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DETECTION OF LEGIONELLA SPP. IN THE SWIMMING POOLS IN THE AREA OF CENTRAL BOSNIA CANTON

DETEKCIJA LEGIONELLA SPP. U BAZENSKIM KUPALIŠTIMA SREDNJOBOSANSKOG KANTONA

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ABSTRACT

Legionella bacteria are microorganisms found in water and moist soil. They are especially favoured by moisture and heat, especially in different types of swimming pools where these bacteria reproduce and survive even for several months. *Legionella* spp. cause human diseases if they are introduced into the body through the respiratory tract. This most often happens by inhalation of aerosols, and less often by microaspiration of water contaminated with *Legionella*. The research aims to examine the presence of *Legionella* spp. bacteria in pool waters and the factors that influence their maintenance in these waters. Water samples were taken from 13 swimming pools that were registered for swimming and recreation in the Central Bosnian Canton in 2022. The presence of legionella was tested, and temperature, pH values (method BAS EN ISO 10523:2013), and residual chlorine concentrations were measured.

The temperature was measured by immersing the probe with a calibrated thermometer until the stabilization of temperature values on the display was reached. The free residual chlorine was measured by the standard colourimetric technique – the N, N-diethyl-phenylenediamine method (HANNA Instruments 96701, Rhode Island, USA). Descriptive statistics and the Chi-square test will be used for data analysis. Statistical analysis of the obtained data will be performed using the basic functions of MS Excel and GraphPad Prism. Of the total number of analyzed samples (n = 13), the presence of legionella was confrmed in 4 samples (31 %). The average water temperature of the samples taken was 30.21 0C and the average residual chlorine value was 0.43 mg/L, while the average pH value in the tested water samples was 6.48. There is a significant statistical difference in temperature, free

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residual chlorine concentration, and pH value with the presence of *Legionella* spp. The correlation between water temperature and the number of isolated bacteria was negative, i.e. as the water temperature increases, the number of isolated bacteria decreases. The correlation between residual chlorine and isolated bacteria indicates that there is a moderate negative association between these two variables. The presence of *Legionella* spp. in the pools in the investigated area is evident. The values of temperature, residual chlorine, and pH values that positively affect its growth and reproduction. To prevent the occurrence of diseases, it is important to regularly monitor and test the hygienic correctness of pool water, including the detection of *Legionella*.

Keywords: Swimming pool, Legionella, Microbiological correctness

SAŽETAK

Bakterije *Legionella* spp. su mikroorganizmi koji se nalaze u vodi i vlažnom tlu. Posebno im pogoduju vlaga i toplina, posebno u različitim vrstama bazena gdje se ove bakterije razmnožavaju i preživljavaju čak i nekoliko mjeseci. *Legionella* spp. izazivaju bolesti ljudi ako se u organizam unesu kroz respiratorni trakt. To se najčešće dešava udisanjem aerosola, a rjeđe mikroaspiracijom vode kontaminirane legionelom. Istraživanje ima za cilj ispitati prisustvo *Legionella* spp. bakterije u vodama bazena i faktori koji utiču na njihovo održavanje u tim vodama. Uzeti su uzorci vode sa 13 bazena koji su registrovani za kupanje i rekreaciju na području Srednjobosanskog kantona u 2022. godini. Testirano je prisustvo *Legionella* spp., te su utvrđena temperatura, pH vrijednosti (metod BAS EN ISO 10523:2013), te koncentracije zaostalog hlora. izmjereno. Temperatura je mjerena uranjanjem sonde sa kalibriranim termometrom dok se ne postigne stabilizacija vrijednosti temperature na displeju. Slobodni rezidualni hlor mjeren je standardnom kolorimetrijskom tehnikom – N,N-dietil-fenilendiamin metodom (HANNA Instruments 96701, Rhode Island, SAD). Za analizu podataka koristit će se deskriptivna statistika i Hi-kvadrat test. Statistička analiza dobijenih podataka vršit će se korištenjem osnovnih funkcija programa MS Excel i GraphPad Prism.

Od ukupnog broja analiziranih uzoraka (n = 13), prisustvo *Legionella* spp. je utvrđeno u 4 uzorka (31 %). Prosječna temperatura vode uzetih uzoraka iznosila je 30,21 0C i prosječna vrijednost rezidualnog hlora 0,43 mg/L, dok je prosječna pH vrijednost u ispitivanim uzorcima vode iznosila 6,48. Postoji značajna statistička razlika u temperaturi, koncentraciji slobodnog ostatka hlora i pH vrijednosti uz prisustvo *Legionella* spp. Korelacija između temperature vode i broja izoliranih bakterija bila je negativna, odnosno kako temperatura vode raste, broj izoliranih bakterija se smanjuje. Korelacija između rezidualnog hlora i izolovanih bakterija ukazuje da postoji umjerena negativna povezanost između ove dvije varijable.

Prisustvo *Legionella* spp. u bazenima na istraživanom području je evidentno. Vrijednosti temperature, rezidualnog hlora i pH vrijednosti koje pozitivno utiču na njegov rast i reprodukciju. Kako bi se spriječila pojava bolesti, važno je redovno pratiti i testirati higijensku ispravnost bazenske vode, uključujući i otkrivanje legionele.

Ključne riječi: bazen, Legionella, mikrobiološka ispravnost

INTRODUCTION

After the first recognition of legionellosis in 1976, when 221 participants of the annual convention of the American Legion contracted pneumonia, and 34 of them died, surveillance systems for these diseases were developed and implemented in a large number of countries (Springston, Yocavitch, 2017). Legionella spp. are widespread microorganisms that live - both in natural aquatic environments (ie lakes, rivers, groundwater, thermal waters) and in man-made aquatic environments, such as water systems of hospitals, hotels, and private houses (Borella et al.2005), cooling towers (Wasler et al. 2014). water systems of dental chairs (Žilić et al. 2019), and recreational (Donati et al. 2015) or therapeutic (Leoni et al. 2015) facilities. Any system or equipment that contains, stores, or recirculates non-sterile water that can be sprayed can be a route of transmission of Legionella (Leoni et al. 2015). The risk of exposure to legionella is often associated with recreation in swimming pools, especially in those with hot water and equipped with hydromassage systems that generate aerosols. The role of these recreational facilities is becoming even more significant due to the growing popularity of private hot tubs as well as the increasing number of people visiting public pools. More recent work shows that 14% of reported legionellosis outbreaks from 2006 to 2017 were associated with swimming pool use (Hamilton et al. 2018).

Legionella disease (LD), which is manifested by symptoms of pneumonia, and Pontiac fever (PF), which has a milder clinical picture similar to flu (Borella et al. 2005). Legionnaires' disease is a severe form of pneumonia, and L. pneumophila serogroup 1 is considered the most virulent of all species and serogroups and causes about 75% of all legionella infections (Springston et al. 2017). So far, more than 52 different *Legionella* species with at least 73 different serogroups have been described, of which about 20 species can cause human disease. For the disease to occur, Legionella must enter the body through the respiratory system by inhaling an aerosol containing the Legionella bacteria. Most often, it is about droplets with a diameter of less than 5 μm (~90% of the aerosol that is created during a shower) that can come into contact with the lower respiratory tract (Darquenne,C.,Prisk, G.K. 2004). Average incubation ranges from 2-10 days, and in rare cases, it can be longer, up to 16-20 days (Seenivasan et al. 2003). Legionnaires' disease has a high mortality rate of 10%-15%.

Legionnaires' disease is often associated with travel, that is, it is most often detected in travelers who become infected in the country they visit, but the symptoms of the disease, due to the long incubation period, appear after returning to the country of residence where the disease is diagnosed and reported. (Mouchtouri, Rudge, 2015).

Access to clean and safe drinking water is crucial for a healthy life and to prevent the spread of infectious diseases (Zucceri, Asproulis, 2012). Hygienic and clear water is also essential for swimming pools. Although swimming pools are typically filled with drinking water that meets hygiene standards, impurities and microorganisms can enter the water from the surrounding environment. The warm water temperature of the pool (between 20°C and 30°C) provides an ideal environment for fungi and bacteria to thrive, making it important to

maintain proper hygiene and cleanliness. The presence of impurities in the water can interfere with the effectiveness of disinfectants, making it critical to keep the water clean and free of contaminants, particularly for preventing the growth and spread of *Legionella*.

The aim of the work: Test for the presence of Legionella spp. in pool waters in the area of SBK and assess the epidemiological risk for the occurrence of legionellosis.

1. MATERIAL AND METHODS

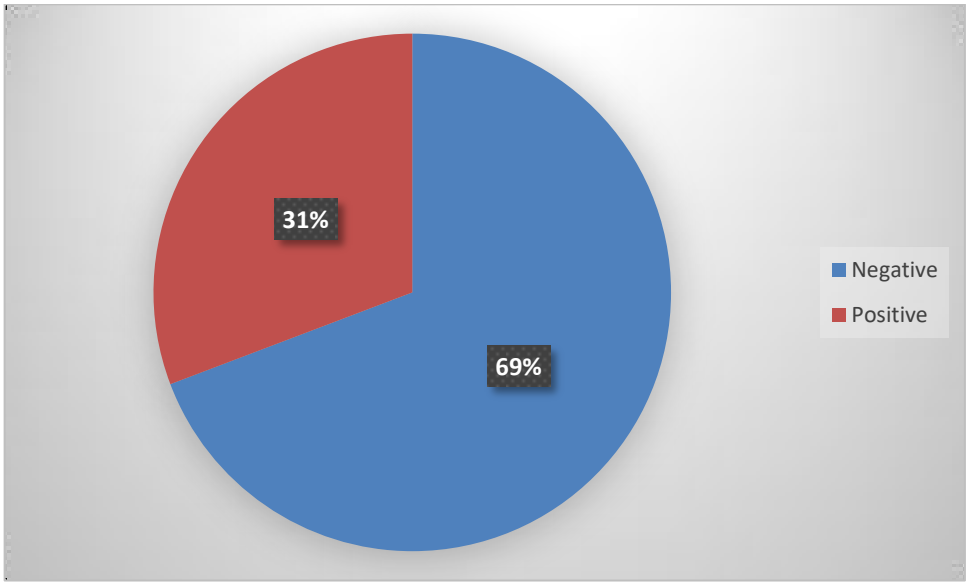
The water samples were taken from 13 swimming pools (open and closed) in the in Central Bosnia Region 2022. The average daily turnover of people in the pools is 50-70. Water samples (1 L) were taken in sterile glass bottles to which, before sterilization, 0.1 mL of standard sodium thiosulfate solution, concentration 0.1 mol/l, was added. Variable parameters were determined at the point of sampling: free residual chlorine and water temperature. After sampling, the samples were stored and transferred in a portable cooler to the laboratory for microbiological and chemical analysis as soon as possible. All steps for quality assurance of laboratory performance for microbiological analyzes are implemented and recorded. The analysis of the presence of *Legionella* in water samples was carried out using the method BAS EN ISO 11731:2018 Water quality - Detection and counting of *Legionella*: membrane filtration method for water with a low number of bacteria.

In addition to legionella detection, the following parameters were measured: temperature, pH values, and measured concentrations of residual chlorine. The temperature was measured by immersing the probe with a calibrated thermometer until stabilization of the temperature value was achieved. The pH value was measured using the BAS EN ISO 10523:2013 method. Free residual chlorine was measured using a standard colourimetric technique – the N, N-diethyl-phenylenediamine method (HANNA Instruments 96701, Rhode Island, USA). Since there is no valid legislation in the area of SBK that regulates the presence of Legionella spp. in water for bathing and recreation, as reference values were used as part of the Rulebook on Amendments to the Rulebook on Sanitary-Technical and Hygienic Conditions of Swimming Pools, and on the Healthiness of Pool Water from 2014, where the reference values are specified both microbiological and physical and chemical indicators of pool water quality. The data obtained from the analysis of samples for the presence of legionella were statistically analyzed with appropriate methods, using the GraphPad Prism 9.3.1 computer program. The existence of statistically significant differences in the frequency of positive and negative samples concerning temperature, residual chlorine, and pH value of pool water was tested.

2. RESULTS AND DISCUSSION

Among 13 samples, *Legionella* spp. was detected in 31% (Graph 1).

Graph 1. Presentation of positive and negative samples for the presence of *Legionella* spp in 2022



Source: authors

Table 1 will present the values of observed statistical indicators and isolated *Legionella* of the total number of samples in the observed period.

Table 1. Overview of the values of basic statistical indicators of the total number of samples in 2022

Sample	Water temperature (°C)	Free residual chlorine (mg/l)	pH value	Isolated legionella
1	32	0.21	7	0
2	28	0.1	7.2	0
3	29	0.6	6.8	0
4	21.3	0.23	7.42	+
5	21.3	0.23	7.42	+
6	30	0.1	6.22	+
7	27.6	0.17	6.2	+
8	31	0,14	7	0
9	32	1.46	7	0
10	35.5	0.47	7	0

11	34	0.35	7	0
12	35.5	0.5	7	0
13	35.5	1.01	7	0

Source: authors

The results showed the presence of *Legionella* spp. in 4 pools. The measured water temperature ranged from 21.3 to 35.5 °C, with an average temperature of 30.21 °C, with a standard deviation of 4.8 °C. The average residual chlorine value was 0.43 (±0.4), while its values ranged from 0.1 to 1.46. The average pH value is 6.48 (±1.9). Table 2. Presents the correlation between observed statistical indicators and samples in which *Legionella* spp. was detected.

Table 2: Correlation between temperatures, residual chlorine, pH value, and number of isolated bacteria

Variable	**Positive samples for the presence of *Legionella* spp**
Water temperature (°C)	-0,744
Free residual chlorine (mg/l)	-0,425
pH value	-0,424

Source: authors

The correlation between water temperatures and the number of isolated bacteria is negative (-0.744), which means that there is a tendency for increasing the water temperature to decrease the number of isolated bacteria and decreasing the water temperature to increase the number of isolated bacteria. This would be consistent with common knowledge that high temperatures can inhibit bacterial growth. The correlation between residual chlorine and isolated bacteria of -0.425 indicates that there is a moderate negative relationship between the two variables, indicating that the higher the concentration of residual chlorine in the water, the lower the number of isolated bacteria in the water. This may indicate that residual chlorine has antibacterial properties and may be useful in maintaining water purity.

According to the results of the research, Bešić et al. on the influence of free residual chlorine on the presence of *Legionella* showed that there were no statistically significant differences in the number of positive and negative results. *Legionella* spp. 8.82% of water samples taken from fountains, pools, and cooling and heating systems were detected (Bešić et al. 2017), which differs from the results of this research. It has been proven that there is a moderate negative relationship between these two variables.

Rakić et al. [14] proved a correlation between temperature and the presence of *L.pneumophila* in water. The average temperature at which the bacterium was present was 47.9 °C compared to the mean temperature of 53.8 °C in waters where *Legionella* spp.was not detected. The correlation between the mentioned two variables was also proven within this research.

The optimal temperature for the growth and development of *Legionella* spp. ranges from 32 °C to 42 °C. Other factors favoring the development of *Legionella* spp. are water stagnation, the presence of biofilms or nutrients, and the presence of other microorganisms. These are the risk factors that are examined to assess the risk of legionellosis (Obradović et

al. 2014). The research carried out in Sveti Martin Spa, Croatia showed that a total of 53 (15.27%) samples were considered defective, of which (4.32%) were due to microbiological defects. (Capan, 2011). The pH value and isolated bacteria have a negative correlation of -0.424. A correlation between pH value and isolated bacteria of -0.424 means that there is a negative relationship between these two variables. This means that when the pH value increases, the number of isolated bacteria decreases, and when the pH value decreases, the number of isolated bacteria increases. However, it is important to keep in mind that correlation does not indicate a causal relationship between variables, i.e. we cannot conclude that a change in pH value causes a change in the number of isolated bacteria.

A two-way t-test was used to determine whether there is a statistically significant difference between the isolated bacteria when it comes to water and air temperature, residual chlorine, and pH values, for a significance level of α=0.05. Table 3 will present the difference between the mean values of the tested parameters of the samples in which the presence of *Legionella* spp was detected and the samples that were negative.

Table 3. The difference between the mean values of the investigated parameters

	Water temperature (°C)	**Free residual chlorine (mg/l)**	**pH value**
Positive samples for the presence of *Legionella* spp	25.05 (±4.43)	0.1825 (±0.061)	5.31 (±3.44)
Negative samples for the presence of *Legionella* spp	32.5 (±2.83)	0.54 (±0.44)	7(±0.1)
t value	-3.39	-3.686	-2.725
P value	0.0056	0.003	0.017

Source: authors

The water temperature of the samples in which *Legionella* spp. was detected ranged from 21.3-30°C, i.e. with an average temperature of 25.5°C, the concentration of residual chlorine ranged from 0.1-0.23 mg/l and the average pH value was 6. 81. The average temperature value for negative samples is 32.5°C, the residual chlorine concentration is 0.54 mg/l, and the pH value is 7. Research by Borello et al. showed that higher temperatures and lower concentrations of residual free chlorine promote the growth of L. pneumophila. Legionella species are more resistant to chlorine than other bacteria (Borella et al., 2004). Based on the data from we can conclude that there are statistically significant differences between samples with isolated and non-isolated bacteria for water temperature, residual chlorine, and pH value, with p-values less than 0.05.

Based on the results obtained related to the presence of legionella and other characteristics of water in pools that are suitable for their growth and reproduction, and based on data on pool users, an attempt was made to make a rough assessment of the epidemiological risk for the occurrence of legionellosis in SBK/KSB. All pools are open throughout the year, but the highest frequency of users is recorded during January and from June to September, that is, during the school holidays. in the school year. The average daily frequency of bathers per pool is 50-70. The capacity of the spa is 500 users, but not all users

of the spa use the pool. When assessing the risk, it is important to pay attention to the characteristics of the users, because the risk is not the same for healthy people who use the pools for recreational purposes and those who are immunocompromised, who are in a spa for rehabilitation, and who have some previous illnesses. In our case, the risk of legionellosis is evident because among the users there is a large number of immunocompromised people. The presence of biofilm, as well as other microbiological parameters, were not investigated in this research, which will be the subject of future research.

CONCLUSIONS

- *Legionella* spp. was detected in 31% of pool water samples in the SBK area in 2022.
- The average temperature of the examined samples in which *Legionella* spp. was detected is 25.5˚C.
- The average residual chlorine concentration of the samples in which *Legionella* spp. was detected is 0.54 mg/l.
- The average pH value of the samples in which *Legionella* spp. was detected was 6.81.
- The correlation between temperature and positive samples for the presence of *Legionella* spp. is negative.
- The correlation between residual chlorine concentration and positive samples for the presence of *Legionella* spp. is moderately negative.
- The correlation between pH values and positive samples for the presence of *Legionella* spp. is negative.
- It is necessary to intensify pool water quality tests while simultaneously raising awareness of all risks and prevention measures.

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