Screening of bacterial kidney disease in the North-West region of the Russian Federation

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Abstract. Our country has all potential for development of an aquaculture as one of sectors of agriculture, due to a huge number of fresh water areas and the extended line of the seas. Bacterial kidney disease, a chronic disease in the Salmonidae family, causes significant damage to aquaculture development. Modern proposals for the use of various antibiotics and chemotherapeutic drugs do not bring a significant effect to control the spread of this disease, and due to the peculiarity of the pathogenesis of the disease, the development of an effective vaccine against bacterial kidney disease is currently difficult, therefore the most effective method of controlling and preventing the disease is early diagnosis of the disease. The article discusses the results of exploratory studies of bacterial kidney disease in fish-breeding enterprises of the North-West region of the Russian Federation for the period from March 2019 to March 2020.

1. Introduction
Aquaculture in the Russian Federation has a complex diversified nature and it is a profitable and branch of agriculture due to the use of the world's largest fund of inland water bodies and coastal waters of the seas.

In 2014, for the first time, the world's population ate more farmed fish than from traditional fishing. In 2019, the volume of farmed fish amounted to 73.8 million tons, and if we add grown algae, the total volume of aquaculture production in 2014 amounted to 101.1 million tons (52% of the total volume of seafood harvested).

In Russia, aquaculture is a rapidly growing agricultural sector. Much attention is paid to the development of aquaculture in Russia in the context of economic reform, both on the part of the leading fisheries organizations of Russia and scientific organizations. At present, the prospects for the development of sturgeon breeding, whitefish breeding, trout breeding and pasture fish breeding have been determined. In commercial aquaculture, lake, pond and industrial fish farming is considered. The main directions are pond, industrial and pasture. Methods of organizing farms are considered.

Trout breeding and salmon breeding are currently considered one of the most developed aquaculture industries in the Russian Federation, as well as in Scandinavian countries, the USA and Japan. These industries are implemented in both freshwater and marine aquaculture (mariculture).

Serious attention should be paid to the prevention and treatment of diseases of salmonids of all ages and physiological groups. The main issue in the cultivation is the prevention and timely diagnosis of salmon fish diseases.
One of the pathologies significantly affecting the development of this industry is bacterial renal disease of salmonids (BKD). This is an infectious disease of a bacterial nature, characterized by the appearance on the body of the fish of small bubbles or nodules filled with pus containing red blood cells and bacteria. Among the clinical signs of BKD, exophthalmia, lethargy, hemorrhages, peritonitis, ascites, pallor of the liver, abscesses of internal organs, kidney damage in the form of multiple granulomas, as well as their presence in the liver and heart, skin swellings of an oblong or oval shape along the lateral line (in rainbow trout and chinook salmon, these lesions can reach a size of 4 mm) [1, 2, 3, 4]. The posterior intestine can be enlarged and hyperemic [4]. When BKD is detected in fish, blood parameters change significantly, there is a decrease in the level of serum proteins and albumin, as well as a drop in the hematocrit level from 52 to 14% [4].

BKD was first described in the 1930s under the name "Dee disease" and was found in Aberdeenshire Dee, in populations of River Dee trout in Scotland [5]. At the same time, BKD was discovered in the USA by Belding & Merrill researchers in Massachusetts, in one of the hatchery fish-seed farms [6]. American scientist - researcher Rucker, referring to previous studies of his colleagues, reported that BKD arose in the western United States among populations of brook, rainbow and brown trout, as well as among populations of chinook salmon, coho salmon and sockeye salmon [7].

The causative agent of BKD – *Renibacterium salmoninarum* - is a small (0.3-1.5 p.m by 0.1-1.0 p.m), strongly Gram-positive, nonsporulating rod that is neither acid-fast, motile, nor encapsulated. The organism often occurs in pairs, and short chains are sometimes observed. Early workers noted pleomorphic forms, some evidence of Chinese letter formation, and metachromatic granules.

BKD is widespread in North America, Europe, Japan and Chile [7]. The disease on the territory of the Russian Federation has not been previously registered [8]; it was first discovered in 2016 by the researches of the laboratory of ichthyopathology of the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV" [9, 10].

BKD is a sluggish chronic infection which leads to difficulties in early diagnosis in the herd, a general decrease in productivity and a deterioration in the marketability of fish, which subsequently leads to the death of the entire livestock.

Vertical transmission of the BKD pathogen from parents to offspring leads to a wide and rapid spread of the disease in fish farms, which is dangerous due to the large-scale purchase of fish fry and caviar from other countries.

There is currently no data available on an effective vaccine against BKD developed. Although the various reports suggest that it might be possible to develop a protective anti-BKD vaccine, they also illustrate the complexity of the problem. Although salmonids are able to produce specific antibody against experimental Renibacterium salmoninarum bacterins, it has not been clearly shown that the presence of such antibodies confers protection. A feature of *Renibacterium salmoninarum* is localization in macrophages. This complicates the development and application of the classic type of vaccines, therefore the most effective means of combating bacterial renal disease is timely laboratory diagnostics carried out on farms and when importing caviar from other enterprises in the Russian Federation and other countries.

In connection with the named features of the pathogenesis of this disease, the life cycle of the pathogen and its transmission mechanism, the aim of the study was to screen for bacterial renal disease in the northwestern region of the Russian Federation.

2. Materials and methods

2.1. Object of study

The present study involved 288 samples from fish and caviar at different stages of development. In total, from March 2019 to March 2020, 8 fish species belonging to the Salmonidae family were studied. Species names are listed in table 1.
The fish used for testing for BKD belonged to different age groups: fry, underyearlings and biennials. Samples were taken for research from 25 fish-breeding enterprises in the North-West region of the Russian Federation.

| English name of the species | Latin name of the species                   |
|-----------------------------|---------------------------------------------|
| 1  Atlantic salmon          | *Salmo salar* Linnaeus                      |
| 2  Chinook salmon           | *Oncorhynchus tschawytscha* Walbaum         |
| 3  Rainbow trout            | *Oncorhynchus mykiss* Walbaum               |
| 4  Brook trout              | *Salvelinus fontinalis*                     |
| 5  Masu salmon              | *Oncorhynchus masu*                        |
| 6  Broad whitefish          | *Coregonus nasus*                          |
| 7  Muksun                    | *Coregonus muksun*                         |
| 8  Peled                    | *Coregonus peled*                          |

2.2. Sampling
Sampling for bacteriological analysis and polymerase chain reaction (PCR) was carried out according to the method proposed by the OIE (2006). The fish was delivered to the FSC VIEV ichthyopathology laboratory chilled and dissected within 12 hours after catching. Samples for the study were taken under strictly sterile conditions.

Before taking the material for research, the instruments were additionally moistened with a 96% alcohol solution and fired on a burner flame.

Fish and caviar that were chilled for more than 12 hours, as well as samples with signs of rotting and decay, were rejected and were not examined.

2.3. Bacteriological examination
For bacteriological examination, samples were taken under strictly sterile conditions in a laminar flow hood (BIO II Advance). After the access to the body cavity of the fish was opened, the state of the internal organs was assessed. The presence of ulcers, abscesses, hyperemic areas and areas of necrosis was noted. First of all, sowing with a microbiological loop was carried out from the affected areas. A sample was taken at the border of the affected and healthy tissue, or the contents of the abscess were taken. Before taking, the puncture site was previously disinfected with a heated metal spatula.

Inoculation was performed from internal organs (liver, kidneys) using a microbiological loop on the recommended selective and storage media: SKDM and KDM-2. Culture media were prepared at the FSC VIEV Ichthyopathology Laboratory.

Incubation of Petri dishes with inoculations was carried out for 30 - 45 days in a refrigeration thermostat at a temperature of 15 °C.

According to the results of incubation, the growth and size of colonies, cultural and tinctorial properties were assessed, and the analysis of the biochemical properties of the isolated pathogen was carried out.

2.4. PCR diagnostics
For the study using the polymerase chain reaction, internal organs were selected: liver, kidneys, spleen, as well as caviar, they were homogenized using a multirotator device and placed in a salt buffer (Eagle MEM medium with Hanks salts and the addition of an antibiotic - gentamicin, which suppresses the growth of secondary microflora in the environment).

DNA isolation from samples was carried out using commercial kits "S-Sorb" (Syntol, Russia) according to the indicated scheme.

Polymerase chain reaction was carried out by the nested method in 2 rounds using 2 pairs of primers. Synthetic oligonucleotide primers were synthesized at Syntol LLC (Moscow, Russia). BKD strains from
the collection of the FSC VIEV Ichthyopathology Laboratory were used as a positive control, and saline buffer was used as a negative control. The thermal cycle included the following stages: 94 °C - 30 sec, 60 °C - 30 sec, 72 °C - 1 min (30 cycles) - for both the 1st and 2nd round.

The results of the reaction were recorded using electrophoretic detection in 2% agarose gel.

3. Results and discussion

For screening for bacterial kidney disease (BKD), 288 samples of fish of different age groups and caviar at different stages of development were taken. In total, 8 species of fish belonging to the Salmon family took part in the study: Atlantic salmon, Chinook salmon, Rainbow trout, Brook trout, Masu salmon, Broad whitefish, Muksun and Peled. The study was carried out from March 2019 to March 2020, fish and caviar were sampled from fish farms in the North-West region of the Russian Federation.

After selection under sterile conditions and sample preparation, each sample was subjected to microbiological inoculation on the recommended media: SKDM and KDM-2, as well as the subsequent study of the cultural, biochemical and tinctorial properties.

The formation of colonies of the causative agent of BKD - *Renibacterium salmoninarum* - on solid media KDM-2 and SKDM was noted on days 15 and 21 (respectively), which indicated an open form of the disease, and in some cases on days 45 and 55 as a latent carriage (figure 1, 2). We have established that *Renibacterium salmoninarum* is a gram-positive small bacillus 0.3 - 1.5 µm in size, located singly or in pairs. The bacterial pathogen was immobile and did not form a capsule.

![Figure 1](image1.png)
![Figure 2](image2.png)

**Figure 1.** Continuous growth of *Renibacterium salmoninarum* along the inoculation line on KDM-2 medium.

**Figure 2.** Spot colonies of *Renibacterium salmoninarum* on KDM-2 medium.

For screening, a test system based on nested polymerase chain reaction using Gail Curat primers was used (figure 3).

![Figure 3](image3.png)

**Figure 3.** Results of nested PCR using copyright primers. From left to right: 1, 7 - mass markers (1000 bp), 2, 4 - positive samples, 3 - negative sample, 5 - positive control, 6 - negative control.
Of the 288 samples tested by polymerase chain reaction, 3 cases were positive, which is 1% of all samples. Positive results obtained using nested PCR with the author's primers were confirmed using microbiological methods.

Such statistics allow us to state that the nested PCR test system used to identify the BKD causative agent is quite effective and does not give false positive and false negative results. In addition, due to the long-term growth of *Renibacterium salmoninarum* colonies on nutrient media, bacteriological examination takes a lot of time and requires significant economic and time costs, while PCR diagnostics gives a quick result within 1 day.

The data obtained allow us to state that bacterial kidney disease continues to be detected on the territory of the Russian Federation, and the most effective method of prevention and control of this disease is timely and early diagnosis, carried out both inside the fish breeding enterprise and when importing eggs and fish seed from farms within the country and their other countries.

In the future, it is necessary to develop and improve existing methods for diagnosing BKD, as well as to develop new research methods.

References

[1] Buller B 2004 *Bacteria from fish and other aquatic Animals* (Cambridge: CABI Publishing) p 45
[2] Takanori M, Toyohiko N and Mamoru Y 2009 Modification of KDM-2 with Culture-spent Medium for Isolation of *Renibacterium salmoninarum* *Fish Pat.* **44**(3) 139-44
[3] Bogdanova E A 1994 *Diseases of salmon and whitefish in aquaculture* (St. Petersburg: NIORCH) pp 96-9
[4] Golovina N A *et al.* 2003 *Ichtyopathology* (Moscow: Mir) pp 61-8
[5] Smith I W 1964 *Freshwater Salmon Fish* **34** 3-12
[6] Belding D L and Metrill B 1935 *Dis. of aq. org.* **65** 76 – 84
[7] Rucker R R, Bernier A F, Whipple W J and Burrows R E 1951 Sulfadiazine for kidney disease *Prog. Fish Cult.* **13** 135-7
[8] Gulukin M I, Zavyalova E A, Droshnev A E and Kolomycev S A 2011 Analysis of the Epizootic Situation in Fish Diseases in Russia *Vet.* **8** 3-7
[9] A E Droshnev et al 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **315** 072007
[10] Zavyalova E A, Droshnev A E, Bulina K Yu and Gulukin A M 2018 Epizootic situation of fish diseases: research methods, trends, prospects *RVJ* **1(25)** 136-42