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Original Scientific Paper

REVIEW OF MICROBIOLOGICAL ANALYSIS OF DRINKING WATER FOR ANIMALS IN THE REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA) DURING THE PERIOD 2018-2020.

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Summary

One of the major challenges for all in the 21st century is safe drinking water and the microbiological control of drinking water should be the norm everywhere. Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrements. The production and distribution of microbiologically safe drinking water should be a non-negotiable goal for water companies with the prospect of providing the same water quality to all consumers. This can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution.
The material represented drinking water samples originating from animal farms at the territory of the Republic of Srpska (Bosnia and Herzegovina) collected in the period of 2018-2020. A total of 645 samples were examined.
The aim of this study was to determine the microbiological status of drinking water for animals intended for human consumption in the Republic of Srpska (Bosnia and Herzegovina), in order to analyse the risks to animal health.
BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1/A1 methods were used for microbiological analyses of drinking water samples.
The obtained results showed that one quarter of drinking water for animals are microbiologically incorrect. The analysis revealed a significantly higher number of unsatisfactory samples of well water in relation to the water supply system. The obtained results indicate fecal contamination of water, especially with intestinal enterococci and coliforms, and less with *E. coli*.

Keywords*:* animals, drinking water, microbiology.

INTRODUCTION

The production and distribution of microbiologically safe drinking water should be a non-negotiable goal for water companies with the prospect of providing the same water quality to all consumers. This can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution (Prest et al., 2016).

Water is essential for life, and a satisfactory (adequate, safe and accessible) supply must be available to all. Improving access to safe drinking-water can result in tangible benefits to health. Therefore, every effort should be made to achieve a drinking-water quality that is as high as possible (WHO, 2008).

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 (10^3 to 10^6 cells/mL), without consequences to human health (Vital et al., 2012a; Hoefel et al., 2005; Hammes et al., 2008).

Water temperature is an important factor that affects the growth and competition of bacteria. Drinking water temperatures typically range between 3 and 25°C in European countries (Uhl and Schaule, 2004; Niquette et al., 2001), 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 (Liu et al., 2013; Francisque et al., 2009), and with higher numbers in indicator organisms such as coliforms or Aeromonas. In addition, water temperature can also affect bacterial community composition, by providing competitive advantages to specific bacterial species in defined temperature ranges, including pathogenic species (Vital et al., 2012; Vital et al., 2007). Vital et al. (2012) showed that the maximum growth rate and competitive ability of *E. coli* grown with an indigenous drinking water community increased with temperature in the range of 12-30°C. So, there is increase chance for problems associated with bacterial growth in summer periods (with higher water temperatures), such as hygienic risks, deterioration of aesthetic aspects of water, malfunctioning of water installations and exceeding of legal guidelines.

The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard (Regulation, 2020). *E. coli*, intestinal enterococci, coliform bacteria and total colony count at 22°C should be monitored in accordance with the monitoring frequencies. *E. coli* and intestinal enterococci are considered as “core parameters”. Minimum requirements for parametric values used to assess the quality of water intended for human consumption is 0/100 ml for *E. coli* and intestinal enterococci. In the Republic of Srpska (Bosnia and Herzegovina) the limit value for total colony count at 22°C (TC 22°C) is 100 CFU/ml and for total colony count at 37°C (TC 37°C) the limit is 20 CFU/ml. Also, coliform bacteria (CB), *Escherichia coli (E. coli)* and intestinal enterococci (EC) must not be detectable in 100 ml/sample of water (Regulation, 2017).

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrements, which become a source of pathogenic bacteria, viruses and parasites (protozoa, parasite eggs) capable of surviving for different periods, and raise the health risk for many people throughout the world. In order to eliminate the risk related to disease transmission, water intended for mass consumption is treated and disinfected before use. Monitoring of water sources involves the determination of important microbiological and physico-chemical parameters which indicate first of all potential organic pollution, particularly pollution originating from animal excrement, storage of waste, natural and artificial fertilizers, and others (Fridrich et al., 2014; Sasakova et al., 2013). Based on the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been main in protecting the public health (Hijnen et al., 2000). These are based upon the principle of detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests. 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 microorganisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, its detection is a useful indication that treatment operations should be investigated (Edberg, 2000).

Key criteria for ideal bacterial indicators of fecal contamination should be universally applied in the feces of humans and other warm-blooded animals. These indicators should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive fecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*. 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* bacteria are the only biotype of the family *Enterobacteriaceae* which can be considered as being exclusively of fecal origin (WHO, 2008; Edberg, 2000) and it can represent up to 95 % of the *Enterobacteriaceae* found in feces. Coliform bacteria belong to the family *Enterobacteriaceae* and share similar cultural characteristics. Typical genera found in water supplies are *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia* and *Yersinia*. It is often useful to determine which species of coliform bacteria are present when coliform bacteria are isolated from drinking water supplies in order to determine the source and significance of the coliform bacteria being recovered. *E. coli* is a coliform bacteria and has been historically regarded as the primary indicator of fecal contamination of both treated and untreated water. *E. coli* occurs in the feces of all mammals, often in high numbers (up to 10^9 per gram of feces). This widespread fecal occurrence, coupled that methods for the recovery and enumeration of *E. coli* are relatively simple to conduct and has contributed to the detection of this bacteria as the cornerstone of microbiological water quality assessment for over 100 years.

Enterococci include a number of species that occur in the feces of humans and warm-blooded 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 fecal contamination and they are regarded as secondary indicators of fecal pollution. Enterococci of fecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and coliform bacteria (WHO, 2008). The species of enterococci that occur in feces are more likely to be found in polluted waters can be divided into two main groups. The first group includes *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans*. These organisms are normally present in the feces of humans and various animals. The second group includes *Streptococcus bovis*, *Streptococcus equinus* and *Enterococcus avium*. These organisms are not normally found in human feces. The identification of species may, therefore, give an indication of the source of contamination. Wild animals and birds can also be natural sources of zoonotic pathogens.

Safe drinking water for all is one of the major challenges of the 21st century and that microbiological control of drinking water should be the norm everywhere (Cabral, 2010). Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *E. coli* by cultural methods. Whenever financial resources are available, fecal coliform determinations should be complemented with the quantification of enterococci.

The territorial and economic organization of the Republic of Srpska is conditionally created at the level of six regions: Banja Luka, Prijedor, Doboj, Bijeljina, East Sarajevo and Trebinje. The distribution of water is such that it is not enough where it is most needed (in the northern, most developed part of Republic of Srpska), and flows are most scarce during periods of the year when the needs are greatest and when the water quality protection problems are most serious.

In study (Kalaba et al., 2015) of microbiological analysis of water on farms in the Republic of Srpska, found that 62.66% of water samples from animal farms were unsatisfactory and that the most common cause of water malfunction was enterococci, *E. coli* and total colony count at 22°C and 37°C. Kalaba et al. (2020) found that 26.20% of water samples from animal farms were unsatisfactory in the Republic of Srpska for the period 2015-2017. Also, they found that 63.40% of unsatisfactory samples were due to the increased total colony count at 22°C and 54.90% due to increased total colony count at 37°C, 58.80% due to presence of intestinal enterococci, 31.40% due to coliform and 19% due to presence of *E. coli*. The lowest risk in presence of coliforms and *E. coli* is in water supply system, and it is significantly higher in well water system.

In Croatia, two studies were conducted to analyze drinking water from different farms (chicken broiler and laying hen farms, cattle and swine farms), where Denžić Lugomer et al. (2019) detected 40% of unsatisfactory samples, while Kiš et al. (2017) detected 20%.

The aim of this study is to determine the microbiological status of drinking water for animals intended for human consumption in the Republic of Srpska, in order to analyse the risks to animal health.

MATERIALS AND METHODS

The material represented water samples originating from the animal farms at the territory of the Republic of Srpska sampled in the period 2018-2020. A total of 645 samples were examined (384 in 2018, 160 in 2019 and 101 in 2020).

Laboratory testing of samples was performed at the Public Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka.

Microbiological examination was carried out according to the Rulebook on the health safety of water intended for human consumption (Regulation, 2017). 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 (ISBIH, 2003a), coliform bacteria (CB) and *E. coli* according to BAS EN ISO 9308-1/A1 (ISBIH, 2018) and intestinal enterococci (EC) according to BAS EN ISO 7899-2 (ISBIH, 2003b).

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

RESULTS AND DISCUSSION

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources are characterized by different microbiological qualities and may be subject to natural or manufactured sources of pollution that may 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 (Regulation, 2020). It is incorrect to state that drinking water distribution and delivery systems should be sterile, the active growth of microorganisms is considered indicative of failures in water processing units or distribution. Adapted bacteria can grow even in oligotrophic systems, such as distilled water (Gottschal, 1992).

The results of the study of LeJeune et al. (2001) demonstrate that drinking water offered to cattle is often of poor microbiological quality. Water troughs are a major source of exposure of cattle to enteric bacteria, including a number of foodborne pathogens. The extent of bacterial contamination observed in the drinking water offered to cattle demonstrates that the animals' daily exposure to *E. coli* from this source alone can be substantial. The association between the water quality parameters and the ecological factors measured suggest that many of the same factors that influence the survival and proliferation of bacteria in natural aquatic ecosystems have parallels in cattle water troughs.

Table 1 shows the results in relation to the total number of samples in % for the period 2018-2020.

Table 1 Test results in relation to the total number of samples in % for the period 2018-2020

Year	Satisfactory	Unsatisfactory
2018.	75.78	24.22
2019.	78.75	21.25
2020.	75.25	24.75

The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Regulation, 2017). A possible explanation for this is dilapidation and damage to water supply installations in farms leading to water contamination. This is in line with observations Interact (2006), which indicate that microbiological quality of drinking water for cattle diminishes once the water has entered the farm. A field survey in the Netherlands has pointed out that at the entry point (130 dairy farms; 285 samples) 98% of water was of suitable microbiological quality, but at the end point (199 samples) this percentage had dropped to 60%, so that 40% of the water samples were unsuitable as drinking water for cattle. For this three-year period, there were on average 76.59±1.89% satisfactory and 23.41±1.89% unsatisfactory samples. The obtained results, in contrast to the results of Kalaba et al. (2015), indicate a much better microbiological status of drinking water on animal farms and are in accordance with the results of Kalaba et al. (2020).

When it comes to the representation of samples in relation to the category, 61.90% of samples was from water supply system and 38.10% was from wells. Table 2 shows the test results of water by categories in relation to the total number of samples in % for the period 2018-2020.

Table 2 Test results of water by categories in relation to the total number of samples in % for the period 2018-2020

Year	Water supply system		Well supply system	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
2018.	79.85	20.15	66.38	33.62
2019.	88.29	11.71	57.14	42.86
2020.	91.49	8.51	61.11	38.89
$\bar{x}$±δ	86.54±6.01	13.46±6.01	61.54±4.64	38.46±4.64

Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well water supplies one or fewer farms, is not under constant control but very rarely, most often once a year as an official control, or in the event of animal health incident. Also, well waters are not flowing but stagnant, so the microbiological status of this water is greatly influenced by the number of animals drinking from the well, i.e. the speed and amount of water consumption from the well. The obtained results differ from the results of Kalaba et al. (2015) who state that 30.50% of samples originating from water supply system and 76.72% of samples from wells are unsatisfactory.

Figure 1 shows the average representation of samples in relation to the region in % for the period 2018-2020 years, and Figure 2 shows the average representation of samples by regions in% in relation to the category for the period 2018-2020 years.

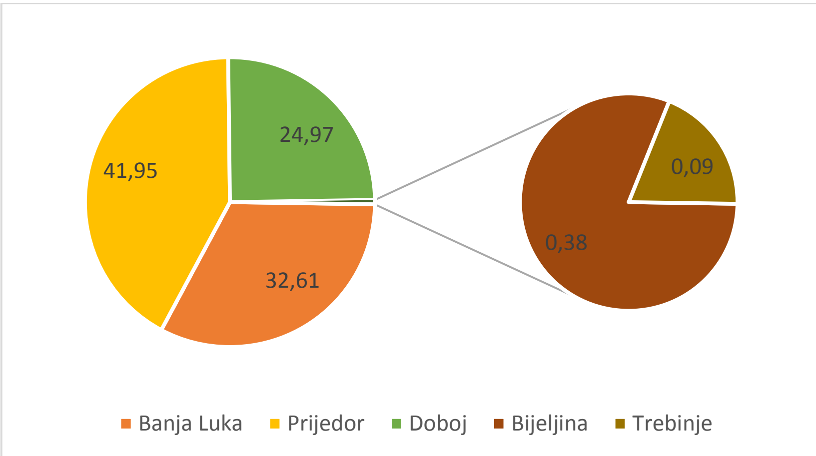

Figure 1 Representation of samples in relation to the region in % for the period 2018-2020

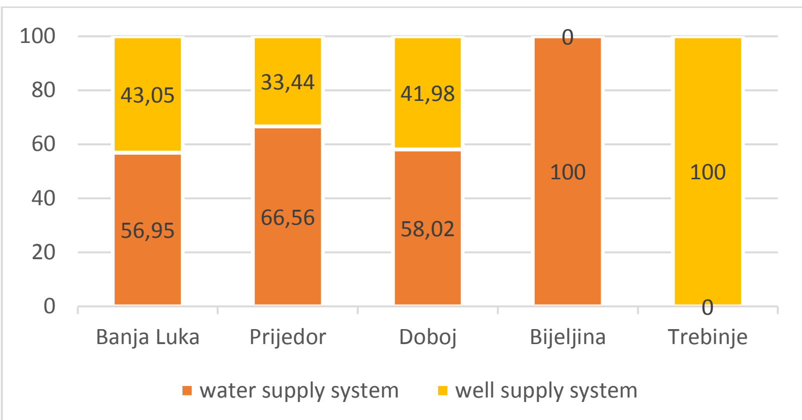

Figure 2 Representation of samples by regions in % in relation to the category for the period 2018-2020

Analysis of the testing samples taking into account the region and category indicates that most samples come from three regions (Prijedor, Banja Luka, Doboj), as well as that two thirds of the sample comes from water supply system and one third from wells.
Table 3 shows the test results by region in relation to the total number of samples in % for the period 2018-2020 years, and Table 4 shows the results by region in % in relation to categories for the period 2018-2020 years.

Table 3 Test results by region in relation to the total number of samples in % for the period 2018-2020

Region	Satisfactory	Unsatisfactory
Banja Luka	68.14±5.83	31.86±5.83
Prijedor	77.13±7.19	22.87±7.19
Doboj	85.73±5.00	14.27±5.00
Bijeljina	25	75
Trebinje	0	100

The obtained results are in accordance with the results of Kalaba et al. (2015), who found that the largest number of unsatisfactory samples were from the region Prijedor and Banja Luka, and that the significantly more favourable microbiological status of drinking water was in the region Doboj.

Table 4 Test results by region in % in relation to categories for the period 2018-2020.

Region	$\bar{x}$±δ			
	Water supply system		Well supply system	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
Banja Luka	83.97±6.55	16.03±6.54	49.69±2.48	50.31±2.48
Prijedor	84.41±4.25	15.59±4.25	62.98±3.39	37.02±3.39
Doboj	92.22±9.10	11.68±8.64	77.16±19.18	22.84±19.18
Bijeljina	25±3.22	75±4.83	0	0
Trebinje	0	0	100	0

The obtained results are in accordance with the results of similar studies (Denžić Lugomer et al., 2019; Kiš et al., 2017; Kalaba et al., 2015). Most of the analyzed samples come from regions with the highest concentration of domestic animal farms (Banja Luka, Prijedor and Doboj). The analyzed number of samples originating from the region of Bijeljina and Trebinje is negligibly small, so it was not included in the discussion, while none of the samples analyzed from the region of East Sarajevo. All types of water sources may be subjected to contamination by agricultural activity. Freerange animals may excrete faeces into water, and animals like cattle have a habit of wading into water and

stirring up sediments. Rainfall can result in the run-off of fecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The potential source of coliform bacteria in water supplies result from sub-optimal operation of water treatment processes or ingress of contamination from breaches in the integrity of the distribution system. These include for example, leaking hatches on service reservoirs, contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross connections and back-flow effects. Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognized sources of these organisms. There are large differences between water and quality control systems for water destined for human or animal consumption (Eenige et al., 2013). For example, testing for presence of microorganisms occurs less frequently in cattle systems; substrates for bacteria are often present in cattle systems; few cattle systems are screened for faecal contamination, even though this is a major source of contaminants; in many cattle systems water can flow in more than one direction, which is not the case for human water systems; the existence and implementation of cleaning and disinfection protocols are poor in cattle systems and biofilms are more often present in the pipelines of cattle systems. The latter phenomenon often leads to the presence of many different bacteria in cattle drinking systems.
Table 5 shows the average test results in % according to test parameter for the period 2018-2020.

Table 5 Test results in % according to test parameter for the period 2018-2020

Year	Test parameter				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
2018.	13.02	15.89	10.94	6.25	6.77
2019.	13.12	16.25	8.75	8.13	12.50
2020.	16.83	20.79	10.89	4.95	8.91
$\bar{x}\pm\delta$	14.33±2.17	17.64±2.73	10.19±1.25	6.44±1.60	9.39±2.90

Legend: TC 22°C – total colony count at 22°C; TC 37°C – total colony count at 37°C; CB – coliform bacteria; *E. coli – Escherichia coli;* EC – intestinal enterococci;

According to the WHO (2008), *E. coli* are the only true indicator of fecal contamination; they are exclusively of intestinal origin and are found in feces. Their presence indicates mostly fresh fecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Fecal streptococci represent evidence of fecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. The heterotrophic plate count includes all of the microorganisms that are capable of growing in or on a nutrient-rich solid agar. Two incubation temperatures and times are used for total colony 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. The test results indicate that the causes of microbiological water malfunction come from animals, having in mind that the largest number of unsatisfactory samples is due to the increased total colony count at 37°C, but we should not ignore the fact of the possibility of contamination from the environment due to the increased total colony count at 22°C. Contamination from animals is very possible due to inadequate drainage of waste and fecal water and consequent contamination of groundwater, because wells are usually not planned and are mostly located near farms. The obtained results are in accordance with the results Kalaba et al. (2015) and Kalaba et al. (2020) and indicate significant fecal contamination of water, especially with intestinal enterococci and coliforms, and less with *E. coli*, with the largest number of unsatisfactory samples due to the increased total colony count at 37°C and 22°C. However, there is a difference in relation to them because of the level of pathogenic bacteria in drinking water is reduced, especially for intestinal enterococci and coliforms, as well as the total colony count at 22°C and 37°C.

CONCLUSION

The obtained results showed that one quarter of the analysed samples of drinking water for animals are microbiologically incorrect. Although the microbiological status of water for animals in the Republic of Srpska (Bosnia and Herzegovina) in the period 2018-2020 was significantly improved compared to previous years, remains a concern given that a significant number of unsatisfactory samples come from water supply system, as well as that one third of farms are supplied with water from wells, which is not under constant supervision. This is especially important given the faecal contamination of water originating from animals. It would be justified to carry out microbiological analysis of water for animals at least once, optimally twice a year, originating from all regions, in proportion to the distribution of farms.

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

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