Assessment of the Level of Legionella Contamination in the Water Systems of the Metropolitan Area

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Abstract. The results of a study of the level of Legionella contamination in the water systems of the Metropolitan metropolis are presented. The study of biological contamination of Legionella pneumophila in water bodies of Moscow (Central administrative district) for social purposes (swimming pools, Jacuzzi, boiler room, fountain, and cooling water for cooling towers) revealed the occurrence of the studied species from 7% to 20%. The most frequent occurrence of Legionella pneumophila was found in the Central water supply of the city (CAO of Moscow), which was 36%. For the control and reduction of the activity of pathogens as Legionella pneumophila in water systems of public facilities the city needs to monitor the temperature changes in water supply in the building (hot water should be at a temperature higher than 60 °C and cold below 20 °C). It is also important to use the filters in the taps and showers and to carry out the ionization of water.

1. Introduction
Water in our life plays a significant role in the occurrence of many invasive and infectious diseases of animals and human ecology [1,2]. Among pathogens that can spread with water, protozoan parasites are of particular importance because they are spread all over the world, and in both developed and developing countries, they are one of the main causes of mass disease (4 billion cases of diarrhea per year), which cause 1.6 million deaths annually. Therefore, they are considered one of the main public health problems worldwide [3,4].

Some water-based bacteria, such as Legionella species, have a negative impact on human health. Currently, great interest is shown in this species because this species has a large distribution in many water places, such as ponds, lakes and streams. Legionella has a bad reputation due to its high pathogenicity [5].

2. Materials and methods
The study was conducted in 2018 on the basis of the branch of the Federal budget healthcare institution "Center for Hygiene and Epidemiology in Moscow” in the Central Administrative District of Moscow. 11 cooling towers of four enterprises (food, chemical, construction and scientific and technological profile) were examined. We also studied 33 cooling towers of industrial plants and 12 hot water systems for public buildings (shopping centers, office centers, hotels, and health care facilities)
When studying the Legionella pneumophila species, we took water samples from cooling towers and hot water supply systems of industrial enterprises and public facilities in Moscow (Central administrative district).

Image filtration equipment was used (a capsule for sampling, a laboratory shaker for mixing sample capsules, a portable continuous flow centrifuge) [5].

For the analysis of Legionella samples, 0.1 ml of concentrated samples were taken heated at 50°C, which were sown in Petri dishes with BUGAR medium with a growth and selective additive. The cups were incubated at 37 °C for up to 10 days in a humid atmosphere at 2.5% CO2.

Polymerase chain reaction (PCR) was used for rapid identification. For setting up PCR, 5 – 10 microl. of bacterial suspension disinfected by boiling is used. PCR is performed using certified PCR test systems based on the detection of gene fragments specific to the Legionella genus and L. pneumophila species [6,7].

To study the level of contamination of the hot water supply for Legionella, selected some buildings with a centralized cold water system. The hot water supply of the facilities was provided by heating cold water in the boiler room heaters to a temperature of 58-65 °C. Water samples with a volume of 500 – 1,000 ml and flushes from shower nets, internal surfaces of pipes and water taps were studied [5,8].

3. Results and discussion

Visual examination revealed biofilms on the surface of cooling towers that showed the level of contamination with Legionella species (table 1).

| № object's | Number of cooling towers | Concentration Legionella (CFU/l) | Presence of biofilms containing Legionella |
|------------|--------------------------|---------------------------------|-------------------------------------------|
|            |                          | min    | max    |                                            |                                          |
| 1          | 1                        | 580    | 2 360  | +                                          |                                          |
| 2          | 2                        | 124    | 150    | -                                          |                                          |
| 3          | 6                        | 860    | 3 600  | +                                          |                                          |
| 4          | 2                        | 11 640 | 96 200 | +                                          |                                          |

To determine contamination, the studied objects were divided into three groups:

1. There is no Legionella pneumophila species in the object under study, and no biofilms were found during visual inspection;
2. Legionella pneumophila in low concentrations (less than 1,000 CFU/l) was detected in the object, no biofilms were detected;
3. Legionella pneumophila was detected in the facility at a concentration exceeding the level of about 1,000 CFU/l and when the concentration of Legionella pneumophila in cooling tower water exceeds the level of 10,000 CFU / l in biofilms.

Out of 50 positive water samples and the presence of biofilms, the results of bacteriological research and PCR gave a positive result in 80% of cases. The number of genomic copies exceeded the number of CFU by 1-2 orders of magnitude. In five cases, positive PCR results were not confirmed by bacteriological tests. In four samples, the concentration of Legionella pneumophila exceeded 10,000 CFU / l, where the results of the bacteriological method and PCR coincided.

Table 2 shows the results of contamination in Central hot water supply systems in public buildings in Moscow (Central Administrative District).

| № object's | Number of cooling towers | Concentration Legionella (CFU/l) | Presence of biofilms containing Legionella |
|------------|--------------------------|---------------------------------|-------------------------------------------|
|            |                          | min    | max    |                                            |                                          |
| 1          | 1                        | 580    | 2 360  | +                                          |                                          |
| 2          | 2                        | 124    | 150    | -                                          |                                          |
| 3          | 6                        | 860    | 3 600  | +                                          |                                          |
| 4          | 2                        | 11 640 | 96 200 | +                                          |                                          |
Table 2. Contamination level for *Legionella pneumophila* in Central heating waters of public buildings in Moscow (Central Administrative District).

| № object’s | Concentration *Legionella* (CFU/l) | Hot water temperature (°C) | Number of sections of the object’s water supply system that pollute *L. pneumophila* |
|-------------|-----------------------------------|---------------------------|-------------------------------------------------------------|
|             | min | max    | min | max |                                   |
| 1           | 133 | 3 580 | 36  | 57  | -                                  |
| 2           | 117 | 8 420 | 42  | 58  | -                                  |
| 3           | 0   | 2 780 | 42  | 56  | -                                  |
| 4           | 0   | 36 190| 36  | 60  | -                                  |
| 5           | 0   | 84 320| 45  | 60  | +                                  |
| 6           | 580 | 118 400| 42  | 58  | -                                  |
| 7           | 0   | 236   | 44  | 60  | -                                  |
| 8           | 0   | 125   | 45  | 58  | -                                  |
| 9           | 0   | 618   | 44  | 58  | +                                  |
| 10          | 62  | 3 598 | 46  | 61  | -                                  |
| 11          | 0   | 1 810 | 52  | 65  | -                                  |
| 12          | 312 | 3 360 | 36  | 57  | +                                  |

We found a correlation between the level of *Legionella pneumophila* contamination and water temperature (Fig. 1).

![Correlation of Legionella with temperature](image)

**Figure 1.** Polynomial regression analysis of water contamination by *Legionella pneumophila* in public buildings’ hot water supply systems.

Our data are consistent with the results obtained by other authors [4-12]. The figure shows that there is a close relationship between water temperature and the concentration of the *Legionella pneumophila* species. Optimal temperatures for the species are the temperature range between 50-60 °C, at which the degree of water contamination by protozoan organisms is high. The concentration of *Legionella pneumophila* in hot water systems in public buildings in Moscow ranged from 62 to 118,400 CFU/l. The concentration of the pathogen exceeding 1,000 CFU/l was detected at 9 sites in the Metropolitan area.

The level of contamination of *Legionella pneumophila* species in cooling towers of industrial enterprises in Moscow and the Moscow region showed the following results. According to the results of bacteriological research and PCR, 44 positive samples of water and biofilms were detected.

Table 3 shows the level of contamination of *Legionella pneumophila* cooling towers of industrial enterprises in Moscow.
Table 3. *Legionella pneumophila* contamination level of industrial cooling towers in Moscow (Central Administrative District).

| № object’s | Number of cooling towers surveyed | Number of contaminated cooling towers | Concentration of *Legionella* (CFU/l) min max | Presence of biofilms containing *L. pneumophila* |
|------------|----------------------------------|--------------------------------------|----------------------------------------------|-----------------------------------------------|
| 1          | 4                                | 3                                    | 587 23980                                    | +                                             |
| 2          | 3                                | 2                                    | 123 844                                      | -                                             |
| 3          | 4                                | 2                                    | 118 148                                      | -                                             |
| 4          | 2                                | 0                                    | 0 0                                          | -                                             |
| 5          | 8                                | 6                                    | 839 358960                                   | +                                             |
| 6          | 2                                | 2                                    | 12350 96120                                  | +                                             |
| 7          | 1                                | 1                                    | 3000 4500                                    | +                                             |
| 8          | 1                                | 1                                    | 50 99                                        | +                                             |
| 9          | 1                                | 1                                    | 5890 1000000                                 | +                                             |
| 10         | 1                                | 1                                    | 1784 1928                                    | -                                             |
| 11         | 1                                | 1                                    | 0 5000                                       | +                                             |
| 12         | 5                                | 4                                    | 1589 225600                                  | +                                             |

In 100 samples of water from cooling towers of industrial enterprises in Moscow, *L. pneumophila* was detected in 52% of cases. In 9% of the Association with *Legionella pneumophila*, other pathogens of nosocomial infections were isolated: *Pseudomonas aeruginosa, Acitenobacter* spp., *Brevibacterium vesicularis, Micrococcus luteus* [11, 13].

In Figure 2, *Legionella pneumophila* water contamination in various places in public buildings is shown.

From the figure, you can see that water contamination was detected above in the water supply system (36%), in the boiler room (20%), in the pool (17%) and in the fountain (15%).

4. Conclusion
1. The study of contamination of *Legionella pneumophila* in water objects of Moscow (CAO) showed the following:
   - in water objects of social use (swimming pools, Jacuzzi, boiler room, decorative fountain, cooling water for cooling towers), the incidence of *Legionella pneumophila* ranged from 7% to 20%;
   - the highest percentage of the *Legionella pneumophila* species was found in the Central water supply of the city (CAO of Moscow), which was 36%.
2. Ways to control and reduce the activity of *Legionella pneumophila* pathogens in the water systems of public use of the Metropolitan metropolis should be based on:
- changes in the temperature regime for water supply in the building (hot water systems must use temperatures greater than 60 °C, and for cold water below 20 °C);
- mandatory use of filters in faucets and showers;
- carrying out the ionization of water.

5. References

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