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Heart and skeletal muscle inflammation – novel dangerous disease of farmed *Salmonidae*

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SUMMARY
Heart and skeletal muscle inflammation (HSMI) is one of the most widespread economically relevant diseases of farmed Atlantic salmon (*Salmo salar*), and it poses serious danger to its aquaculture. The disease was first reported in Norway in 1999. In 2006, the Norwegian researchers demonstrated its viral etiology. Heart and skeletal muscle inflammation is a novel and understudied highly contagious transboundary disease of *Salmonidae* characterized by erythrocyte damage, blood circulation failure, jaundice and aggregated signs of heart and skeletal muscle inflammation. The disease associated economic damage to aquaculture is enormous. Total cumulative mortality can reach 30% and morbidity can amount to 100%. Loss of quality of the commercial fish products due to melanised foci in the salmons' muscles further increases the disease-associated economic losses. Aquacultured Atlantic salmon is the most susceptible to HSMI. Rainbow trout, chub salmon and bull trout are also susceptible species and the list is still being continued. The disease is caused by the virus belonging to genus *Orthoreovirus* in the family *Reoviridae*. Currently Piscine orthoreovirus demonstrates the tendency towards its global spread. The virus-induced disease is reported in Norway, Scotland, Ireland, Iceland, France, Germany, Italy, Denmark, the Faroe Islands, Chile, Canada, Atlantic coast of the USA and Alaska. The majority of the outbreaks are registered in Central and Northern parts of Norway, which borders the Murmansk Oblast. The vicinity of the affected areas to Russia, the Gulf Stream passing the Norwegian shore while moving towards the Murmansk Oblast as well as wild *Salmonidae* migration to the Barents Sea, White Sea and Pechora Sea through the Norwegian territorial waters coupled with high stability of the virus compose high threat of *Piscine orthoreovirus* introduction to the Russian Federation from the adjacent countries.

Key words: *Piscine orthoreoviruses*, heart and skeletal muscle inflammation (HSMI), epidemic situation.

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Воспаление сердечных и скелетных мышц – новое опасное заболевание культивируемых лососевых рыб

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РЕЗЮМЕ
Воспаление сердечных и скелетных мышц в настоящее время является одним из самых распространенных, экономически значимых заболеваний культивируемого атлантического лосося и представляет серьезную угрозу его аквакультуре. Впервые болезнь выявлена в 1999 г. в Норвегии. В 2006 г. норвежские ученые доказали ее вирусную этиологию. Воспаление сердечных и скелетных мышц является трансграничной, высококонтагиозной, новой, еще недостаточно изученной вирусной болезнью лососевых рыб, характеризующейся поражением эритроцитов, симптомокомплексом воспаления сердечных и скелетных мышц, нарушением кровообращения и желтухой. Экономический ущерб, наносимый этим заболеванием аквакультуре, чрезвычайно велик. Общая кумулятивная смертность может достигать 30%, а уровень заболеваемости составлять 100%. Снижение качества товарной рыбной продукции из-за меланизированных участков в мышцах лососевых еще больше повышает экономические потери от этого заболевания. Наиболее чувствителен к воспалению сердечных и скелетных мышц выращиваемый в аквакультуре атлантический лосось. Восприимчивыми видами также являются радужная форель, чавыча и кумжа, и этот список продолжает пополняться. Возбудителем заболевания является вирус, относящийся к роду *Orthoreovirus* семейства *Reoviridae*. На сегодняшний день ортореовирус рыб имеет тенденцию к глобальному распространению. Заболевание, вызванное этим вирусом, регистрируют в Норвегии, Шотландии, Ирландии, Исландии, Франции, Германии, Италии, Дании, на Фарерских островах, в Чили, Канаде,

на Атлантическом побережье США и на Аляске. Наибольшее количество очагов отмечают в Средней и Северной Норвегии, пограничной с Мурманской областью. Близость неблагополучных территорий к России, протекающий мимо норвежских берегов в сторону Мурманской области Гольфстрим, а также миграция диких лососевых через норвежские территориальные воды в Баренцево, Белое и Печорское моря в совокупности с высокой стабильностью вируса создают высокий уровень угрозы заноса ортореовируса рыб с территории сопредельных стран на территорию Российской Федерации.

Ключевые слова: ортореовирусы рыб, воспаление сердечных и скелетных мышц, болезни рыб, эпизоотическая ситуация.

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DISEASE BACKGROUND

Heart and skeletal muscle inflammation (HSMI) was first reported in the Atlantic salmon (*Salmo salar*) grown on an aquaculture farm in Norway in 1999 [1]. For a long time the disease cause remained unknown. The virus etiology was supposed only in 2004, and later the infectious nature of the disease was confirmed by the Norwegian researchers [1, 2]. In 2006, the virus was observed during electronic microscopy [3]. In 2010, the virus was identified as reovirus using molecular and biological methods [4], and it was later attributed to genus *Piscine orthoreovirus* (PRV) [5]. In 2014, O. W. Finstad et al. demonstrated that PRV replicated in erythrocytes [6]. In 2016, Japanese scientists proved that erythrocytic inclusion body syndrome (EIBS) known since 1970-s was caused by PRV genetic variant in a coho salmon in Japan [7], and the orthoreoviruses were subdivided into three types. The latest evidence of the orthoreovirus being the HSMI agent was demonstrated by O. Wessel et al. in 2017 [8].

CLASSIFICATION

Heart and skeletal muscle inflammation is caused by *Piscine orthoreovirus*. PRV belongs to genus *Orthoreovirus*. PRV greatly differs from aquareoviruses. M. J. T. Kibenge et al. performed the phylogenetic analysis of S1 gene segment of many available virus isolates that allowed for grouping the Norwegian PRV strains in one genotype (PRV-1) including subgenotypes Ia and Ib. The Canadian PRV strains belonged to genotype Ia, and the Chilean PRV strains isolated from the Atlantic salmon belonged to subgenotype Ib [5].

Two more PRV genetic variants were described over the past few years that are adapted not to the Atlantic salmon but to other salmonids. One of such variants designated as PRV-2 is an EIBS agent on coho salmon aquaculture farms in Japan [7].

Another genetic variant designated as PRV-3 (also called Y virus or PRV-*Om*) induces HSMI-like disease in rainbow trout [9]. The same genotype includes PRV strains isolated from coho salmon in Chile [10]. PRV-3 was demonstrated to be closer related to PRV-1 than to PRV-2 [11]. PRV-3 also replicates in the Atlantic salmon but pathogenicity of this virus genotype for this fish species is lower as compared to the rainbow trout [12].

VIRUS STABILITY

Orthoreoviruses are stable against disinfecting agents. The virions are stable at pH 2–9 and up to 55 °C; they are also stable against fat-dissolving agents and detergents. UV irradiation reduces the virus infectivity.

EPIDEMIC DATA

Heart and skeletal muscle inflammation is a severe disease characterized by high morbidity and duration. Progressive increase of the severity of the lesions during the months before the clinical signs' onset is indicative of the fact that the subclinical infection can be present on the farm for a very long time [2]. In Norway, the HSMI morbidity of the aquacultured Atlantic salmon amounts up to 100% with 20% mortality. In Chile, the mortality rate does not usually exceed 5% during freshwater culture. During seawater culture two mortality peaks are reported in fish: the first one – in two months after transfer from the freshwater to the seawater and it amounts to 2–10%; the second peak starts in six months after the first one. During the second peak the mortality reaches 30% and it is associated with secondary infections. HSMI outbreaks in the Atlantic salmon are reported both in winter and in summer. This suggests that seasonality and water temperature have no impact on the disease development. HSMI was long considered to occur only in fish in seawater, usually 5–9 months after the Atlantic salmon is transferred to seawater cages from the freshwater cages. Over the recent years (2016–2019) the disease has been also reported on the freshwater farms [13]. In freshwater pools PRV infects young fish before smoltification [14]. According to the latest data PRV-1 is present in nearly every batch of cultivated Atlantic salmon during the seawater phase [8]. Adult fish is more often infected as compared to young fish; males are more often diseased than females. The disease outbreaks associated with high mortality last for several weeks. The amount of the virus detected using real-time reverse-transcription polymerase chain reaction (PCR) directly correlates with the disease development [15]. The virus is isolated from the wild fish, but no clinical signs are reported. This is due to the fact that in natural environment the diseased fish quickly dies or it is captured by the predators. PRV is preserved in tissues of convalescent fish for over one year [16]. A. B. Kristoffersen et al. demonstrated that HSMI risk increases with the increase of the fish lifespan, growth of the virus concentration in the environment and increase of the fish stocking density [17].

Geographic distribution and susceptible species. In spite of the vast PRV spread in the cultured Norwegian salmon [18] and in wild Atlantic salmon [4], HSMI is reported

only in cultured fish. In addition to Norway, PRV is widely spread in aquacultured Atlantic salmon and coho salmon in Chile [5, 10], Scotland, Ireland, Iceland and on the Faroe Islands [19], in cultured and wild Atlantic salmon in Denmark [20], Atlantic salmon, cutthroat trout, steelhead trout, and chum salmon in Canada [5], cultured chinook salmon and coho salmon along the US Atlantic coast [21], aquacultured chinook salmon and wild coho salmon on Alaska [22]. K. A. Garver et al. experimentally infected coho salmon, chinook salmon and sockeye salmon with PRV [16], but they identified no heart lesions or immune response pathology. Orthoreovirus was also isolated from 3% of andromous sea-trout tested, but it was not detected in Arctic char [23]. L. Bigarre et al. described increased PRV-induced mortality in brown trout population in France [24]. The virus was isolated from a farmed Atlantic salmon escaped from the cage that was considered as one of the routes of the disease spread [25]. During PCR-screening of the samples collected from the marine fish captured along the Norwegian seacoast, C. R. Wiik-Nielsen et al. detected low PRV concentrations in Atlantic capelin, European horse mackerel and great silver smelt . The authors suggested the interactions to be more complex and involving several virus carriers and reservoirs as no positive samples were collected from fish captured near the aquaculture farms, where HSMI outbreaks were reported [26].

For Norway HSMI is a serious problem due to a great number of epidemic outbreaks; in 2014 the disease was reported on 181 farms [27]. Chile reported 44 PRV cases in the Atlantic salmon in the first half of 2015 [28]. In British Columbia (Canada) 75% of tested Atlantic salmon were PRV-positive [29]. Retrospective molecular and genetic tests of archived clinical samples collected in 1974-2013 on Alaska and in British Columbia demonstrated the virus presence in the samples collected from different salmonid species in 1970-s, i.e. before the establishment of Atlantic salmon aquaculture.

The anemia-accompanied disease designated as EIBS was first reported in rainbow trout in 1977 [30] and in 1987 in chinook salmon from the Pacific Northwest of the USA [31]. In 2016, orthoreovirus was isolated from EIBS-infected coho salmon in Japan and it was classified as PRV-2 [7]. Nevertheless, while the cases occurred in 1977 and 1987 precede the evidence of the etiological role played in EIBS, it is still unknown, what etiological agent (PRV-1 or PRV-2) is the cause of the above mentioned erythrocyte lesions. Spread of PRV-2 outside of Japan has not been evidenced so far.

A new HSMI-like disease of freshwater rainbow trout was reported in Norway in 2013. Its agent was designated as PRV-3 [9]. PRV-3 was reported in salmonids with cardiac signs in Chile [10] and in clinically healthy adult rainbow trout in Norway [32]. Over the recent years, in several European countries including Scotland, German, Italy and Denmark, during the disease outbreaks the virus has been detected in rainbow trout both with and without clinical manifestations of the disease [11]. In 2017, H. Hauge et al. published results of the experiments on contact transmission of PRV-3 and demonstrated that the virus of this genotype extensively replicates in the blood of the rainbow trout and it is easily transmitted to a naïve host, while its replication capacity in the Atlantic salmon is limited [12]. PRV-3 is also supposed to be associated with proliferative darkening syndrome (PDS) reported in brown trout in the European pre-alpine countries [33].

Transmission. Horizontal virus transmission was confirmed during the laboratory experiment on infected and contact fish [2]. Based on these experiments the researchers concluded that the virus is excreted and transmitted with water. H. Hauge et al. isolated the virus from the feces of the experimentally infected fish [34]. The maximal amount of the virus was excreted two-three weeks after the experimental infection that agrees with the peak levels of the virus accumulation in blood [6]. Vertical virus transmission is less studied but, though it is unlikely to be the basic one, it should be still taken into account until proven otherwise [26]. The route of the virus introduction into the susceptible organism is not yet known. The possibility of the PRV transmission with the marine species cannot be excluded. In 2012, C. R. Wiik-Nielsen et al. detected PRV genome in greater argentine (*Argentina silus*), capelin (*Mallotus villosus*), Atlantic horse mackerel (*Trachurus trachurus*), and Atlantic herring (*Clupea harengus*) [26]. Therefore, PRV poses a potential threat to the survival of the wild Atlantic salmon.

PATHOGENESIS

PRV target cells include myocytes, erythrocytes and macrophages. At the early stage of the infection the virus affects the salmon's erythrocytes, herewith at the acute phase of the infection the affection may reach 50% over a short period of time [6]. Inclusion bodies similar to those observed at EIBS were detected in the erythrocytes. Unlike the mammalian erythrocytes the fish ones are nucleated cells capable of protein production [35], and therefore they are able to maintain the virus replication. D. Morera and S. A. MacKenzie demonstrated that fish erythrocytes are involved in the immune response [36].

PRV infection of the erythrocytes results in their dysfunction, decrease of the hemoglobin levels and anemia. The fish becomes more sensible to stress factors and hypoxic conditions [37]. Cardiac workload increases and in combination with the virus replication in the cardiac muscle cells it results in the damage and dysfunction of the organ. Heart failure leads to circulation deficiency (stagnation). Post-mortem examination of dead salmon most often reveals the circulation deficiency. The liver fails to perform its function that results in its lesions. PRV replication in the erythrocytes leads to the infectious hemolytic anemia and subsequent jaundice [10]

According to the published data, PRV is the cause of black (melanised) foci in red and white skeletal muscles of cultivated Atlantic salmon in Norway [38]. Poikilothermal animals including fish have a population of melanin-producing leukocytes being a strong anti-oxidant. Focal discoloration of muscles is associated with melanin-producing macrophages (melanomacrophages), which are normal for the lymphoid organs of fish [39]. Their function is protection against oxidative damage. Pathogenesis of the melanised foci is the following: cytotoxic immune cells employ oxidation for the inactivation of pathogens. The virus replication in myocytes (chronic antigen stimulation) results in chronic inflammatory responses. Melanomacrophages concentrate in those inflammation sites where chronic inflammation occurs. Therefore, melanised foci in muscle tissue appear to be the result of autoimmune reactions.

PRV is ubiquitous; however HSMI does not always occur. This suggests that the key triggers of the disease are stress and environmental factors.

CLINICAL SIGNS

HSMI usually occurs in 5–9 months after the Atlantic salmon is transferred from the freshwater cages to the seawater ones. By appearance the diseased fish does not differ from the healthy ones. Ruffled scales, pale gills and exophthalmos are reported in case of acute disease. Behavioral changes are also observed. The diseased fish are lethargic, reduce feed intake and become poor swimmers. They usually swim near the cage net facing the water current that is indicative of the lack of oxygen; mortality also increases [1].

In natural environment HSMI is complicated with co-infection with other viral pathogens (salmonid alphavirus – agent of pancreas disease (PD), infectious hematopoietic necrosis virus (IHNV), piscine myocarditis virus (PMCV) etc.) as well as with bacterial and fungal diseases (*Piscirickettsia salmonis*, *Flavobacterium psychrophilum*, *Saprolegnia* sp. etc.) thus making the clinical presentation unclear [40].

POST-MORTEM LESIONS

Post-mortem examination of the Atlantic salmon demonstrates signs of anemia (pale gills and heart, jaundice), hemodynamic abnormalities and cardiac failure (ascite, enlarged and deformed heart, hemorrhages in the pericardium, swim bladder and visceral fat), liver lesions (hepatomegaly, discoloration and hemorrhages), swollen spleen and kidneys (Fig. 1). One of the most frequent post-mortem lesions is hemopericardium (hemorrhages in pericardial cavity) that induces cardiac tamponade and can result in internal bleeding and death [1, 3, 10, 19]. Pancreas is not damaged in case of HSMI. HSMI post-mortem lesions are indicative of circulatory deficiency (stagnation) due to cardiac failure.

Main post-mortem HSMI-like lesions in Chilean farmed Atlantic coho salmon include pale gills, hemopericardium, pale heart and yellow liver [10].

Muscle lesions are often reported at later stages of the disease. Spread of melanised foci in the rainbow

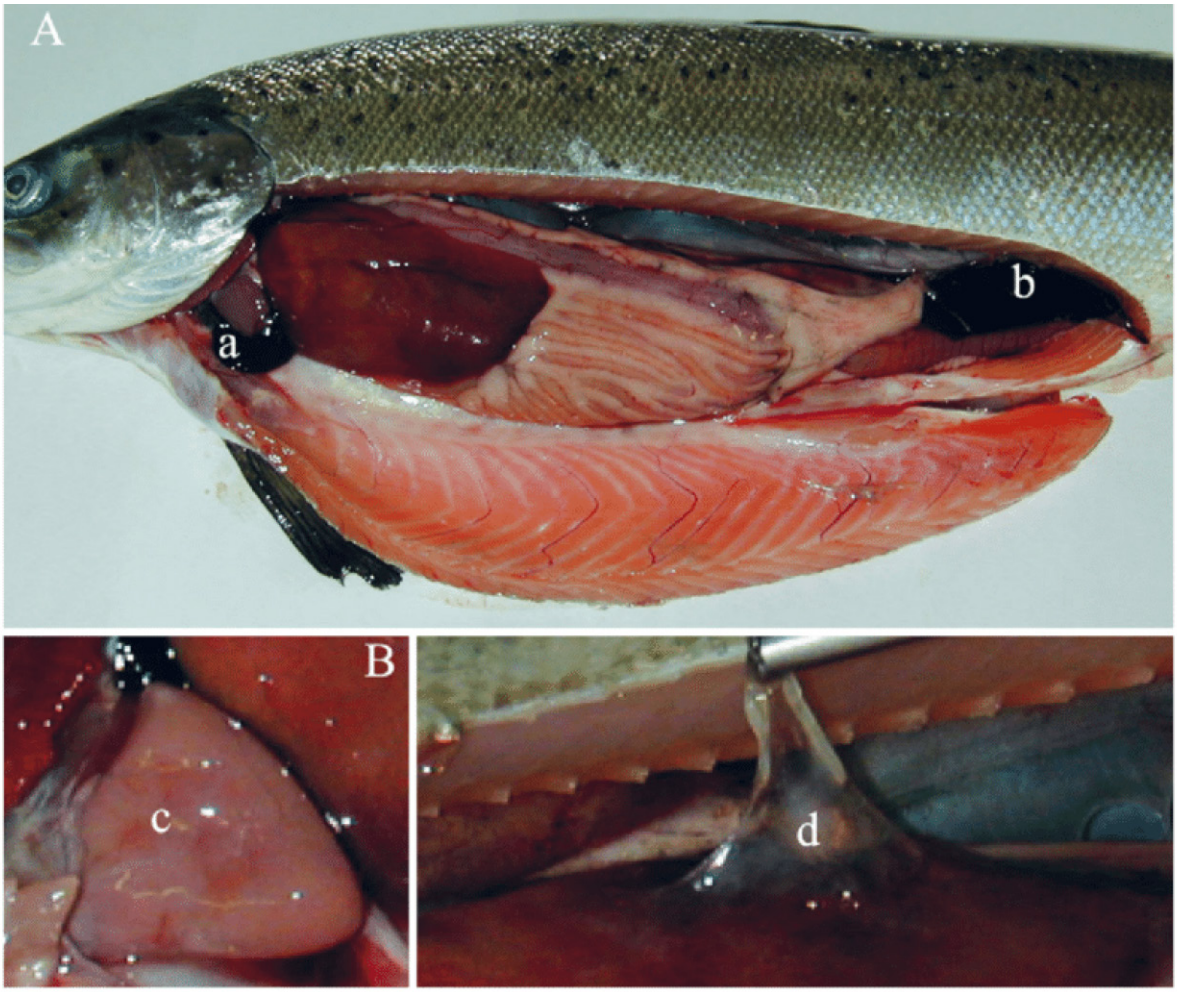

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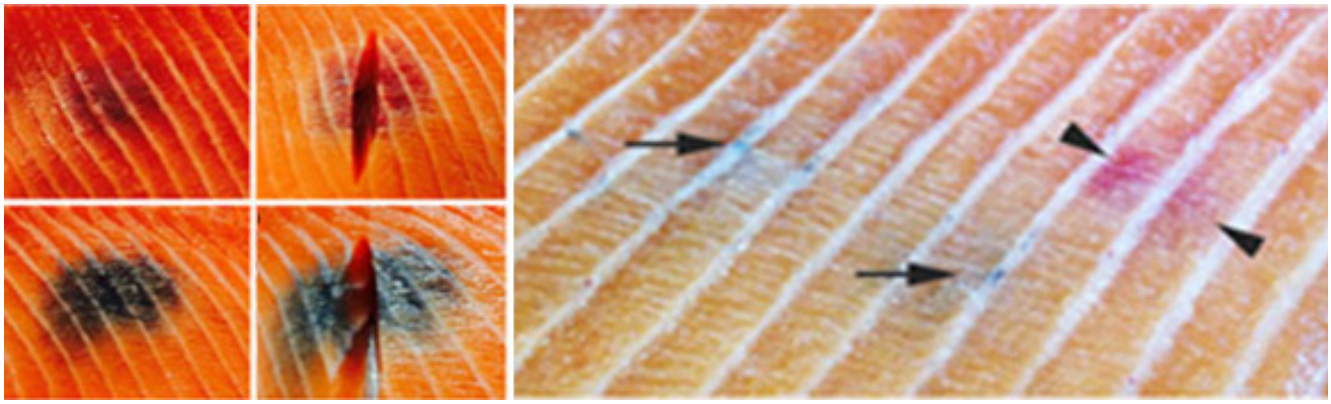
Fig. 1. Salmo salar. Macroscopic lesions in heart and skeletal muscle inflammation
A – haemopericardium (a), swollen spleen (b);
B – pale heart (c), fibrinous layer on the liver (d).

Рис. 1. Макроскопические изменения при воспалении сердечных и скелетных мышц (HSMI) у атлантического лосося
A – гемоперикард (a), увеличение селезенки (b);
B – бледное сердце (c), фибриновая пленка на печени (d).



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(https://veterinaryresearch.biomedcentral.com/track/pdf/10.1186/s13567-015-0244-6)

Fig. 2. Muscle lesions in case of aggregated signs of heart and skeletal muscle inflammation (HSMI)

Рис. 2. Изменения в мышцах при симптомокомплексе воспаления сердечных и скелетных мышц (HSMI)

trout muscles is insignificant in spite of aquaculture environment similar to the one of the Atlantic salmon (Fig. 2). There are no reports of similar lesions in wild salmonids [38].

One more HSMI sign is jaundice syndrome in chinook salmon and coho salmon. The jaundice syndrome is associated with the infectious hemolytic anemia due to PRV replication in erythrocytes [10].

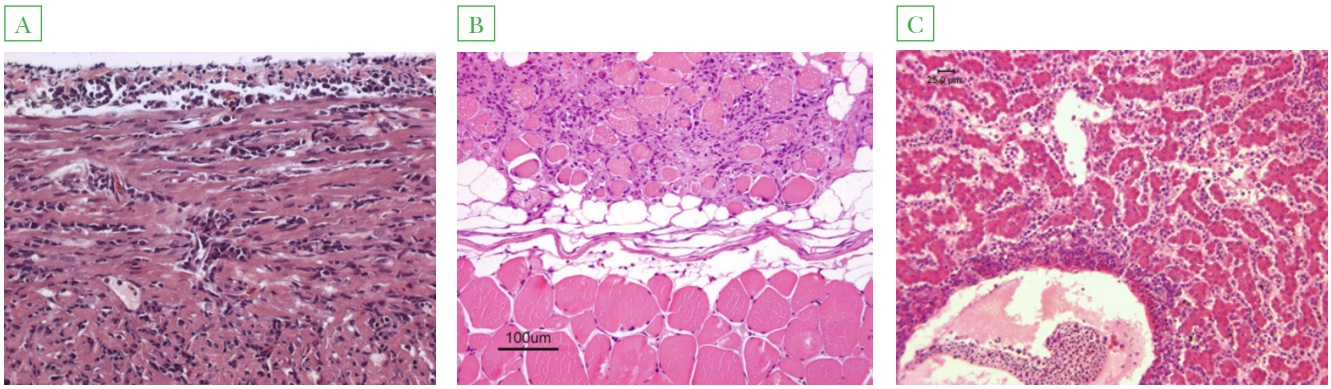
HISTOPATHOLOGY

Histopathological HSMI lesions are reported in the heart and red skeletal muscle (myocarditis and red skeletal muscle necrosis), Fig. 3. During the degeneration and necrosis of cardiac muscle cells both compact and spongy myocardiums are involved in the extensive inflammation. Infiltrates are composed of monocytes. Extensive epicarditis is reported that is generally closely related to myocarditis. Myodegeneration and necrosis are observed at later stages of the disease. Red muscle inflammation occurs similarly to the one in the heart: degeneration signs, loss of cross striation, eosinophilia, vacuolization and karyorrhexis appear in the affected myocytes. Focal hepatic necrosis, circulatory deficiency, edema and erythrocyte accumulation are observed in some organs [1, 2, 3, 6, 27]. Histopathological lesions in coho salmon with PRV-3-associated disease were different from those reported in orthoreovirus-infected Atlantic salmon and rainbow trout in Norway. In the Chilean coho salmon the heart inflammation was diffuse and as a rule involved the spongy myocardium; red muscle lesions were absent or insignificant [10].

The key histological signs of jaundice include hemorrhages in all organs and severe hemosiderosis in combination with erythrophagocytosis in kidneys and spleen [10].

IMMUNITY

PRV infection activates both cellular and antibody immune responses [41]. As it was mentioned above, cardiac tissues are the site of the virus replication. The immune response to the virus replication involves emergence of



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(https://link.springer.com/content/pdf/10.1007%2F978-94-007-2010-7.pdf)

Fig. 3. HSMI histopathological lesions
A – heart inflammation;
B – red muscle degeneration and inflammation (top);
C – liver inflammation.

Рис. 3. Гистопатологические поражения при ВССМ
А – воспаление в сердце;
В – дегенерация и воспаление красных мышц (вверху);
С – воспаление в печени.

cytotoxic T-lymphocytes (CD8+) in the heart that is indicative of this immune response to be directed to the virus-infected cells. O. W. Finstad et al. noted that cytotoxic cellular responses to the infected cells play an important role in HSMI immunity. However, such responses are directly accountable for the development of lesions in the heart and skeletal muscles. Atlantic salmon erythrocytes are the target cells for PRV replication [6], and they can play an immediate role in the immune response [36]. The erythrocytes react to the infection by means of the interferon-mediated immune response [42], which is capable of further protection against IHNV, salmonid alphavirus and, probably, other infectious agents [40, 43].

DIAGNOSTIC TOOLS

HSMI diagnosis is based on the presence of histologically recovered signs of myocardium inflammation extending to epicardium and endocardium, myocardium necrosis and red skeletal muscle myositis and necrosis [1] in combination with reverse transcription quantitative PCR (RT-qPCR) results. The target tissue for diagnosis usually involves the heart as the virus presence is to be related to the cardiac muscle pathology. HSMI should be differentiated from pancreas disease and cardiomyopathy syndrome (CMS) [45].

PREVENTION AND CONTROL

Heart and skeletal muscle inflammation (HSMI) is not subject to notification to the World Organization for Animal Health (OIE). This PRV-associated disease was officially reported only in Norway [4, 46] and Chile [10]. HSMI was excluded from National List-3 and in Norway it is currently registered as a non-notifiable disease [27].

In spite of specific antibodies produced after the PRV infection [41], the virus is ubiquitous in the populations of the virus-susceptible fish. This is indicative of the immune system's failure to eliminate the virus completely, and this poses the challenge of the effective vaccine development. Some production companies and research institutions have officially targeted on the development of HSMI vaccines using various strategies (whole-virion vaccines, recombinant subunit vaccines and DNA-vaccines). No vaccines have been developed so far.

The diseased fish is extremely sensitive to stress factors and hypoxic conditions. PRV diagnostic testing of the fish on aquaculture farms is needed. In case of PRV-positive fish detection and in order to reduce HSMI-associated losses one should abandon from immunosuppressive and stressful treatments, limit the fish transportation and take maximal care of the fish. All these will decrease the cardiac workload and result in the reduction of mortality.

Use of disinfectants reducing the amount of the viruses and opportunistic bacteria in the environment is appropriate for the disease prevention. Anti-inflammatory agents also demonstrated benefits during HSMI control. There are reports on functional feeds having antioxidant and anti-inflammatory actions that aid to the reduction of HSMI-associated losses and share of the fish discarded due to melanised spots in the muscle [47].

One more effective HSMI control tool involves breeding and culture of the disease-resistant fish.

CONCLUSION

Heart and skeletal muscle inflammation is an infectious fish disease with high morbidity and long disease course. During fish farming intensification of the culture process is inevitable and it involves increased stocking thus aiding to the spread of the pathogen, increase of stress and reduction of the disease resistance. Currently, there is a tendency towards the wide spread of the disease (European countries, North and South Americas); new virus variants are detected and the range of the disease susceptible fish species is expanded. The PRV-induced disease is the third significant disease of cultured Atlantic salmon in Norway. Heart and skeletal muscle inflammation is ubiquitous in cultured and wild Atlantic salmon in Norway. Since 2010, from 100 to 200 HSMI affected sites have been annually reported in Norway [13]. The highest number of the disease outbreaks is reported in the middle and northern parts of Norway that are bordering on the Murmansk Oblast. Up to 20% of the Atlantic salmon fillet produced at the Norwegian fish processing plants demonstrate melanised foci [48] thus impairing the product quality and increasing the economic losses born by the aquaculture sector due to the disease. All that is indicative of the fact that PRV-infection consequences can be graver as it was previously supposed.

In spite of the above mentioned HSMI data, in 2014 the Norwegian Food Safety Authority delisted the disease from National List-3 of notifiable diseases. Therefore, the decrease of the disease-affected sites being reported since 2015 is unlikely to fit the reality. Furthermore, there is no any national HSMI control program is Norway, and no positive impact on the situation can be envisaged.

Piscine orthoreovirus is reported in migratory wild Atlantic salmon [38] that is indicative of natural long-distance spread of the virus. This fact is supported by high genetic similarity of the PRV variants isolated on different continents [10].

The above mentioned data reflect a major threat of PRV introduction to the Russian Federation from the neighboring countries.

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