Microorganisms of Lake Baikal and their pathogenic properties

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Abstract. The article presents the results of microbiological studies of water from Lake Baikal in the spring period, and identifies a wide range of microorganisms that are tested for pathogenicity. It was established that many microorganisms of the enterobacteriaceae were pathogenic for laboratory animals. In 100 % of cases, pathogenic, sporogenous, anaerobic microorganisms were isolated - Cl. perfringens that multiply without access to oxygen, so, in the water mass.

1. Introduction

The problem of water quality control has always attracted the attention of government agencies and specialists responsible for public health and environmental protection. It is especially true for Lake Baikal, a part of the world's natural heritage. Lake Baikal is the main source of fresh drinking water in the region and in the surrounding areas, as the formation of the water quality of the Angara river is primarily determined by the water quality of Lake Baikal. The anthropogenic impact on the Lake is increasing every year, especially in its southern part. This is due not only to the relatively high density of the population living here, but also to the development of intensive domestic and international tourism in this area, the construction of private hotels, baths, saunas, and holiday villages. The littoral zone of the Lake in the village of Listvyanka, located at the source of the Angara river, is experiencing a very strong anthropogenic load. The village is located at the closest distance from the city of Irkutsk (70 km), so it is accessible to Irkutsk residents and guests of the city and is attractive for mass tourism – both summer and winter.

Lake Baikal is one of the most popular tourist destinations in Russia. Every year, tens of thousands of tourists come here from different parts of the Russian Federation and around the world. The region is rich in attractions, flora, fauna and nature mysteries. Many interesting facts are connected with Lake Baikal, and it is also included in the list of wonders of Russia.

The microflora of Lake Baikal is represented by a wide range of microorganisms that can be pathogenic to laboratory animals and affect the sanitary condition of the reservoir. For example, fecal bacteria enter the environment with animal and human excrement. They are included in the category of so-called coliform bacterias, an indicator of water purity.
Of the microorganisms, thermotolerant coliform bacteria (TCB) are particularly indicative, which are able to feed and multiply rapidly at high temperatures. These include Escherichia coli – the main component of the intestinal microflora. However, some strains of coliform bacteria can cause severe food poisoning.

The purpose of this research was to conduct research on water from an open reservoir on Lake Baikal, allot a pure culture of microorganisms and check pathogenic properties.

The object of the study was water from an open reservoir on Lake Baikal near the village of Listvyanka. Water samples were taken in the spring. The material for allotment of microorganisms was surface and deep (140 meters) water. Isolation of microbial cultures and their identification were carried out according to generally accepted methods based on the Department of diagnostics of bacterial and parasitic diseases of Irkutsk Central Veterinary Laboratory [1-9].

The virulence of the tested microorganisms in vivo (intraperitoneal test) was determined by the ability to cause the death of white mice. Laboratory animals (white mice) were intraperitoneally injected with 0.5 ml of 1 billion suspensions in the number of three for each bacterial culture. Laboratory animals were observed at the following exposures: 24, 48, and 72 hours and 10 days later [10]. The animals were kept at Irkutsk Central Veterinary Laboratory. The experiments were performed in accordance with the "Rules for conducting work using experimental animals” approved by the Ministry of health of the USSR (1977), "International recommendations for conducting biomedical research using animals" (1986).

Statistical processing of these results was carried out using generally accepted statistical criteria.

2. Research results and discussion

We have conducted studies of the surface and deep (140 meters) water layer of Lake Baikal in the area of the village of Listvyanka, located on the Western shore of the reservoir. The results of the study are shown in table 1.

| №  | Type of material | Time of year | Type of microorganisms                           | Research result     | Samples location                                      |
|----|------------------|--------------|-------------------------------------------------|---------------------|-------------------------------------------------------|
| 1  | Water            | Spring       | Total bacterial count                           | 1.5 CFU/ml          | Lake Baikal, on the freezing border, opposite the limnological museum in Listvyanka |
|    |                  |              | Clostridium perfringens                         | Not detected        |                                                       |
|    |                  |              | Coliform bacteria                               | Detected            |                                                       |
|    |                  |              | Thermotolerant coliform bacteria                 | Detected            |                                                       |
|    |                  |              | Leclercia adecarboxylata                         | Detected            |                                                       |
|    |                  |              | Enterococcus faecium                            | Detected            |                                                       |
|    |                  |              | Bacillus cereus                                 | Detected            |                                                       |
|    |                  |              | Bacillus weihenstephanensis                     | Detected            |                                                       |
|    |                  |              | Enterobacter kobei                              | Detected            |                                                       |
|    |                  |              | Bacillus thuringiensis                          | Detected            |                                                       |
|    |                  |              | Salmonella spp.                                 | Not detected        |                                                       |
| 2  | Water            | Spring       | Total bacterial count                           | 0 CFU/ml            | Lake Baikal, at the beginning of Listvyanka village   |
|    |                  |              | Clostridium perfringens                         | Detected            |                                                       |
|    |                  |              | Coliform bacteria                               | Detected            |                                                       |
|    |                  |              | Thermotolerant coliform bacteria                 | Detected            |                                                       |
|    |                  |              | Escherichia coli                                | Detected            |                                                       |
|    |                  |              | Salmonella spp.                                 | Not detected        |                                                       |
| 3  | Water            | Spring       | Total bacterial count                           | Permanent growth     | Lake Baikal, 2 km after entering                     |
|    |                  |              | Clostridium perfringens                         | Not detected        |                                                       |
|    |       |                                |                                   |                                         |                                      |                                    |
|----|-------|--------------------------------|-----------------------------------|----------------------------------------|--------------------------------------|-----------------------------------|
|    | Water | Spring                         | Total bacterial count             | 68 CFU/ml                              | Lake Baikal, 3 km after entering     | Listvyanka                        |
|    | Coliform bacterias                | detected                        |                                   |                                        |                                      |                                    |
|    | Thermotolerant coliform bacteria  | detected                        |                                   |                                        |                                      |                                    |
|    | Escherichia coli                  | detected                        |                                   |                                        |                                      |                                    |
|    | Staphylococcus aureus             | detected                        |                                   |                                        |                                      |                                    |
|    | Salmonella spp.                   | not detected                    |                                   |                                        |                                      |                                    |
|    | Clostridium perfringens           | detected                        |                                   |                                        |                                      |                                    |
|    | Coliform bacterias                | not detected                    |                                   |                                        |                                      |                                    |
|    | Thermotolerant coliform bacteria  | detected                        |                                   |                                        |                                      |                                    |
|    | Escherichia coli                  | not detected                    |                                   |                                        |                                      |                                    |
|    | Staphylococcus aureus             | not detected                    |                                   |                                        |                                      |                                    |
|    | Salmonella spp.                   | not detected                    |                                   |                                        |                                      |                                    |
|    | Total bacterial count             | 9 CFU/ml                        | Lake Baikal, at a depth of 140     |                                        |                                      |                                    |
|    | Water | Summer                         | Total bacterial count             | 60 CFU/ml                              | Lake Baikal, at the beginning of      | Listvyanka village                |
|    | Coliform bacterias                | not detected                    |                                   |                                        |                                      |                                    |
|    | Thermotolerant coliform bacteria  | not detected                    |                                   |                                        |                                      |                                    |
|    | Escherichia coli                  | not detected                    |                                   |                                        |                                      |                                    |
|    | Staphylococcus aureus             | not detected                    |                                   |                                        |                                      |                                    |
|    | Salmonella spp.                   | not detected                    |                                   |                                        |                                      |                                    |
|    | Clostridium perfringens           | detected                        |                                   |                                        |                                      |                                    |
|    | Coliform bacterias                | not detected                    |                                   |                                        |                                      |                                    |
|    | Thermotolerant coliform bacteria  | not detected                    |                                   |                                        |                                      |                                    |
|    | Escherichia coli                  | not detected                    |                                   |                                        |                                      |                                    |
|    | Staphylococcus aureus             | not detected                    |                                   |                                        |                                      |                                    |
|    | Salmonella spp.                   | detected                        |                                   |                                        |                                      |                                    |
|    | Total bacterial count             | 110 CFU/ml                      | Lake Baikal, 2 km after entering   |                                        |                                      |                                    |
|    | Water | Summer                         | Total bacterial count             | 65 CFU/ml                              | Lake Baikal, 3 km after entering     | Listvyanka                        |
|    | Coliform bacterias                | not detected                    |                                   |                                        |                                      |                                    |
|    | Thermotolerant coliform bacteria  | not detected                    |                                   |                                        |                                      |                                    |
|    | Escherichia coli                  | detected                        |                                   |                                        |                                      |                                    |
|    | Staphylococcus aureus             | not detected                    |                                   |                                        |                                      |                                    |
|    | Salmonella spp.                   | detected                        |                                   |                                        |                                      |                                    |
When studying surface and deep water (140 meters), the following families of microorganisms were identified: enterobacteria, staphylococci, clostridiums and enterococci.

All the isolated microorganisms were tested on the time-of-flight mass spectrometer Maldi Biotyper, a revolutionary development in the field of microbiology by Bruker. The system evaluates the presence of a unique set of proteins of an unknown microorganism in a few seconds.

As the markers by which microorganisms are identified are proteins, the result does not depend on the cultivation method and is not linked with the growth of microorganisms. The method provides successful identification for traditionally complex objects in microbiology.

We also studied isolated microorganisms using the classical method. All isolated microorganisms in 100% of cases coincided with the express method.

When studying surface and deep water (140 meters), clostridium was isolated, which was colored by Gramm – gramm positive, clostridium botulinum. On a nutrient medium Kitt - Torozzi – formed a heavy turbidity and a strong vaporization.

We conducted studies on the pathogenicity of isolated microorganisms to laboratory animals (white mice). The results of the study are shown in table 2.

Table 2. Results of the study of pathogenicity of isolated microorganisms from the surface and deep (140 meters) water layer of Lake Baikal.

| Sample of water No. | Isolated microorganism       | Pathogenicity (virulence) of microorganisms                                      |
|---------------------|------------------------------|---------------------------------------------------------------------------------|
| 1                   | Coliform bacterias           | The mice died within 48 hours                                                    |
|                     | Thermotolerant coliform bacteria | The mice died within 48 hours                                                   |
|                     | Leclercia adecarboxylata     | Mice are alive for 10 days                                                      |
|                     | Enterococcus faecium         | The mice died within 24 hours                                                   |
|                     | Bacillus cereus              | The mice died within 48 hours                                                   |
|                     | Bacillus weihenstephanensis | Mice are alive for 10 days                                                      |
|                     | Enterobacter kobei           | Mice are alive for 10 days                                                      |
|                     | Bacillus thuringiensis       | Mice are alive for 10 days                                                      |
| 2                   | Clostridium perfringens      | The mice died within 18 hours                                                   |
|                     | Coliform bacterias           | The mice died within 48 hours                                                   |
|                     | Thermotolerant coliform bacteria | The mice died within 48 hours                                                   |
|                     | Escherichia coli             | Mice are alive for 10 days                                                      |
| 3                   | Coliform bacterias           | The mice died within 48 hours                                                   |
|                     | Thermotolerant coliform bacteria | The mice died within 48 hours                                                   |
|                     | Escherichia coli             | Mice are alive for 10 days                                                      |
|                     | Staphylococcus aureus        | Plasma coagulation on rabbit plasma is positive                                |
| 4                   | Coliform bacterias           | The mice died within 48 hours                                                   |
|                     | Thermotolerant coliform bacteria | The mice died within 48 hours                                                   |
|                     | Escherichia coli             | Mice are alive for 10 days                                                      |
| 5                   | Clostridium perfringens      | The mice died within 12 hours                                                   |
| 6                   | Clostridium perfringens      | The mice died within 16 hours                                                   |
| 7                   | Clostridium perfringens      | The mice died within 12 hours                                                   |
|                     | Salmonella spp.              | The mice died within 10 hours                                                   |
| 8                   | Escherichia coli             | Mice are alive for 10 days                                                      |
|                     | Citrobacter freundii         | Mice are alive for 10 days                                                      |
|                     | Aeromonas hydrophila         | Mice are alive for 10 days                                                      |
|                     | Aeromonas caviae             | Mice are alive for 10 days                                                      |
We studied the pathogenic properties of microorganisms gotten from surface and deep water (140 meters). The table shows that in 100% of cases of clostridiums isolation, the biological test was positive, and laboratory mice died within 18 hours.

Microorganisms of the enterobacteriaceae - Escherichia coli, were not pathogenic for laboratory animals in 100% of cases.

Microorganisms of the staphylococcus family were tested for pathogenicity by setting up a plasma coagulation reaction on rabbit blood plasma. In sample No. 3, staphylococcus showed pathogenic properties.

Microorganisms - Citrobacter freundii, Aeromonas hydrophila, Aeromonas caviae in water sample No. 8 did not have pathogenic properties.

Indicators: Common coliform bacteria, Thermotolerant coliform bacteria caused the death of laboratory animals within 48 hours.

3. Conclusions
We found that when the water was isolated from the surface and deep (140 meters) Escherichia coli was not pathogenic for laboratory animals.

We found that all microorganisms of the Clostridiaceae family, Clostridium genus, kind perfringens were pathogenic to laboratory animals in 100% of cases.

We found that the microorganisms Citrobacter freundii, Aeromonas hydrophila, Aeromonas caviae did not have pathogenic properties.

The isolated microorganism of the staphylococcus family was a pathogenic strain.

We have identified a microorganism of the family Enterobacteriaceae-Salmonella, which had pathogenic properties.

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