

DOI 10.7251/VETJEN2401088G

UDK 636.52/.58.082.47:579.68

Original Scientific Paper

MICROBIOLOGICAL STATUS OF DRINKING WATER FROM BROILER FARMS AND BIOFILM-FORMING ABILITY OF ISOLATED PATHOGENIC BACTERIA

Bojan GOLIĆ^{1*}, Aleksandra ŠMITRAN², Ljiljana BOŽIĆ², Dragan KNEŽEVIĆ¹, Dejana KRNETA¹, Goran VASILIC¹, Dejan LAUŠEVIĆ³, Željko CVETNIĆ⁴, Drago N. NEDIĆ⁵

¹PI Veterinary Institute of the Republic of Srpska “Dr. Vaso Butozan” Banja Luka, Bosnia and Herzegovina

²University of Banja Luka, Faculty of Medicine, Banja Luka, Bosnia and Herzegovina

³Specialist Veterinary Laboratory, Podgorica, Montenegro

⁴Croatian Academy of Sciences and Arts, Zagreb, Croatia

⁵University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia

*Corresponding author: Bojan Golić, bojan.golic@virs-vb.com

Summary

Water is an essential nutrient for the development of broiler chickens, which consume it in large quantities. Providing safe and high-quality water is crucial for maintaining the health of the individual. Biofilm is a protective mechanism for bacteria to group together and survive adverse conditions in the external environment. It is most often formed at the transition between the aquatic environment and air or the aquatic environment and a solid substrate in nature. The study was conducted during 2023 and included 150 water samples originating from broiler chicken farms. Laboratory testing of water was performed using the BAS EN ISO 6222, BAS EN ISO 9308 and BAS EN ISO 7899-2 methods. The aim of the study is to determine the microbiological status of water originating from poultry farms, as well as to examine the ability of isolated pathogenic bacteria from water to produce biofilms, which bacteria very easily and efficiently create in the aquatic ecosystem, and which can significantly contribute to the creation of antimicrobial resistance. Microbiological testing determined 64.67% satisfactory and 35.33% unsatisfactory drinking water samples. Drinking water on broiler chicken farms has a very unfavorable microbiological status, especially from the point of view of the adverse impact of pathogenic bacteria on this sensitive category of poultry. The water disinfection process needs to be considered and significantly improved, especially considering the significant use of well and spring water, and favorable conditions for the formation of biofilms in water supply systems. *Escherichia coli* strains showed significantly better biofilm formation at 25°C compared to 37°C, as well as compared to *Enterococcus spp* isolates.

Key words: water, microbiology, broilers, pathogens, biofilm.

INTRODUCTION

Birds are small animals that mature early, and their lower resistance make them more susceptible to infections, mainly caused by pathogens of intestinal origin, which may be present in water, so Macari (1997) and Englert (1998) recommend that drinking water intended for humans should be used for birds.

Water is the most critical nutrient for poultry (Bell, 2002) which support metabolism and excretion (Jafari et al., 2006). Compared to other nutrients, birds consume large amounts of water. Water consumption can be twice the amount of feed consumed and can vary depending on the age and species of the bird, activity level, air temperature, humidity, respiratory rate, and existing diseases, as well as environmental conditions such as heat stress.

Any increase or decrease in the normal water consumption of poultry may indicate a health disorder in the bird (Butcher et al., 1999). The availability and quality of water play a significant role in the overall health and productivity of birds. However, the role of providing safe and quality water for optimal performance is largely overlooked.

Bacteria are present in drinking water, even in relatively high numbers (10^3 to 10^6 CFU/ml), without consequences for human health, as long as no pathogens are present (Hoefel et al., 2005; Hammes et al., 2008; Vital et al., 2012).

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excreta, which becomes a source of pathogenic bacteria, viruses and parasites and increases health risks (Sasakova et al., 2013; Fridrich et al., 2014).

The use of indicator organisms, especially the coliform group, to assess the potential presence of waterborne pathogens is crucial for the protection of public health (Hijnen et al., 2020).

Escherichia coli is a coliform bacterium, considered a primary indicator of faecal contamination of treated and untreated water and occurs in the faeces of all mammals, often in high numbers (Edberg, 2000; WHO, 2008).

Enterococci include numerous species that occur in the faeces of humans and warm-blooded animals and are considered secondary indicators of faecal pollution (WHO, 2008).

The development of the gut microbiota in animals is profoundly influenced by their early life environment (Ballou et al., 2016). Reducing the bacterial load in the surrounding environment, and thereby reducing the intake of pathogens, has the advantage of establishing a healthy gut microbiota ecology.

Meza (1989) states that there should be better bacteriological control of water given to birds during the initial phase of development, as there is rapid bacterial growth and an increased health risk for birds from 1 to 21 days of age.

It must be noted that the water supplied to birds on many farms is contaminated at the source. It has been reported that samples from water sources and tanks were contaminated with *Escherichia coli* in 10 broiler and laying hen farms, which is

evidence of faecal contamination of the samples (Amaral et al., 1999; Amaral et al., 2001).

Diseases caused by bacteria, viruses and protozoa are among the most common diseases in poultry farming, in which drinking water plays an important role (Gama, 1995).

The presence of bacteria, fungi, minerals and water additives in poultry water systems and drinkers hinders effective management practices aimed at achieving optimal performance (Oviedo, 2006).

Microbial contamination in poultry drinking water threatens the general health and performance of birds (Maharjan et al., 2016). Poultry water supply systems can be a suitable habitat for bacteria, which pose a health risk to birds.

Biofilm is a sessile lifestyle of microorganisms and is formed when bacteria, after adhering to a substrate and successfully multiplying, begin to synthesize an adhesive polysaccharide matrix shell, which plays a role in additional fixation of the biofilm to the substrate, as well as in mechanical protection of bacteria within the biofilm from external environmental conditions, the effects of antimicrobial substances and the host immune system, if the biofilm is formed in a living host (Liu et al., 2016). If the biofilm is formed in a non-living environment, biocorrosion and damage to the substrate on which the biofilm was formed occurs. Biofilm is a way in which bacteria overcome unfavorable environmental conditions and survive and spread from an inanimate environment to a host (humans or animals).

The formation of a community within a biofilm is one of the most important mechanisms contributing to the development of antimicrobial resistance, as it prevents the passage of antibiotics to bacteria and allows bacteria to transit to persistent forms with reduced metabolism and division (Bowler et al., 2020). Therefore, the eradication of bacteria formulated in this way requires doses of antibiotics that are 10-1,000 times higher than those tested classically for individual planktonic strains. Biofilm is a protective mechanism for bacteria to group together, forming a protective shell of exopolysaccharides, and survive unfavorable conditions in the external environment. Biofilm, or a sessile community of microorganisms, is most often formed at the transition between the aquatic environment and air or the aquatic environment and a solid substrate in nature. Within the biofilm, bacteria are protected, but due to limited nutrients, bacteria begin to die, which weakens the sessile community and the protective coating, leading to the weakening and damage of the biofilm. In this way, bacteria that have the ability to form biofilms disperse and seek new substrates and niches in which there are more favorable conditions for regrouping and spreading biofilms in nature. Biofilms are very important due to the long-term survival and survival of bacteria in artificial substrates and environments such as water supply networks, filters, instruments used during washing and handling food, which can serve as a significant reservoir of bacteria for constant contamination of water and food, i.e. they can cause infections and intoxications. Due to the association of biofilm with possible contamination of water systems and indirect contamination of food during

washing with such water, it is very important to examine the ability of bacteria to form biofilm at ambient temperatures and temperatures of warm-blooded organisms. Higher water temperatures and stagnant flow in the poultry water system increase bacterial growth and biofilm formation (Maharjan et al., 2017).

Studies have reported increased biofilm formation at higher water temperatures (Kadam et al., 2013; Bonsaglia et al., 2014).

Poimenidou et al. (2016) observed that 20°C was more supportive to biofilm formation than 37°C, which is the house temperature during the second half of the bird growth period.

Untreated drinking water on farms may be more susceptible to the presence of pathogens in the drinking system. Even treated drinking water was susceptible to biofilm formation in the poultry drinking system during the flock growth period. Therefore, cleaning the drinking system between two rotations becomes a mandatory practice for improvement (Raut et al., 2024).

The poor efficacy of disinfectants against the polymer matrix of biofilms favors better protection of bacteria on their surfaces, despite disinfection practices (Muhterem-Uyar et al., 2015). Flushing the supply system with disinfected water releases substances and thus removes the developed biofilm.

The aim of the study is to determine the microbiological status of water originating from poultry farms, as well as to examine the biofilm production ability of isolated pathogenic bacteria from water, which bacteria very easily and efficiently create in the aquatic ecosystem, and which can significantly contribute to the creation of antimicrobial resistance.

MATERIALS AND METHODS

The material for testing is 150 water samples originating from broiler farms located on the territory of the Republic of Srpska, which were sampled in 2023, of which 93 samples (62%) originated from the water supply, 44 samples (29.33%) from well water and 13 samples (8.67%) from spring water. Most of the well and spring water samples were treated with disinfectants immediately before being released into the water supply system.

Sampling was carried out in such a way that all fittings were removed from the tap, the water was allowed to flow for 2-3 minutes, after which the tap was closed and the tap was disinfected with alcohol and sterilized with a flame, and the water was allowed to flow again for 2-3 minutes. After that, the water was poured into darkened sterile glass bottles with a volume of 1 liter, with water being poured into them in an amount of $\frac{3}{4}$ of the bottle's volume. The samples were transported at a temperature of $5\pm 3^\circ\text{C}$, and the testing was carried out within 6 hours of sampling.

Laboratory water testing was performed using the following standard methods:

- number of microorganisms at 22°C and 37°C according to BAS EN ISO 6222 (ISBIH, 2003a),
-

- number of intestinal enterococci according to BAS EN ISO 7899-2 (ISBIH, 2003b),
- the number of coliform bacteria and *Escherichia coli* according to BAS EN ISO 9308 (ISBIH, 2018).

The Rulebook on the Health Safety of Drinking Water Intended for Human Consumption (Regulation, 2017) establishes testing parameters and microbiological criteria (Table 1).

Table 1 Test parameters and microbiological criteria

Parameter	Purified water	Unpurified water
	Criterion	
Number of microorganisms at 22°C	100CFU/1 ml	300CFU/1 ml
Number of microorganisms at 37°C	20CFU/1 ml	100CFU/1 ml
Intestinal enterococci	0CFU/100 ml	0CFU/100 ml
<i>Escherichia coli</i>	0CFU/100 ml	0CFU/100 ml
Coliforms	0CFU/100 ml	0CFU/100 ml

The biofilm production assay was performed according to the method of Stepanovic et al. (2000), with a modification in the concentration of the tested inoculum of 10^6 CFU/ml, which was prepared in brain-heart infusion broth (BHI). Three tests were performed for each strain, i.e. three wells were inoculated with 100 μ l of BHI for each strain. The microtiter plates were then incubated for 24 hours at 25°C and 37°C. After incubation, the contents of the plate were removed by pipetting, and each well was washed three times with buffer. Adherent bacteria were fixed by air drying. After drying, 100 μ l of crystal violet dye from a Gram staining kit (Biomérieux, France) was pipetted into each well. Staining lasted 15 minutes, after which the plates were washed three times with buffer and air-dried. To resuspend the stain, 100 μ l of 30% acetic acid (Zorka, Šabac) was added to each well. The absorbance was read on an automated ICN Flow Titrek Multiscan Plus reader at λ of 570 nm. Three negative samples were used on each microtiter plate, i.e. three wells were inoculated with sterile TXY broth. Based on this, the cut-off value of the ODc absorbance was determined, which was calculated as the mean value of the three negative samples increased by three standard deviations. Isolates were designated as non-biofilm producers, and weak, moderate and strong producers based on the following formulas:

- $OD < OD_c$ (non-producers)
- $OD_c < OD < 2 \times OD_c$ (weak producers)
- $2 \times OD_c < OD < 4 \times OD_c$ (moderate producers)
- $4 \times OD_c < OD$ (strong producers).

The reference strain *Staphylococcus epidermidis* ATCC 14990 was used as a positive control.

In the analysis of the obtained microbiological test results, we used descriptive statistical parameters (Excel, Micosoft Office 2019). To compare the degree of biofilm production between the two groups of bacteria, Fisher's exact probability test was used. To compare the biomass of the formed biofilm in the two groups of tested bacteria, T test was used. The value $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Microbiological testing of water samples

Microbiological water quality is defined as the maximum acceptable number or concentration of bacteria that do not pose a health hazard (Regulation, 2020), and *Escherichia coli* and intestinal enterococci are considered basic parameters. The production and distribution of water with biologically stability can only be achieved by adequate monitoring and control of microbiological processes during water treatment and distribution (Prest et al., 2016). The assessment of microbiological compliance of drinking water samples was carried out in relation to the Rulebook on the Health Safety of Drinking Water Intended for Human Consumption (Regulation, 2017). Microbiological testing determined 64.67% satisfactory and 35.33% unsatisfactory drinking water samples, and the results by water category are shown in Table 2.

Table 2 Microbiological test results by category

Water category	Satisfactory samples %	Unsatisfactory samples %
Water supply network	74.19	25.81
Well water	54.55	45.45
Spring water	30.77	69.23

Water is essential for organisms to maintain their basic metabolic activities, and the quality of drinking water is undoubtedly linked to animal health and performance (El-Katcha et al., 2018; Guo et al., 2022). Kalaba et al. (2018) found that 26.20% of water samples obtained from animal farms were unsatisfactory in the Republic of Srpska for the period from 2015 to 2017, and Golić et al. (2023) found that 45.82% of water samples were unsatisfactory during 2022. The results obtained are within the scope of previous research, and indicate variations from year to year, which indicates a constant significant microbiological contamination of drinking water, and both the inadequacy of the disinfection process and inconsistency in its application. Table 3 shows unsatisfactory samples according to test parameters.

Table 3 Unsatisfactory samples according to test parameters

Parameter	Unsatisfactory samples %
Number of microorganisms at 22°C	12.67
Number of microorganisms at 37°C	15.33
Intestinal enterococci	13.33
<i>Escherichia coli</i>	20
Coliforms	20

The results indicate that apart from *Escherichia coli*, there were no other coliforms in the tested water samples. In a study of microbiological analysis of water on farms in the Republic of Srpska (Kalaba et al., 2015), it was found that 62.66% of water samples obtained from animal farms were unsatisfactory and that the most common causes of irregularities were related to enterococci, *Escherichia coli* and the number of microorganisms at 22°C and 37°C. Similar results were obtained by Golić et al. (2023). The results obtained in our study confirm the previous ones, only with a significantly lower percentage per tested parameter.

Table 4 shows the unsatisfactory results of microbiological testing in relation to the total number of samples by category, according to the test parameter.

Table 4 Unsatisfactory microbiological test results in relation to the total number of samples by category according to the test parameter

Parameter	Unsatisfactory samples in %		
	Water supply network	Well water	Spring water
Number of microorganisms at 22°C	8.60	13.64	38.46
Number of microorganisms at 37°C	11.83	18.18	30.77
Intestinal enterococci	9.68	13.64	38.46
<i>Escherichia coli</i>	13.98	18.18	69.23
Coliforms	13.98	18.18	69.23

In the period 2018-2020, it was found that 23.41% of water samples originating from animal farms were unsatisfactory (Golić et al., 2021), of which 10.19% of samples were due to the presence of intestinal enterococci, and 6.44% of samples were due to the presence of *Escherichia coli*. In our study, similar results were obtained for intestinal enterococci, but significantly more favorable results were obtained for *Escherichia coli*. The highest percentage of unsatisfactory samples refers to spring water, possibly because it is not disinfected, unlike tap and well water.

Table 5 shows the results of microbiological testing by category and testing parameter in relation to unsatisfactory samples.

Table 5 Microbiological testing results by category and testing parameter in relation to unsatisfactory samples

Parameter	Unsatisfactory samples in %		
	Water supply network	Well water	Spring water
Number of microorganisms at 22°C	33.33	30	55.56
Number of microorganisms at 37°C	45.83	40	44.44
Intestinal enterococci	37.50	30	55.56
<i>Escherichia coli</i>	54.17	40	100
Coliforms	54.17	40	100

Faecal coliforms were reported in water samples from 20 farms (50%), which were above the maximum acceptable level (Jafari et al., 2006). Amaral et al. (1995) found that 90% of well water samples and 100% of spring water samples had bacteria indicative of faecal contamination. Kalaba et al. (2018) found that 63.40% of unsatisfactory samples were due to increased the number of microorganisms at 22°C, 54.90% were due to increased the number of microorganisms at 37°C, 58.80% were due to the presence of intestinal enterococci, 31.40% were due to the presence of coliforms and 19% were due to the presence of *Escherichia coli*. Also, our study found a high percentage of unsatisfactory drinking water samples in relation to the testing parameters.

Biofilm formation testing of *Escherichia coli* and *Enterococcus spp.* isolates

A total of 30 strains of *Escherichia coli* and 20 strains of *Enterococcus spp.*, which were isolated from all samples during the basic microbiological analysis, were tested. The test was performed in a way that the total amount of biofilm biomass was determined based on the extinction of the dye. The strains were marked as non-biofilm producers, and as weak, moderate and strong producers based on negative controls. Among *Escherichia coli* strains, a statistically significant difference was observed in the biomass of the formed biofilm, as well as in the degree of biofilm production at the tested incubation temperatures (Table 6). At room temperature, more than half of the isolates (17/30, i.e. 56.7%) showed strong biofilm production, while at the temperature of warm-blooded organisms, moderate producers were the most common (16/30, i.e. 53%). When it comes to the biomass of the biofilm created, it was observed that the isolates at room temperature created a biomass of 0.63 ± 0.71 , compared to a statistically highly significantly lower biofilm production at the temperature of warm-blooded organisms of 0.21 ± 0.12 ($p=0.003$)

Table 6 Biofilm production of *Escherichia coli* isolates at two tested temperatures in %

Strain	25°C	37°C	<i>p</i>
Non-producer	3 (10%)	2 (6.7%)	0.001*
Weak producer	7 (23.3%)	7 (23.3%)	
Moderate producer	3 (10%)	16 (53.3%)	
Strong producer	17 (56.7%)	5 (16.7%)	

On the other hand, enterococci showed an equal ability to produce biofilm, whether the degree of biofilm production (Table 7) or biomass was examined. At both temperatures, they produced almost the same biofilm biomass (0.07 at the lower and 0.09 at the higher temperature) ($p>0.05$).

Table 7 Biofilm production of *Enterococci spp.* isolates at two tested temperatures

Strain	25°C	37°C	<i>p</i>
Non-producer	8 (40%)	2 (10%)	0.06
Weak producer	8 (40%)	8 (40%)	
Moderate producer	3 (15%)	9 (45%)	
Strong producer	1 (5%)	1 (5%)	

When comparing the biofilm-forming ability of these two bacteria at the above temperatures, it is observed that *Escherichia coli* was a significantly better biofilm producer in terms of both biomass and biofilm production rate at 25°C ($p=0.001$, $p=0.00018$) (Figure 1-4).

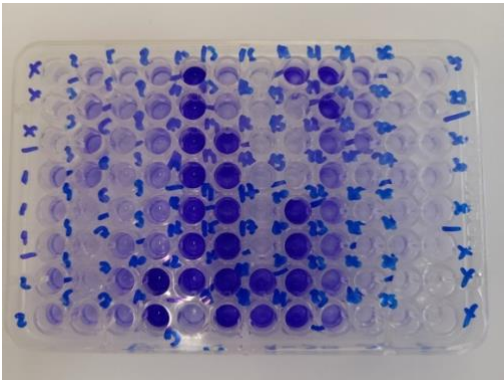


Figure 1 *Escherichia coli* biofilm production at 25°C

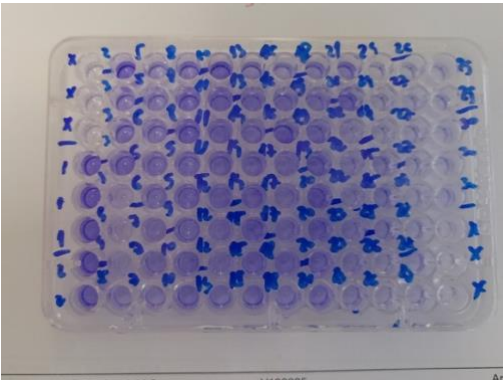


Figure 2 *Escherichia coli* biofilm production at 37°C

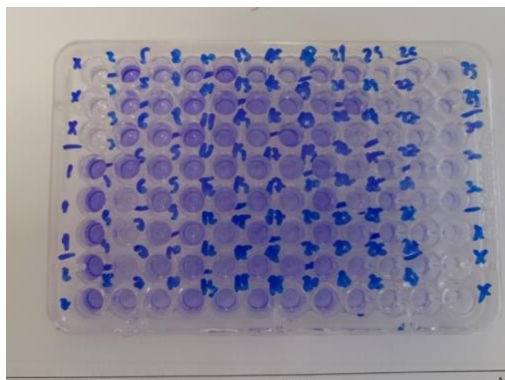


Figure 3 Biofilm production of *Enterococcus* spp. at 25°C

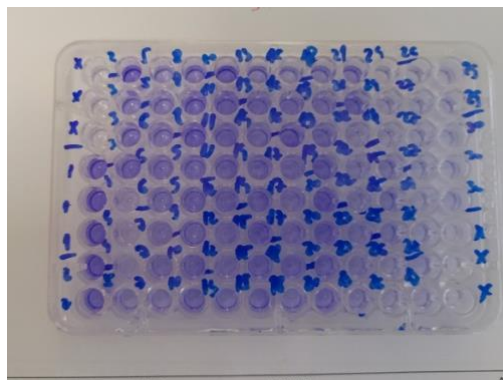


Figure 4 Biofilm production of *Enterococcus* spp. at 37°C

At room temperature, most *Escherichia coli* isolates were moderate and strong producers (20/30, i.e. 66.7%), while most *Enterococcus* isolates were non-producers and weak producers (16/20, i.e. 80%). This is clearly visible when looking at the biofilm biomass, as the extinction of the dye was tenfold higher at 25°C. At the temperature of warm-blooded organisms, both tested microorganisms produced biofilm equally well when comparing the degree of biofilm production, as well as the biomass created ($p > 0.05$, $p > 0.05$).

Biofilm formation is a universal protective mechanism for microorganisms in adverse conditions. Aquatic ecosystems in nature or in the water supply network represent an excellent environment for the formation of microbial communities in the form of biofilms, which bacteria themselves create after reaching a critical number on a certain substrate or medium (Liu et al., 2016). In our study, *Escherichia coli* strains showed significantly better biofilm formation at lower temperatures compared to higher incubation temperatures, as well as compared to enterococcal isolates. Our results are in agreement with the work of Ingle et al. (2011) and Maltholouti et al. (2018) who also demonstrated better biofilm production at 25°C, especially if rich nutrient media with neutral pH were used, as was the case in our case. Important virulence factors that contribute to biofilm formation are curly pili and cellulose production. Pili are important both as an adhesion factor and as a structural protein element, and cellulose is the dominant polysaccharide component. The gene for cellulose production is inactivated at 37°C, while it is activated at temperatures below 30°C (Uhlich et al., 2014). This could also explain our results. On the other hand, temperature had no effect on biofilm formation of enterococcal strains, even when temperatures in the range of 10-45°C were used (Marinho et al., 2013), which is in agreement with our study. The same authors showed that the addition of higher glucose concentrations led to differences in biofilm formation at different temperatures. In future work, it would be interesting to determine whether the strains used in our study will also exhibit similar behavior. Also, Jahan et al. (2014) showed that better biofilm production at 25°C compared to 37°C is primarily species-dependent and that *Enterococcus faecalis*

isolates were better producers compared to *Enterococcus faecium*, which poses an important task for us to perform speciation of our isolates in order to determine which species were dominant in our study.

CONCLUSION

Drinking water on broiler farms has a very unfavorable microbiological status, especially from the point of view of the adverse impact of pathogenic bacteria on this sensitive category of poultry. The water disinfection process needs to be considered and significantly improved, especially considering the significant use of well and spring water, and favorable conditions for biofilm formation in water supply systems. *Escherichia coli* strains showed significantly better biofilm formation at 25°C compared to 37°C, as well as in relation to *Enterococcus spp.* isolates. Since we did not examine the effect of nutrients on the effect of biofilm production and did not have complete identification of the isolates, we cannot give a definitive answer as to why the enterococcal isolates formed significantly less biofilm, except to assume that they, as gram-positive bacteria, are less voluminous and were not induced to produce biofilm to the extent that gram-negative *Escherichia coli* isolates did. Similar studies have not been conducted and no data are available, so in future studies we need to detect virulence factors in both species that are important during biofilm formation, in order to clarify their behavior in a sessile community in aquatic ecosystems.

Acknowledgment

The study was supported by the Ministry of Scientific and Technological Development and Higher Education of Republic of Srpska (contract No. 19.032/961-101/23).

Conflict of interest statement: The authors declare that there is no conflict of interest.

REFERENCES

- Amaral L. A., Nader Filho A., Isa H., Barros L. S. S. (2001): Qualidade higiênico-sanitária e demanda de cloro da água de dessedentação de galinhas de postura coletadas em bebedouros tipo nipple e taça. *Revista Brasileira de Ciência Avícola*, 3(3):249-255.
- Amaral L. A., Nader Filho A., Rossi Junior O. D., Penha L. H. C. (1995): Características microbiológicas da água utilizada no processo obtenção do leite. *Pesquisa Veterinária Brasileira*, 15(2/3):85-88.
- Amaral L. A., Rossi Junior O. D., Cardoso V. (1999): Qualidade higienico-sanitária da água de bebedouros pendular e nipple utilizados na criação de frangos de corte. *Revista Brasileira de Ciência Avícola*, 1(2):145-148.
- Ballou A. L., Ali R. A., Mendoza M. A., Ellis J. C., Hassan H. M., Croom W. J., Koci M. D. (2016): Development of the chick microbiome: how early exposure influences future microbial diversity. *Front. Vet. Sci.*, 3:(2).
- Bauer, A. W., Kirby W. M. M., Sherris J. C., Turck M. (1966): Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 36:493-496.
-

- Bell D. D. (2002): Consumption and quality of water. In Commercial Chicken Meat and Egg Production, 5th edition. Eds. D. D. Bell., W. D. Weaver, Jr. Springer, 411-430.
- Bonsaglia E. C. R., Silva N. C. C., Fernandes A. J., Araujo J. P. J., Tsunemi M. H., Rall V. L. M. (2014): Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. *Food Control*, 35:386-391.
- Bowler P., Murphy C., Wolcott R. (2020): Biofilm exacerbates antibiotic resistance: Is this a current oversight in antimicrobial stewardship? *Antimicrobial Resistance & Infection Control*, 9(1), 1-5.
- Butcher G. D., Jacob J. P., Mather F. B. (1999): Common poultry diseases, Fact Sheet PS-47. University of Florida, 1-19.
- Edberg S. C., Rice E. W., Karlin R. J., Allen M. J. (2000): *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*, 88:106-116.
- El-Katcha M., Soltan M. A., El-Shobokshy S. A., Kasser M. (2018): Impact of water acidification or magnetic treatment on growth performance, health and oxidative status of broiler chicks challenged by *Salmonella enteritidis*. *Alex. J. Vet. Sci.*, 59:154-168.
- Englert S. (1998): Produção de frangos de corte. In: Avicultura: tudo sobre raças, manejo e alimentação. 7^ª. Eds. S. Englert, Guaíba: Agropecuária, 94-151.
- Fridrich B., Krcmar D., Dalmacija B., Molnar J., Pesic V., Kragulj M., Varga N. (2014): Impact of wastewater from pig farm lagoons on the quality of local groundwater. *Agricultural Water and Management*, 135:40-53.
- Gama M. S. Q. (1995): Água, que cura, que nutre, que mata. *Aves & Ovos*, 30-33.
- Golić B., Kalaba V., Nedić D. (2021): Review of microbiological analysis of drinking water for animals in the Republic of Srpska (Bosnia and Herzegovina) during the period 2018-2020. *Veterinary Journal of Republic of Srpska, Banja Luka*, 21(1-2):37-49.
- Golić B., Knežević D., Pećanac B. (2023): Assessment of the microbiological status of drinking water on animal farms. In XIV International Scientific Agricultural Symposium „Agrosym 2023“. Proceedings, 1086-1093.
- Guo Y. J., Wang Z. Y., Wang Y. S., Chen B., Huang Y. Q., Li P., Tan Q., Zhang H. Y., Chen W. (2022): Impact of drinking water supplemented 2-hydroxy-4-methylthiobutyric acid in combination with acidifier on performance, intestinal development, and microflora in broilers. *Poult. Sci.*, 101(3).
- Hammes F., Berney M., Wang Y., Vital M., Koster O., Egli T. (2008): Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Res.*, 42:269-277.
- Hijnen W. A. M., van Veenendaal D. A., van der Speld W. H. M., Visser A., Hoogenboezem W., van der Kooij D. (2000): Enumeration of faecal indicator bacteria in large volumes using in site membrane filtration to assess water treatment efficiency. *Water Research*, 34:1659-1665.
- Hoefel D., Monis P. T., Grooby W. L., Andrews S., Saint C. P. (2005): Profiling bacterial survival through a water treatment process and subsequent distribution system. *J. Appl. Microbiol.*, 99:175-186.
-

- Ingle, D. J., Clermont, O., Skurnik, D., Denamur, E., Walk, S. T., & Gordon, D. M. (2011). Biofilm formation by and thermal niche and virulence characteristics of *Escherichia* spp. *Applied and environmental microbiology*, 77(8):2695-2700.
- ISBIH (2003a): Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium. The Institute for Standardization of Bosnia and Herzegovina, BAS EN ISO 6222.
- ISBIH (2003b): Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method. The Institute for Standardization of Bosnia and Herzegovina, BAS EN ISO 7899-2.
- ISBIH (2018): Water quality – Enumeration of *Escherichia coli* and coliform bacteria – Part 1: membrane filtration method for waters with low bacterial background flora. The Institute for Standardization of Bosnia and Herzegovina, BAS EN ISO 9308-1.
- Jafari R. A., Fazlara A. M. (2006): Govahi An investigation into *Salmonella* and fecal coliform contamination of drinking water in broiler farms in Iran. *Int. J. Poult. Sci.*, 5:491-493.
- Jahan M., Holley R. A. (2014): Incidence of virulence factors in enterococci from raw and fermented meat and biofilm forming capacity at 25°C and 37°C. *International Journal of Food Microbiology*, 170:65-69.
- Kadam S. R., den Besten H. M. W., van der Veen S., Zwietering M. H., Moezelaar R., Abee T. (2013): Diversity assessment of *Listeria monocytogenes* biofilm formation: Impact of growth condition, serotype and strain origin. *Int. J. Food Microbiol.*, 165:259-264.
- Kalaba V., Golić B., Ilić T. (2018): Microbiological safety of water in primary production. In 29. Counselling of veterinary of Serbia, Book of Article and Abstracts, 326.
- Kalaba V., Golić B., Kasagić D., Nedić D., Dojčinović S. (2015): Determining the microbiological safety of water on farms in the Republic of Srpska. *Veterinary Journal of Republic of Srpska*, 15(2):259-271.
- Liu S., Gunawan C., Barraud N., Rice S. A., Harry E. J., Amal R. (2016): Understanding, monitoring, and controlling biofilm growth in drinking water distribution systems. *Environmental science & technology*, 50(17), 8954-8976.
- Macari M. (1997): Qualidade da água e bebouros para frangos de corte: tipos, vantagens e desvantagens. In: Conferência Apinco'97 de Ciência e Tecnologia Avícolas, Campinas: Facta, 121-143.
- Maharjan P., Clark T., Kuenzel C., Foy M. K., Watkins S. (2016): On-farm monitoring of the impact of water system sanitation on microbial levels in broiler house water supplies. *J. Appl. Poult. Res.*, 25:266-271.
- Maharjan P., Dey S., Huff G., Zhang W., Phillips G. K., Watkins S. (2017): Effect of chlorine treatment on inhibition of *E coli* serogroup O2 incorporation into 7-day-old biofilm on polyvinylchloride surface. *Poult. Sci.*, 96:2862-2870.
- Mathlouthi A., Pennacchietti E., De B. D. (2018): Effect of temperature, pH and plasmids on in vitro biofilm formation in *Escherichia coli*. *Acta Naturae*, 10(4(39)):129-132.
- Marinho A. R., Martins P. D., Ditmer E. M., d'Azevedo P. A., Frazzon J., Van der Sand S. T., Frazzon A. P. G. (2013): Biofilm formation on polystyrene under different
-

- temperatures by antibiotic resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from food. *Brazilian Journal of Microbiology*, 44:423-426.
- Meza H. (1989): Controle de qualidade na produção de frangos de corte. *Avicultura & Suinocultura Industrial*, 80:38-44.
- Muhterem-Uyar M., Dalmasso M., Bolocan A. S., Hernandez M., Kapetanakou A. E., Kuchta T., Manios S. G., Melero B., Minarovicova J., Nicolau A. I., Rovira J., Skandamis P. N., Jordan K., Lazaro D. R., Stessl B., Wagner M. (2015): Environmental sampling for *Listeria monocytogenes* control in food processing facilities reveals three contamination scenarios. *Food Control*, 51:94-107.
- Oviedo E. O. (2006): Important factors in water quality to improve broiler performance. *N. Carolina Poult. Industry Joint Area Newsletter*, 1:7-8.
- Poimenidou S. V., Chrysadaku M., Tzakoniati A., Bikouli V. C., Nychas G. J., Skandamis P. N. (2016): Variability of *Listeria monocytogenes* strains in biofilm formation on stainless steel and polystyrene materials and resistance to peracetic acid and quaternary ammonium compounds. *Int. J. Food Microbiol.*, 237:164-171.
- Prest E. I., Hammes F., van Loosdrecht M. C. M., Vrouwenvelder J. S. (2016): Biological Stability of Drinking Water: Controlling Factors, Methods, and Challenges. *Front. Microbiol.*, 7:45.
- Raut R., Kilonzo Nthenge A., Aniume T., Basnet A., Watkins S., Maharjan P. (2024): Impacts of on-farm water sanitation practices on microbial hygiene in poultry waterlines and efficacy of sodium hypochlorite-based product on foodborne pathogens. *Journal of Applied Poultry Research*, 33(3).
- Regulation (2017): Rulebook on the Health Safety of Drinking Water Intended for Human Consumption. The Official Gazette of Republic of Srpska, 88/17.
- Regulation (2020): Directive (EU) 2020/2184 of the European Parliament and of the Council on the quality of water intended for human consumption (recast). Official Journal of European Union, L435.
- Sasakova N., Veselitz-Lakticova K., Hromada R., Chvojka D., Koscco J., Ondrasovic M. (2013): Contamination of individual sources of drinking water located in environmentally polluted Central Spis Region (Slovakia). *Journal of Microbiology, Biotechnology and Food Sciences*, 3:262-265.
- Stepanovic S., Vukovic D., Dakic I., Savic B., Svabic-Vlahovic M. A. (2000): Modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods*, 40(2):175-9.
- Uhlich G. A., Chen C.-Y., Cottrell B. J., Nguyen L.-H. (2014): Growth media and temperature effects on biofilm formation by serotype O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli*. *FEMS Microbiology Letters*, 354(2):133-141.
- Vital M., Dignum M., Magic-Knezev A., Ross P., Rietveld L., Hammes F. (2012): Flow cytometry and adenosine tri-phosphate analysis: alternative possibilities to evaluate major bacteriological changes in drinking water treatment and distribution systems. *Water Res.*, 46:4665-4676.
- WHO (2008): Guidelines for Drinking-water Quality, 3rd ed., Vol. 1. World Health Organisation.
-