish strain (NLF-15, cultured at 12°C) and a Chilean Atlantic salmon strain (Ch-2402, cultured at 14°C).

Recently, genomic analysis of BC-farmed Atlantic salmon isolates identified four known species *T. ovolyticum*, *T. dicentrarchi*, *T. finnmarkense* and *T. maritimum* and two proposed novel species *T. pacificus* and *T. retecalamus* (Nowlan *et al*., 2022). These findings suggest a high species diversity in BC water as previously believed. Long-read genomic analysis predicted hemolysins in all sequenced isolates with additional putative toxins and type-IX secretion systems as well as antimicrobial resistance genes such as oxytetracycline resistance (Nowlan *et al*., 2022). Further research is needed to investigate the presence of the genes in relation to virulence and antimicrobial resistance phenotype (Nowlan *et al*., 2022; Britney *et al*., 2022; Britney, 2023).

TREATMENT

Treatment primarily relies on antibiotics, administered at varying frequencies, ranging from an average of 0.75 treatments per cage to 5.41 treatments per cage, depending on the specific region (Hewison, 2019). The current mitigation strategy for tenacibaculosis is treatment with antibiotics such as florofenicol or potentiated sulfonamides during the first two months after seawater entry. The use of antibiotics in BC waters remains a challenge for salmon farming companies because it impacts certification by the Aquaculture Stewardship Council (https://www.asc-aqua.org/). Indeed, tenacibaculosis is one of the last uses of antibiotics in BC salmon farming. Between 2018 and 2020, the recommended effective dose for treating tenacibaculosis with florfenicol in Canada is 10 mg/kg/day, while oxytetracycline is approximately 75 mg/kg/day (Government of Canada, 2022a, 2022b). However, the frequent use of a limited range of antimicrobials for treating infectious diseases poses a continuous risk of developing antimicrobial resistance. To date, none of the 80 tested isolates surpassed the wild-type cut-off (16 µg/mL) in British Columbian waters (Britney *et al*., 2022; Britney, 2023). Given the limited research on antimicrobial resistance among *Tenacibaculum* species, a coordinated, interlaboratory effort is imperative to establish regional and global thresholds and monitor collected bacteria for signs of developing antimicrobial resistance.

Recent studies have shown that a formalin-inactivated bacterin vaccine, either for *T. mar* (TmarCan15-1, TmarCan16-2, and TmarCan16-5) (Frisch *et al*., 2018a) or *T. finn* (HFJT) (Småge *et al*., 2018b), resulted in an increased *Tenacibaculum*-specific antibody response in Atlatic salmon. However, following experimental bath-exposure, mortality rates were comparable to non-vaccinated fish, providing limited to no protection (Frisch *et al*., 2018b; Småge *et al*., 2018b). In British Columbia, a concerted research effort on vaccines should be explored, leveraging advanced genome analysis and the selection of potential target antigens.

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MORITELLA VISCOSA, THE CAUSATIVE AGENT OF WINTER ULCER IN FARMED ATLANTIC SALMON

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INTRODUCTION

Winter ulcer, first described by Lunder *et al*. (1990), is a particular problem in Norwegian aquaculture, but also impacts the salmon farming industries of Scotland, Ireland, Iceland, the Faroe Isles and the eastern seaboard of USA/Canada (Benediktsdóttir, Verdonck, Spröer, Helgason, & Swings, 2000; Bruno, Griffiths, Petrie, & Hastings, 1998; Lunder, Evensen, Holstad, & Hastein, 1995; Whitman, Backman, Benediktsdottir, Coles, & Johnson, 2001). The disease is primarily associated with and can be recreated by exposure to, the bacterium *Moritella viscosa* (previously *Vibrio viscosus*). Winter ulcer remains, despite vaccination against *M. viscosa*, the most financially significant bacterial disease in Norwegian salmon farming and is a severe animal welfare issue (Sommerset, 2022). In a report from 2015, a conservative estimate of 1.1 – 2.5% overall losses were estimated due to direct mortality or downgrading at slaughter as a result of winter ulcer in Norway (Takle *et al*., 2015). As skin infections in farmed fish are non-notifiable and are relatively easily diagnosed at the local level, they are almost certainly underreported. Therefore, official statistics of the prevalence of this type of disease do not exist, but there is a consensus within the industry that the situation has considerably worsened in Norway in recent years (Sommerset 2022).

While the term “winter ulcer” is mainly associated with *Moritella viscosa* infection, which results in development of open ulcers, primarily on the flanks of affected fish (see Figure 1), the term “tenacibaculosis” is used for another cold-water associated skin condition associated with *Tenacibaculum spp*., which affects primarily the head, fins, and tail of the fish. There is, however, often no clear delineation between these two conditions, as mixed infections with *M. viscosa* and *Tenacibaculum spp*. are common. As tenacibaculosis is covered in a separate chapter in this report, this disease will not be discussed further here.

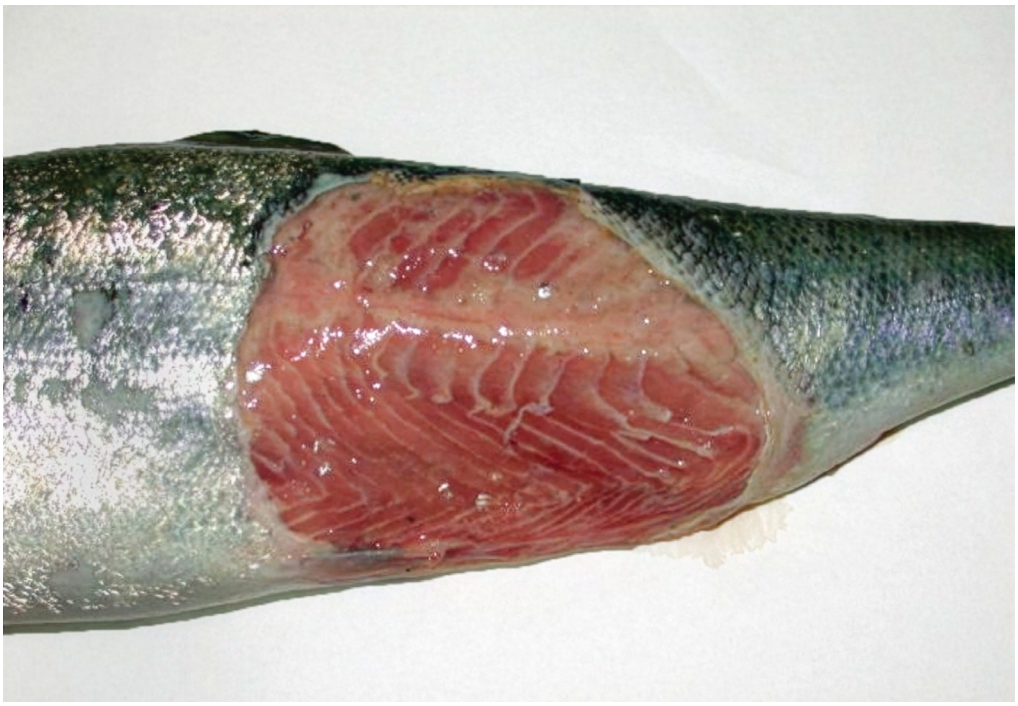


Figure 1: Atlantic salmon with large *Moritella viscosa*-associated "winter ulcer".
Photo: Duncan J. Colquhoun

M. viscosa-related winter ulcer in Atlantic salmon farming is primarily associated with low water temperatures, normally below 8°C in Norway (Lunder *et a*l. 1995), although outbreaks may occur at any time of the year in any location along the coast. *M. viscosa*-associated ulcer outbreaks are, however, regularly reported at higher temperatures in Eastern Canada (Mackinnon *et a*l. 2019).

Bacteriological investigation of exposed skin ulcers often results, unsurprisingly, in identification of a mixed flora. Although *M. viscosa* and/or *Tenacibaculum spp*. may be isolated alone or as mixed infections, other bacteria such as *Aliivibrio wodanis*, *Aliivibrio logei* and *Vibrio splendidus* are commonly co-isolated. Although early infection trials with *A. wodanis* failed to recreate winter ulcer, isolation of this bacterium, alone or in mixed culture with *M. viscosa* from the ulcers and internal organs of fish with winter ulcer, is not uncommon (Lunder 1992). The significance of such infections has not been clarified, but it cannot be ruled out that *A. wodanis* has a role in the development of winter ulcer. Interestingly, both *M. viscosa* and *A. wodanis* have also recently been reported from farmed salmonids in the Northwest region of the Russian Federation (Droshnev *et a*l. 2019).

TAXONOMY/DIVERSITY

Initially named *Vibrio viscosus* (Lunder *et al.* 2000), *Moritella viscosa* (Benediktsdóttir *et al.*, 2000) was described as a genetically and phenotypically homogenous species based on the study of isolates originating almost exclusively from farmed salmon in Norway (Lunder *et al.*, 2000). However, a degree of genetic (Benediktsdóttir *et al.*, 2000) and antigenic (Heidarsdóttir, Gravningen, & Benediktsdóttir, 2008) heterogeneity was quickly identified following comparison of geographically disparate isolates from different fish species. Later work identified a specific genotype (then termed "typical" *M. viscosa*) almost exclusively associated with salmon displaying winter ulcer (Grove *et al.*, 2010; Karlsen *et al.*, 2014) within a restricted geographical area (Norway, Scotland and the Faroe Isles), while genetically diverse isolates predominated amongst other fish species and in salmon farmed in Iceland and Canada.

Initial whole-genome sequencing (WGS) of a small collection of isolates confirmed the existence of several distinct phylogenetic lineages amongst clinical *M. viscosa* strains (Karlsen, Hjerde, Klemetsen, & Willassen, 2017).

A multi-locus variable number tandem repeat analysis (MLVA) genotyping scheme was recently published for the epidemiological study of *M. viscosa* (Sørgaard *et al.* 2023). Analysis of nearly 750 isolates, mostly from farmed Norwegian salmon, but also covering various other fish species, resulted in identification of three major clonal complexes (CC1, CC2 and CC3) alongside a number of minor clusters and singletons (Figure 2).

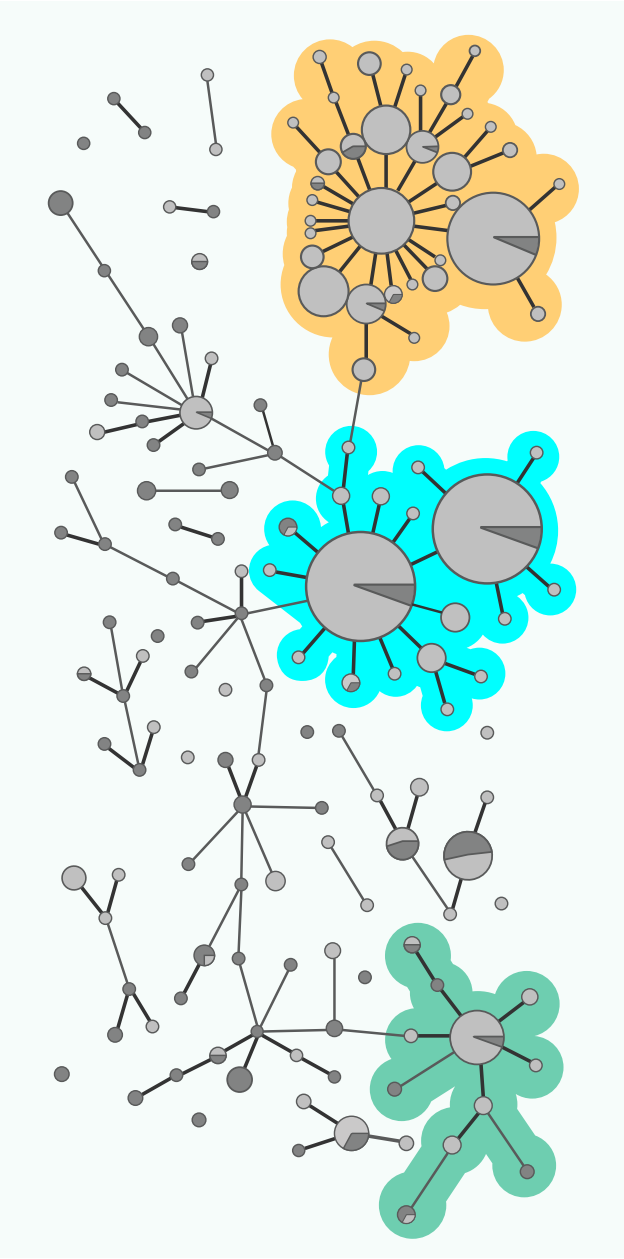


Figure 2: MLVA clustering of *M. viscosa* from Atlantic salmon and other species of fish.

Comparative genomics involving representative genomes from each clonal complex supported the relationships identified by MLVA. While nearly all (90%) of the salmon isolates belonged to CC1, CC2 or CC3, only 20% of isolates recovered from other fish species did so. This indicates a considerable degree of specificity within the major clonal complexes for Atlantic salmon as a host. Further, a number of shifts in the dominating clonal complex affecting Norwegian farmed salmon were identified over the 35-year sampling period. Until the emergence of CC2 in 2003, only CC1 had been identified from Norwegian salmon. After about a decade, during which both CC1 and CC2 shared dominance, CC3 then emerged in Norwegian aquaculture in 2012 and dominated until 2021 when CC1 appeared to be making a comeback. Apparently, these shifts have rapidly swept the entire Norwegian coastline and conceivably, as suggested by typing of a small number of non-Norwegian isolates, the Northeast Atlantic region as a whole. Interestingly, both CC2 and CC3 had been isolated from Icelandic salmon as early as 1992 and 1994, respectively.

>10°C

MACKINNON *ET AL.* (2019; 2020) DESCRIBED ULCER DEVELOPMENT AS A RELATIVELY COMMON SUMMER AND AUTUMN PROBLEM IN FARMED ATLANTIC SALMON IN ATLANTIC CANADA AT WATER TEMPERATURES >10°C.

MORITELLA VISCOSA AS A PATHOGEN OF FARMED ATLANTIC SALMON

Koch's postulates confirming the aetiological role of *Moritella viscosa* in winter ulcer were rapidly established (Lunder *et al*., 1995; Bruno *et al*., 1998). Salmon of all sizes farmed in seawater may be affected, including fish held in juvenile production units utilising seawater (Lunder 1992). The disease is typically characterized by wounds of varying size, often on the flanks, which penetrate the skin and expose underlying muscle tissue (Lunder *et al*. 1995). *M. viscosa*, (in contrast to *Tenacibaculum spp*., in Norwegian aquaculture) may establish systemic infections with considerable pathology in affected internal organs (Lunder *et al*., 1995, Bruno *et al*., 1998). Løvoll *et al*. (2009) identified the early and consistent presence of *M. viscosa* in salmon gill tissues following bath exposure to the bacterium and suggested that the gills may be an important point of entry, while Mackinnon *et al*. (2020) concluded that systemic *M . viscosa* infections are probably most commonly the result of initial skin infection. This is consistent with the findings of Karlsen *et al*. (2012) who found the invasive ability of *M. viscosa* to be greater in the head and gill area compared to other areas.

Although acute mortality episodes may occur, *M. viscosa*-associated winter ulcer outbreaks usually extend over long periods of moderately elevated mortality. They generally involve water temperatures below ~8°C (Lunder *et al* 1995) and the infection may resolve in surviving fish on increase in water temperature. However, Mackinnon *et al*. (2019; 2020) described ulcer development as a relatively common summer and autumn problem in farmed Atlantic salmon in Atlantic Canada at water temperatures > 10°C. Although there may be underlying differences in the epidemiology of winter ulcer in the western and eastern Atlantic areas, injection of Atlantic salmon with extracellular products (ECP) from a Canadian *M. viscosa* isolate resulted in

pathology consistent with that identified during outbreaks of Canadian ulcer disease, thus supporting a causal relationship between the bacterium and the disease (Mackinnon *et a*l., 2020). *M. viscosa*-associated winter ulcer has not yet been reported in the North-eastern Pacific area (Jia *et a*l. 2020), but environmental DNA (eDNA) based investigations suggest that *M. viscosa* is present in that area and that the highest concentrations of both *Tenacibaculum maritimum* and *M. viscosa* eDNA correlate with proximity to domestic salmon populations (Nekouei *et a*l. 2018; Shea *et a*l. 2020).

While there are, as yet, no reports of *M. viscosa*-associated ulcerous disease in Chilean farmed salmon, 16S rRNA gene sequences with significant similarity to *M. viscosa* have been associated with the parasitic salmon louse *Caligus rogercressyi* in Chile (Morales-Ribera *et a*l. 2022).

In the Atlantic Canadian situation at least, there appears to be little evidence of horizontal spread of winter ulcer between cages and farms (Mackinnon *et a*l. 2019). On investigation of the diversity of *M. viscosa* isolates involved in individual outbreaks in Norway, Sørgaard *et a*l. (2023) found that in many cases, more than one clonal complex could be isolated from individual fish within an affected population.

These authors reflected that already damaged skin may conceivably allow colonisation with isolates of (possibly) varying virulence and that the variety of clonal complexes identified may result from a complex epidemiological situation influenced by bacterial virulence, underlying skin lesions (e.g. from physical delousing) and the fluctuating presence of various genotypes in the bacterioplankton.

Development of winter ulcer may thus result from a complex interplay between environmental factors and the general health status of the fish. While infection trials indicate that exposure to *M. viscosa* alone is sufficient to instigate an outbreak of winter ulcer, there is little doubt that management procedures such as handling and delousing result in skin damage and thereby predispose the treated fish to winter ulcer. Carvalho *et a*l. (2020) concluded that co-infestation with high numbers of the salmon louse *Lepeopththeirus salmonis* can enhance the impact of *M. viscosa* infection and significantly reduce the ability of lesions to resolve, resulting in increased mortality. There are indications that triploid Atlantic salmon may be more susceptible than diploid to *M. viscosa* associated winter ulcer (Sindre 2018; **https://www.fhf.no/prosjekter/prosjektbasen/901076/**, in Norwegian).

A number of putative virulence factors have been identified in *M. viscosa.* Bjørnsdottir *et a*l. (2009) characterised a previously unknown vibriolysin, a metallopeptidase produced in late exponential growth, which was non-lethal to salmon but caused considerable tissue necrosis and haemorrhage at the site of injection, thereby possibly playing a role in invasion and dissemination. Bjørnsdottir *et a*l. (2011) concluded that virulent isolates secrete a lethal toxic factor that may reflect host adaptation, and subsequently identified two type VI secretion systems (Bjornsdottir *et a*l., 2012). Relatively little has been published regarding the genetic background for the host specificity of various clonal complexes proposed by Sørgaard *et a*l. (2023). Karlsen *et a*l. (2014) found a large number of tentative virulence factors common across a panel of 38 *M. viscosa* isolates independent of origin and host species (including a selection of other *Moritella spp*.). They did, however, identify a putative

insecticidal toxin complex (mitABC) almost exclusively amongst members of MLVA CC1 (Sørgaard *et al*, 2023). CC1 is synonymous with the "typical" group proposed by Grove *et al*. (2010), which induced high mortality in Atlantic salmon but not in rainbow trout, *Oncorhynchus mykiss* (Karlsen *et al*., 2014).

Molecular investigations have shed some light on the pathogenesis of winter ulcer. Hjerde *et al*. (2015) found that *A. wodanis* impeded the growth and altered gene expression in *M. viscosa* when present as a co-infection. Eslamloo *et al*. (2022) found that *M. viscosa* caused massive dysregulation in Atlantic salmon skin gene transcription, including immune effectors, focused to the site of infection. Krasnov *et al*. (2021) identified increased immune gene expression in populations of salmon previously exposed to low dissolved oxygen levels, but found that this was actually ensued by higher mortality in this group following *M. viscosa* exposure.

MORITELLA VISCOSA AS A PATHOGEN OF OTHER FISH SPECIES

M. viscosa has been isolated and appears capable of causing disease, albeit not to the same extent as that found in Atlantic salmon farming, in a number of other fish species. These include farmed rainbow trout (Grove *et al*., 2010), Atlantic cod, *Gadus morhua* (Colquhoun *et al*., 2004), lumpfish, *Cyclopterus lumpus* (Einarsdottir *et al*., 2018), various wrasse species, Atlantic halibut, *Hippoglossus hippglossus*, whiting, *Merlangius merlangus* (Sørgaard *et al*. 2023) and plaice, *Pleuronectes platessa* (Lunder *et al*., 2000). As Sørgaard *et al*. (2023) have shown, the strains found from these species (including rainbow trout) generally do not belong to those complexes dominating Atlantic salmon farming in Norway.

DIAGNOSIS OF *MORITELLA VISCOSA*

M. viscosa infections are relatively easily confirmed by a combination of bacteriological culture, histopathological investigation and immunohistochemistry. Diagnostic PCRs have been developed (Grove *et al*., 2008; Netzer *et al*., 2021) and are offered by many commercial diagnostic laboratories.

CULTURE AND CLASSIFICATION

The culture of *M. viscosa* is dependent on the supplementation of culture media with NaCl. While good growth is achieved on most general-purpose agars containing a final NaCl concentration of 2% incubated aerobically at 15°C, for diagnostic purposes and detection of subtle differences in colony pigmentation and hemolysis production, blood-agar with 2% NaCl (BAS) is recommended. On BAS, *M. viscosa* produces flat, entire, grey to grey-yellowish colonies of 2-4 mm after 48-72 hrs incubation. Initially named due to the viscous nature of its colonies, it seems this characteristic may be less pronounced than in previous years and, in Norway at least, there seems to be an increasing number of non-viscous isolates identified. As mentioned previously, isolation of mixed flora is quite common from fish displaying winter ulcer, especially on culture from ulcers. *M. viscosa* is a relatively slow-growing bacterium and many of the more "opportunistic" taxa, often co-cultured from ulcers, grow faster and may therefore overgrow *Moritella* colonies. Additionally, *A. wodanis* may directly inhibit growth of *M. viscosa.* It has been demonstrated that the addition of the vibriostatic agent 0129 (2, 4-Diamino-6,7-di-iso-propylpteridine phosphate, to which *M. viscosa* is relatively resistant) to diagnostic agar may result in reduced growth of contaminating bacteria and greater recovery of *M. viscosa*. Although the fastest growth was identified at 15°C, Benediktsdottir and Heidarsdottir (2007) found that cultures reached a maximum density at 4°C.

These authors hypothesized that differences in resistance to cell membrane lysis at 4 and 15°C may be related to their relatively high omega-3 polyunsaturated fatty acid content.

RESERVOIRS AND TRANSMISSION

The reservoirs of *M. viscosa* remain largely unknown, but they are, as other members of the genus *Moritella*, considered natural inhabitants of deep, cold, marine waters. This suspicion is substantiated by the fact that fish farms pumping deep seawater often experience winter ulcer problems. Therefore, it is likely that infections at least initially arise from environmental sources/ bacterioplankton. This is further supported by single winter ulcer outbreaks, and even individual affected fish, often displaying co-infection with multiple *M. viscosa* strains (Sørgaard *et al*. 2023), which points toward colonization from the surrounding seawater as perhaps more important than direct fish-to-fish transmission for spreading the disease. The high degree of genetic (micro) diversity evidently existing within the species (Sørgaard *et al*. 2023), with only a few specific strains regularly recurring in salmon ulcers, points towards diverse marine *M. viscosa* populations that are possibly subject to niche partitioning. Although not yet proven, it is thus conceivable that shifting conditions in the marine environment including e.g., temperature and nutrient availability, may to some extent influence the risk for winter ulcer development via "sculpting" of the *M. viscosa* strain composition in the bacterioplankton. The temporally shifting dominance of various clonal complexes in relation to winter ulcer in Norway may, in part, have arisen due to such processes.

TREATMENT AND PROPHYLAXIS

In severe cases there is some use of antibacterial treatment, but the effect is variable and uncertain. Management routines that may aggravate the situation, e.g., grading, physical delousing, should be avoided as much as possible. Removal of affected and dead fish from the population is advisable. Emphasis should be placed on good smolt quality, optimal conditions around sea-transfer and reduced stress. A commercial product based on *M. viscosa*-specific bacteriophages, intended for eliminating the bacterium, e.g., from treatment water during delousing, was also recently announced.

Greger and Goodrich (1999) reported good protection against i.p. challenge in both Atlantic salmon and rainbow trout utilising a multi-valent, intraperitoneal oil-adjuvanted vaccine including a *M. viscosa* (then *V. viscosus*) component. Vaccination of Norwegian farmed salmon against *M. viscosa* is normal practice and most if not all sea-farmed salmon in Norway are vaccinated against this bacterium. Whilst providing relatively good protection in controlled laboratory trials, *M. viscosa*-associated winter ulcer continues to be problematic in vaccinated fish in the field. It is quite possible that a combination of natural infection pressure, stress, and development of management-related skin lesions overcomes, in many cases, the protection afforded by vaccination. Most currently available vaccines are based on an *M. viscosa* CC1 component although in recent years many outbreaks have been linked to a different genotype, i.e., CC3 (Sørgaard *et al*. 2023). The degree of cross-protection awarded by current vaccines against the various genotypes involved has not been clearly established.

Antibodies specific to bacterial antigens, including *M. viscosa*, appeared as early as one week following vaccination of salmon par with a multi-component oil-based vaccine (Lund *et al*. 2019). Interestingly, gene expression studies also indicated a number of significant non-vaccine specific immune responses that may play an important role early in the post-vaccination period (Lund *et al*. 2019). Chukwu-Osazuwa *et al*. (2022) used a reverse-vaccinology approach to identify a total of 154 antigens common to *Moritella viscosa*, *Piscirickettsia salmonis*, *Aeromonas salmonicida*, *Yersinia ruckeri* and *Vibrio anguillarum*. Low sequence identity but good structural homologies suggest that this could represent a promising future avenue for vaccine development.

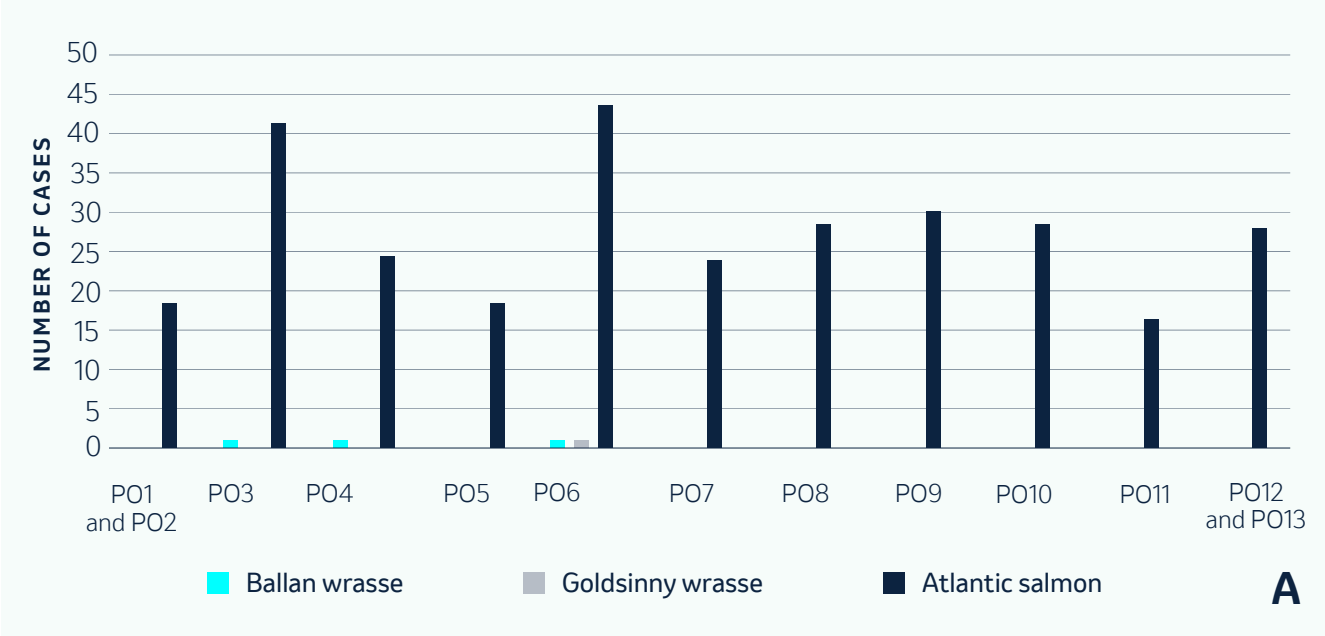
Probiotic treatment has been proposed as having a beneficial effect towards many different diseases. Klakegg *et al*. (2020a) concluded that pre-exposure of juvenile farmed lumpfish to a probiotic mix of *Aliivibrio* species resulted in increased resistance to natural *M. viscosa* infection and reported lower mortality and lower ulcer development in Atlantic salmon similarly treated under field conditions (Klakegg 2020b). De O. Roberti Filho and co-workers (2019) found that dietary supplementation with Beta-glucans increased resistance in both vaccinated and unvaccinated Atlantic salmon to challenge with *M. viscosa* and infectious salmon anaemia virus (ISAV).

Biosecurity in all fish diseases is important and in land-based farms UV treatment of intake water is the norm. Greater than 3log inactivation of *M. viscosa* was achieved using low pressure (3mJ/cm^2) and medium pressure (2.3mJ/cm^2) UV (Justad 2021).

THE NORWEGIAN SITUATION

As previously mentioned, the situation regarding winter ulcer in Norway seems to be worsening. This is probably largely linked to less than adequate vaccine protection and the increasingly stressful farming environment, with smolts being transferred to sea under non-favourable water temperatures and subjected to regular physical delousing, which has increased since ca. 2015 (Sommerset *et a*l., 2023).

Combined data from the Norwegian Veterinary Institute and private diagnostic laboratories revealed that winter ulcer, irrespective of underlying causes, was diagnosed in 433 Norwegian salmon farms throughout 2022 (Sommerset *et a*l., 2023). *M. viscosa*, either in pure or mixed culture (e.g. together with *Tenacibaculum spp*.), was isolated from 296 salmon farms spread across all production areas, but primarily occurring during the colder months of the year (Figure 3). Our internal results from MLVA genotyping shows that the Norwegian trend of increasing CC1 dominance (at the cost of CC3) was reinforced throughout 2022. In the annual survey issued to fish health personnel in Norway, *M. viscosa*-related disease was ranked third amongst the most important health challenges in sea-farmed Atlantic salmon (Sommerset *et a*l., 2023). In this regard, high overall rankings were also awarded to the closely connected problems with delousing-related injuries (2nd), *Tenacibaculum spp*. (6th) and non-specific skin lesions (7th).



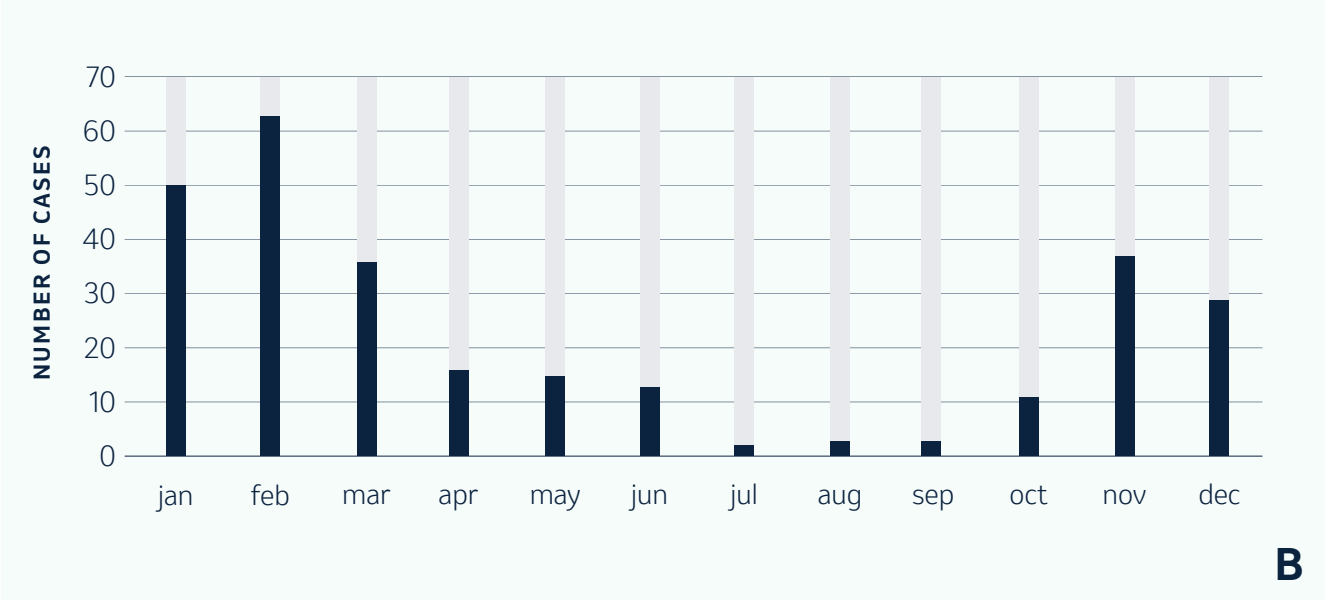


Figure 3: Number of Norwegian salmon farms with *Moritella viscosa* diagnoses made throughout 2022 (Norwegian Veterinary and private laboratories), per production area/PO (A) and per month (B). Diagrams sourced from Sommerset *et al*., 2023.

MORITELLA VISCOSA PREVALENCE IN BRITISH COLUMBIA (CANADA)

To date, research studies on winter ulcer disease and its associated etiological agent, *Moritella viscosa*, have been limited in British Columbia (BC), Canada. The occurrence of winter ulcers in Atlantic salmon farms was initially reported in December 2011, initiating Fish Health Audit and Surveillance Program's *M. viscosa* screening activities. From 2012 to 2018, winter ulcer diagnoses were confirmed in 17 out of 715 (2.4%) audits conducted on Atlantic salmon farms in BC. During this period, 13 Fish Health Events were linked to winter ulcer in Atlantic salmon farms in BC, with one mortality event attributed to winter ulcer (Wade and Weber, 2020).

While molecular testing using qPCR-based techniques (Fluidigm Biomark) detected *M. viscosa* in two out of 2,006 juvenile Sockeye Salmon samples collected in June 2013, there have been no documented cases of winter ulcer or isolation of *M. viscosa* in Pacific salmon (Nekouei *et al*., 2018).

An analysis of 40 distinct strains obtained from various geographic regions and hosts, including Canadian Atlantic salmon, has identified two phenotypic and genotypic clades of the bacterium, classified as "typical" and "variant" (Grove *et al*., 2010). The "variant" form, which exhibits higher hemolysin activity, has been isolated from Canadian farmed Atlantic salmon (isolates Vvi-7; Vvi-11) (Grove *et al*., 2010).

Significant knowledge gaps exist regarding the epidemiology and prevalence of winter ulcer in BC. Nevertheless, the presentation of winter ulcer due to *M. viscosa* infection in BC appears to align with descriptions from farmed Atlantic salmon in Norway, Scotland and Iceland. This presentation is characterized by onset at temperatures below 8-10°C, a decrease or minimal incidence of infection at temperatures above 10°C, the presence of superficial wounds, and the isolation of *M. viscosa* (Wade and Weber, 2020).

Unfortunately, the virulence of *M. viscosa* strains isolated from British Columbia's Atlantic salmon remains a subject of limited investigation, with a significant gap in our understanding of the molecular mechanisms of this bacterium. There is an urgent need to assess susceptibility levels among different isolates to antibiotics, study isolates genetic diversity and genomic makeup, examine the biological factors influencing infection and disease progression and evaluate the applicability of findings from studies conducted on Atlantic salmon along the East Coast of Canada (MacKinnon *et al*., 2019). Addressing these research topics is key to enhancing our understanding of *M. viscosa*-related infections in British Columbia and developing effective strategies for prevention and management.

> ***The virulence of M. viscosa strains isolated from British Columbia's Atlantic salmon remains a subject of limited investigation, with a significant gap in our understanding of the molecular mechanisms of this bacterium.***

DISCUSSION

There remains much to be identified regarding the pathogenesis of winter ulcer, host specificity of the various *M. viscosa* clonal complexes, the relevance of variability in colony viscosity, and genetic background for developing improved vaccines. Host-related factors affecting the skin barrier are poorly documented. There is also much to be learned regarding the natural infection pressure surrounding open-water fish farms. This includes questions surrounding *M. viscosa* demographics and ecology outside of commercial salmon farming. Is the diversity identified during diagnostic investigations merely a reflection of the *Moritella* population composition in the surrounding water bodies?

The association between the increasing prevalence and impact of *M. viscosa* infections and stressful management procedures, particularly delousing, appears strong. Although there is no doubt that existing vaccines could be further developed in terms of protection, possibly in terms of increased mucosal immunity, it is clear that the industry must primarily focus on the reduction of physical injury and stress resulting from present management procedures.

The association between the increasing prevalence and impact of M. viscosa infections and stressful management procedures, particularly delousing, appears strong.

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