Review of microbiological analysis of water in meat, milk and fish production in the Republic of Srpska (Bosnia & Herzegovina) in the period 2018-2020

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Abstract. Water is essential for life, and a satisfactory supply must be available to all. Improving access to safe drinking water can result in tangible benefits to health. This study analysed samples of water from meat, milk and fish production from Republic of Srpska (Bosnia & Herzegovina) sampled in the period 2018-2020. A total of 390 samples were examined. The aim of the study was to determine the microbiological status of water used in meat, milk and fish production in the republic, in order to identify the risks to food safety. Microbiological testing used methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1/A1. The microbiological status of water used in the production of meat, milk and fish in the Republic of Srpska in the period 2018-2020 has significantly improved compared to previous years. It is important that the presence of pathogenic bacteria in the water is at a low level. However, there are concerns that almost one-fifth of the water comes from wells, which are not under constant surveillance. This is especially important given the possibility of well water contamination and consequent food contamination.

1. Introduction
The production and distribution of biologically stable drinking water should be a non-negotiable goal for water utilities, with the perspective of providing the same water quality to consumers as is produced at the treatment facility. This can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution [1]. Water supplies within food production premises should be subject to risk and hazard assessments to ensure that appropriate water quality is maintained throughout the production process [2, 3].

The presence of bacteria in drinking water per se is not an issue, as long as no pathogenic organisms are present: there are bacteria in drinking water, even in relatively high numbers (10³ to 10⁶ cells/mL), without consequences on human health [4, 5]. Water temperature is an essential factor influencing bacterial growth kinetics and competition processes. Drinking water temperatures typically range between 3 and 25°C in European countries [6], and fluctuate seasonally within this temperature range even within a single drinking water distribution system. Elevated water temperatures have often been associated with increased bacterial abundance in drinking water distribution systems [7, 8], and with higher numbers of indicator organisms such as coliforms or Aeromonas.

The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard [9]. Escherichia coli (E. coli), intestinal

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enterococci (EC), coliform bacteria (CB) and total colony count at 22°C are monitored in accordance with set monitoring frequencies. *E. coli* and intestinal enterococci are considered core parameters. To assess the quality of water intended for human consumption, the minimum level is 0 CFU/100 ml for *E. coli* and intestinal enterococci. In Republic of Srpska (Bosnia & Herzegovina), the limit value for total count at 22°C (TC 22°C) is 100 CFU/ml, and for total count at 37°C (TC 37°C), the limit is 20 CFU/ml. Also, CB, *E. coli* and intestinal EC must not be detectable in 100 ml sample of water [10].

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites (protozoa, parasite eggs) capable of surviving for different periods, and raises the health risk for many people throughout the world. In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use [11, 12]. On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection. Indicator organisms are used to assess the microbiological quality of water. Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated [13].

The use of indicator bacteria, in particular *E. coli* and coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health [14]. *E. coli* is a coliform bacterium and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. *E. coli* occurs in the faeces of all mammals, often in high numbers (up to 10^8 per gram of faeces). This widespread occurrence in faeces, coupled with methods for the recovery and enumeration of *E. coli* that are relatively simple to conduct, has contributed to the detection of this bacteria being the cornerstone of microbiological water quality assessment for over 100 years. The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin [13, 15] and this species can make up to 95% of the Enterobacteriaceae found in faeces.

The water used during handling and processing of milk products can be potential sources of microbial contamination with possible negative consequences on food safety. Especially, the water used in maintaining the hygiene of milking and milk storage equipment is crucial to the quality and safety of the products. *E. coli* was isolated in 39.20% of samples of water [16]. Enterococci include a number of species that occur in the faeces of humans and warmblooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution [15]. Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *E. coli* by culture methods [17].

In the period 2015-2017, 26.20% of 584 water samples were of unsatisfactory quality [18]. In a study by [19], it was determined that all water samples used in the production of milk and milk products were microbiologically safe.

The aim of this study was to determine the microbiological status of water used in meat, milk and fish production in the Republic of Srpska (Bosnia & Herzegovina), in order to identify the risks to food safety.

2. Materials and Methods

Samples of water from meat, milk and fish production were from the Republic of Srpska (Bosnia & Herzegovina), sampled in the period 2018-2020. A total of 390 samples were examined (122 in 2018, 127 in 2019 and 141 in 2020).

Laboratory testing of waters was performed at the Dr Vaso Butozan Public Veterinary Institute of the Republic of Srpska, Banja Luka. Microbiological examination was carried out according to the
Regulation [10]. This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C (TC 22°C) and 37°C (TC 37°C) according to BAS EN ISO 6222 [20], CB and E. coli according to BAS EN ISO 9308-1/A1 [21] and intestinal EC according to BAS EN ISO 7899-2 [22].

In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables and figures.

Results and DiscussionTable 1 shows the test results in relation to the total number of water samples (% satisfactory or unsatisfactory) for 2018-2020.

Table 1. Percentage of waters classified as satisfactory and unsatisfactory, 2018-2020

| Year | Satisfactory | Unsatisfactory |
|------|--------------|----------------|
| 2018 | 91.80        | 8.20           |
| 2019 | 81.89        | 18.11          |
| 2020 | 90.78        | 9.22           |

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and could be subject to natural or manufactured sources of pollution that can result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination. Zero-probability level of microbiological contamination of drinking water does not exist [9]. It is incorrect to state that drinking water distribution and delivery systems should be sterile, but the active growth of microorganisms is considered indicative of failures in water processing units or distribution [23]. Water is used in various ways in milk production and dairy industry, thereby becoming part of the food intentionally, inevitably or accidentally. The contamination of the food by water-borne microorganisms occurs directly much more often, but indirectly too, after multiplication of these microorganisms on the cleaned surfaces of the equipment used [24].

The presence of pathogenic bacteria in the water supply system is a particularly worrying fact given that water must be microbiologically acceptable, which means that it must not contain pathogens [10]. A possible explanation for this is dilapidation and damage to water supply installations leading to water contamination. For this three-year period, there were on average 88.16±5.45% satisfactory and 11.84±5.45% unsatisfactory water samples. The results, compared to previous research [18], indicate the much better microbiological status of water in the republic. Use of contaminated water in the handling and processing of milk products can cause a higher potential health risk than the risk through direct drinking. This is due to the fact that multiplication of pathogenic microorganisms can occur in milk and milk products with amplification of the load of the pathogens [25].

When it comes to water contamination in relation to the supply system, 82.41% of samples was from a public water supply system and 17.59% was from wells. Table 2 shows the test results (satisfactory/unsatisfactory categories) of water by supply system in relation to the total number of samples for the period 2018-2020.

Table 2. Percentage of waters classified as satisfactory or unsatisfactory by supply system in relation to the total number of samples, 2018-2020

| Year | Water supply system | Well supply system |
|------|---------------------|--------------------|
|      | Satisfactory | Unsatisfactory | Satisfactory | Unsatisfactory |
| 2018 | 94.78        | 5.22             | 42.86        | 57.14          |
| 2019 | 83.48        | 16.25            | 66.67        | 33.33          |
| 2020 | 92.05        | 7.95             | 88.68        | 11.32          |
| \(\bar{x} \pm \delta\) | 90.10±5.90 | 9.81±5.75 | 66.07±22.92 | 33.93±22.92 |
Comparing the results of water testing in relation to the supply category, the significantly higher number of unsatisfactory samples of well water than reticulated water supply is noticeable, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well waters do not flow, stagnate and are not under constant control or are controlled very rarely, usually once a year as an official control.

Some studies revealed that wash water can be source of bacterial contamination for milk and further compromise the quality and safety of milk or milk products [26, 27]. Table 3 shows the average test results in % according to test parameter for the period 2018-2020.

Table 3. Percentage of wash water classified as unsatisfactory according to microbiological tests, 2018-2020

| Year | TC 22°C | TC 37°C | EC | E. coli | CB |
|------|---------|---------|----|---------|----|
| 2018 | 5.74    | 5.74    | 1.64| 1.64    | 1.64|
| 2019 | 14.96   | 14.19   | 1.57| 1.57    | 3.14|
| 2020 | 8.51    | 7.80    | 3.55| 3.55    | 4.26|
| \(\bar{x}_{\pm \delta}\) | 9.74±4.73 | 9.24±4.41 | 2.25±1.12 | 2.25±1.12 | 3.01±1.31 |

According to [15], E. coli are the only true indicators of faecal contamination; they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and, thus, points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Faecal streptococci provide evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. Total colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations can include bacteria with water environment as a natural habitat or those that have originated from soil or vegetation. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources. In a study of testing water at milk collection points, 20.40% of unsatisfactory samples were found, of which E. coli and coliforms were detected in 10.20%, and faecal streptococci in 12.24% [28]. Analysis of water from milk collection points, originating from wells, showed 63.90% of samples were unsatisfactory [29]. The current results differ from these results of similar research and indicate the significantly more favourable microbiological status of water.

3. Conclusions

Research shows that the microbiological status of water used in the production of meat, milk and fish in the Republic of Srpska (Bosnia & Herzegovina) in the period 2018-2020 has significantly improved compared to previous years. It is especially important that the presence of pathogenic bacteria in the water is at a low level. However, there are concerns that almost one-fifth of the water comes from wells, which are not under constant microbiological surveillance. This is especially important given the possibility of well water contamination and consequent food contamination. For this reason, it is recommended that microbiological control of well water be performed more frequently for the purpose of timely disinfection, as well as consideration of the possibility of connecting facilities used for food production with water supply system.

References

[1] Prest E I, Hammes F, van Loosdrecht M C M and Vrouwenvelder J S 2016 Front. Microbiol. 7 45
[2] Dawson D 1998 Water Quality for the Food Industry: An Introductory Manual CCRFA Guideline No. 21 Campden and Chorleywood Food Research Association Chipping Campden UK
[3] Dawson D 2000 Water Quality for the Food Industry: Management and Microbiological Issues
CCRFA Guideline No. 27, Campden and Chorleywood Food Research Association Chipping Campden UK

[4] Hoefel D, Monis P T, Grooby W L, Andrews S and Saint C P 2005 J. Appl. Microbiol. 99 175–86
[5] Hammes F, Berney M, Wang Y, Vital M, Koster O and Egli T 2008 Water Res. 42 269–77
[6] Niquette P, Servais P and Savoir R 2001 Water Res. 35 675–82
[7] Francisque A, Rodriguez M J, Miranda-Moreno L F, Sadiq R and Proulx F 2009 Water Res. 43 1075–87
[8] Liu G, Verberk J Q and Van Dijk J C 2013 Appl. Microbiol. Biotechnol. 97 9265–76
[9] EU 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 1–62
[10] Republic of Srpska 2017 Rulebook on the health safety of water intended for human consumption Official Gazette of Republic of Srpska No 88/17
[11] Sasakova N, Veselitz-Lakticova K, Hromada R, Chvojka D, Koscco J and Ondrasovic M 2013 Journal of Microbiology, Biotechnology and Food Sciences 3 262–5
[12] Fridrich B, Krcmar D, Dalmacija B, Molnar J, Pesic V, Kragulj M and Varga N 2014 Agricultural Water and Management 135 40–53
[13] Edberg S C, Rice E W, Karlin R J and Allen M J 2000 Journal of Applied Microbiology, 88 106–16
[14] Hijnen W A M, van Veenendaal D A, van der Speld W H M, Visser A, Hoogenboezem W and van der Kooij D 2000 Water Research 34 1659–65
[15] WHO 2008 Guidelines for Drinking-water Quality, 3rd ed., Vol. 1. World Health Organisation
[16] Amenu K, Shitu D and Abera M 2016 Springerplus 5 (1) 1195
[17] Cabral J P S 2010 Int. J. Environ. Res. Public Health 7 (10) 3657–703
[18] Kalaba V, Golici B and Ilic T 2020 Veterinary Journal of Republic of Srpska (Banja Luka) 20 (1-2) 66–80
[19] Habes S, Hasanagic E, Aldzic A, Smjecanin E and Jukic H 2015 Balkan Journal of Health Science 3 (2) 34–8
[20] BAS EN ISO 2003 6222:2003 Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium
[21] BAS EN ISO 2018 9308-1/A1:2018 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora
[22] BAS EN ISO 2003 7899-2:2003 Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method
[23] Gottschal J C 1992 J. Applied Bacteriol. Symp. Suppl. 73 39–48
[24] Terplan G 1980 Zentralbl Bakteriol Mikrobiol Hyg. B. 172 (1–3) 96–104
[25] Amenu K 2013 Assessment of water sources and quality for livestock and farmers in the Rift Valley area of Ethiopia: implications for health and food safety Sierke Verlag Göttingen
[26] Kivaria F M, Noordhuizen J P T M and Kapaga A M 2006 Trop Anim. Health Prod. 38 (3) 185–94
[27] Perkins N R, Kelton D F, Hand K J, MacNaughton G, Berke O and Leslie K.E 2009 J Dairy Sci. 92 3714–22
[28] Denzić M, Sokolović J, Iaki-Tkalec V, Majnarić D and Pavlček D 2013 Book of Abstracts, VII Scientific-professional Conf. Water and public water supply Lopar 139–40
[29] Jaki V, Majnarić D and Lukačić M 2010 Book of Abstracts, 39th Croatian Symp. of Dairy Experts with International Participation Opatija 56–57