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Assessment of occurrence of somatic coliphages in drinking water in Poland

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Abstract

Introduction and Objective. The use of Escherichia coli and enterococci as indicators of the presence of pathogenic viruses or parasitic protozoa is limited. In order to increase the control of enteric pathogens in drinking water, Directive (EU) 2020/2184 of the European Parliament and of the Council incorporates into microbiological analysis the determination of somatic coliphages as a new operational parameter in raw water for controlling the effectiveness of treatment processes. The goal was to assess the occurrence of somatic coliphages in raw water samples collected at groundwater and surface water intakes, and in treated water samples fed into the distribution system.

Materials and Method. The study included 7 groundwater intakes and 6 surface water intakes at Water Treatment Stations. A total of 52 raw water samples and 40 treated water samples were assessed. Somatic coliphages were determined according to PN-EN ISO 10705–2 and PN-EN ISO 10705–3 (with modifications).

Results. The results showed the presence of somatic coliphages in low numbers in 8% of water samples collected at groundwater intakes and in 89 % of samples collected at surface intakes. In 44 % of the water samples tested, the number of somatic coliphages was higher than 50 pfu/100 ml. Somatic coliphages were not detected in any of the treated water samples.

Conclusions. Somatic coliphages can be a useful operational monitoring parameter and a tool for strengthening the control of waterborne pathogens in assessing the effectiveness of water treatment processes. The implementation of somatic coliphage determination for water intakes where there is a risk associated with faecal contamination, should be part of ensuring adequate drinking water quality.

Key words

water quality, drinking water, coliphages

INTRODUCTION AND OBJECTIVE

Drinking water can be supplied from surface water and groundwater intakes. To ensure an adequate quality of drinking water, including its microbiological safety, it is necessary to identify potential adverse incidents and associated risks. Risk management includes the use of a system of barriers to prevent hazards or mitigate their consequences at every stage of the water supply chain, from water resources and intakes through distribution systems to consumer taps.

Despite all the actions taken to ensure the safety of drinking water, it can become a source of infection [1]. Inadequate protective barriers may allow viruses and pathogenic microorganisms, such as bacteria, protozoa and helminths, to penetrate the water [2]. Campylobacteriosis, giardiasis, hepatitis A and shigellosis are infections very often recorded in European countries [3]. The most commonly reported infections include epidemics of viral gastroenteritis, infectious hepatitis (caused by the hepatitis A virus, among others), diarrhoea caused by pathogenic *Escherichia coli* and legionellosis. Approximately 18% of these epidemics are related to water, and most of them originate from water supplied by public mains [3]. Globally, waterborne diarrhoea accounts for about 4 billion cases of disease annually, killing 1.8 million people [4].

Technological water treatment and disinfection processes are an important barrier against microbiological hazards which can be severe in the case of consumption of water contaminated with human or animal faeces. One of the mainstays of ensuring adequate microbiological quality of drinking water is the monitoring of faecal contamination indicators, such as *E. coli* and intestinal enterococci [2, 5, 6]. However, the effectiveness of *E. coli* as an indicator organism for pathogenic viruses or parasitic protozoa has its limitations [7]. Parasitic protozoa and viruses are more resistant to treatment processes than bacteria, which means that they can be found in treated water even if no *E. coli* bacteria are detected [2, 5, 8]. To enhance the effectiveness of monitoring, somatic coliphages, as a new operational parameter determined in raw water, have been incorporated in microbiological analyses by Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020, to monitor the effectiveness of treatment processes, including assessment of the risk of penetration of treatment barriers by pathogenic viruses [5, 9].

Somatic coliphages are a group of viruses (bacteriophages) that includes representatives of different families, including: *Myoviridae, Siphoviridae, Podoviridae, Microviridae* [1, 10]. However, they mostly infect *Escherichia coli* [2], although

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some of them can use other bacteria as hosts for replication, including *Klebsiella* spp. and *Shigella* spp. [10, 11]. Somatic coliphages are not pathogenic to humans, they replicate in the gastrointestinal tract of people and animals, and are excreted into the environment with faeces [2, 8, 11, 12].

The aim of the study was to assess the presence of somatic coliphages in raw water samples collected from groundwater and surface water intakes, and samples of treated water supplied into the distribution system.

MATERIALS AND METHOD

The study tests were carried out from 2020–2021. Water samples were collected from the following sites:

- 7 groundwater intakes (referred to as Nos. 1–7) with Water Treatment Plants (WTPs) located in the Masovian Province, the largest and most populous province, located in East-Central Poland;
- 6 surface water intakes with WTPs – 4 in the Masovian Province (referred to as Nos. I – IV), and 2 intakes with WTPs in the Subcarpathian Province (V – VI), located in the south-eastern part of Poland.

The water samples were collected directly at the intakes (raw water) and at the water treatment plant, at the point where the water was supplied into the distribution system (treated water). The samples were collected in the spring season (March – April), summer season (July – August) and autumn season (October – November).

Water samples were collected into sterile bottles made of polypropylene with added sodium thiosulphate according to PN-EN ISO 19458:2007. The water samples were transported in cold storage and kept at 5±3 °C until the time of the test, not longer than 48 hours.

Tests of sanitary indicators. Water samples from groundwater intakes and WTPs were tested for the following microbiological parameters: total microbial count at 22 °C, acc. to PN-EN ISO 6222:2004, *E. coli*, bacteria of the coli group, acc. to PN-EN ISO 9308–1:2014/A-1:2017, and intestinal enterococci, acc. to PN-EN ISO 7899–2:2004. Water samples from surface water intakes were tested for *E. coli,* acc. to PN 9308–1:1999 and intestinal enterococci, acc. to PN-EN ISO 7899–2:2004.

Tests of somatic coliphages. The somatic coliphage count was determined ac. to PN-EN ISO 10705–2 using the *E. coli* ATCC 700078 host strain and the ssMSA medium, with added nalidixic acid. For samples with an expected large phage count from surface intakes, somatic coliphages were determined directly in a volume of 1 ml, and in samples concentrated from 100 ml. In water samples from groundwater intakes and samples of treated water (downstream of the WTP, regardless of the intake type), coliphages were determined in samples concentrated from 100 ml. Water samples were concentrated by membrane filtration acc. to PN ISO 10705–3 with modifications (unpublished data). Magnesium chloride was added to 100-ml samples until the final concentration of 0.05 mol/litre. The samples were filtered through cellulose ester filters.

Water samples from groundwater intakes and samples of water after treatment were filtered through filters with a pore diameter of 0.22 μm (Millipore MZGSWG101), and the filtration of water samples from surface intakes was performed using filters with a pore diameter of 0.45 μm (Millipore EZHAWG474). After filtration, the filter was cut into 8 parts and placed in eluate. The phages deposited on the filter were washed with an ultrasonic cleaner for 4 minutes and vortexed for 1 minute. The results are given in the form of the number of plaque-forming units (result can also be given as plaque-forming particles (pfp)).

RESULTS

Groundwater intakes. The tests that covered 4 groundwater intakes (1–4) with the WTPs were conducted in the summer and autumn seasons of 2020. The analyses of water samples from these intakes were continued in the spring and autumn seasons of in 2021; at the same time, 3 new intakes (5–7) were added, together with WTPs. Overall, samples were collected for tests in 2020–2021 from 7 groundwater intakes, including 38 raw water samples and 22 treated water samples. Of all the tested intakes, only 2 (of 7) results of analyses showed the presence of somatic coliphages. Somatic coliphages were detected in 2 samples collected in 2020 from one of the deep wells supplying intake No. 2. The determined number of somatic coliphages per sample was 3 pfu/100 ml (collected in the summer season) and 11 pfu/100 ml in the second sample (collected in the autumn season). At the same time, the number of coli bacteria detected in these samples was 33 cfu/100 ml in the summer season and 27 cfu/100 ml in the autumn season, with a total microbial count of 28 cfu/1 ml – sample collection in the autumn season. In 2021, no somatic coliphages were detected in that intake, but bacteria of the coli group were detected in an amount of 100 cfu/100 ml in the autumn season, with the total microbial count in the spring and autumn seasons at 6 cfu/1 ml and 1.6×10^2 cfu/1 ml, respectively. In the second well supplying that intake, neither somatic coliphages nor indicator bacteria were detected in any of the tests. The other heterotrophic bacteria (total microbial count) were not detected in the summer of 2020 and the autumn of 2021, but in the autumn of 2020 and the spring of 2021, they were 1.4×10^2 and 2.6×10^2 cfu/1 ml, respectively. Somatic coliphages in an amount of 1 cfu/100 ml were detected in the raw water sample collected in the autumn season at intake No. 6 incorporated in the tests in 2021. In the tested samples from intake No. 6, no indicator bacteria were detected. The total microbial count at 22 °C determined in water samples from that intake in the autumn and spring seasons was 2.4×10^2 cfu/1 ml and 1.3×10^3 cfu/1 ml, respectively. The test results are given in Table 1. In the remaining intakes, no somatic coliphages and faecal contamination indicators were detected in raw water samples, nor were they detected in any of the treated water samples.

Surface water intakes. In 2021, the tests covered 6 surface water intakes with WTPs. 18 raw water samples and 18 treated water samples were tested. The tests of treated water samples did not show the presence of somatic coliphages, *E. coli* or intestinal enterococci. The tests of all water samples collected directly from the intakes, in turn, confirmed the presence of somatic coliphages. The determined count of somatic coliphages in raw water was in the ranges of 14–99

Table 1. Results of the analysis of raw water samples from groundwater intakes where somatic coliphages were detected (study results for 2020–2021)

Water Intake No.	Examination Date	Somatic Coliphages	Coliform bacteria	E. coli	Intestinal Enterococci	TMC* 22 °C/72 hrs.
		Pfu/100 ml	cfu/100 ml	cfu/100 ml	cfu/100 ml	cfu/1 ml
2 Well A	Summer 2020	**3**	**33**	nd	nd	nd
	Autumn 2020	**11**	**27**	nd	nd	**28**
	Spring 2021	nd	nd	nd	nd	**6**
	Autumn 2021	nd	**100**	nd	nd	**1.6×10^2**
2 Well B	Summer 2020	nd	nd	nd	nd	nd
	Autumn 2020	nd	nd	nd	nd	**2.6×10^2**
	Spring 2021	nd	nd	nd	nd	**1.4×10^2**
	Autumn 2021	nd	nd	nd	nd	nd
6	Spring 2021	nd	nd	nd	nd	**1.3×10^3**
	Autumn 2021	**1**	nd	nd	nd	**2.4×10^2**

nd = not detected; *TMC – Total Microbial Count

pfu/100 ml (intake No. I), 9–172 pfu/100 ml (intake No. II), 48–265 pfu/100 ml (intake No. III), 4–96 pfu/100 ml (intake No. IV), 64–9.0×10^4 pfu/100 ml (intake No. V) and 1–8 pfu/100 ml (intake No. VI).

Tests of sanitary indicators performed at the same time confirmed the presence of *E. coli* and intestinal enterococci in all intakes where they were present in most raw water samples (8/18 – *E. coli*, 17/18 – intestinal enterococci), whereas somatic coliphages were found even in the samples where no indicators were detected (Tab. 2).

Table 2. Results of the analysis of raw water samples from surface intakes (study results for 2021)

Water Intake No.	Examination Sezon	Somatic Coliphages	*E. coli*	Intestinal Enterococci
		pfu/100 ml	cfu/100 ml	cfu/100 ml
I	Spring	99	nd	+
	Summer	14	nd	+
	Autumn	24	+	+
II	Spring	65	nd	+
	Summer	9	+	+
	Autumn	172	nd	+
III	Spring	nd	+	+
	Summer	48	+	+
	Autumn	265	+	+
IV	Spring	49	nd	+
	Summer	4	+	+
	Autumn	96	nd	+
V	Spring	64	+	+
	Summer	$9{,}0\times10^4$	nd	+
	Autumn	245	+	+
VI	Spring	nd	nd	nd
	Summer	8	nd	+
	Autumn	1	nd	+

Nd = not detected; (+) detected/present

Comparison of results of tests of groundwater and surface water intakes. Somatic coliphages were present at 2 out of 7 groundwater intakes and 3 out of 38 samples, which amounted to 8%. The somatic coliphage count in these samples was low, amounting to 1- 11 pfu/100 ml. In the case of surface waters, somatic coliphages were detected at all tested intakes, in 16 of 18 samples, which amounts to 89%; in 44 % of samples, the somatic coliphage count was higher than 50 pfu/100 ml (Fig. 1 and 2).

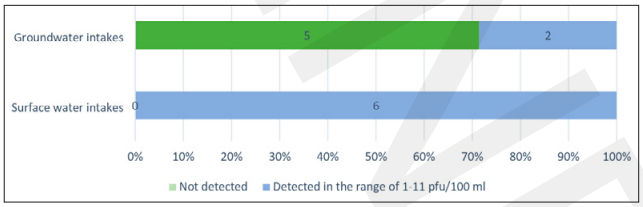

Figure 1. Occurrence of somatic coliphages at various types of intakes

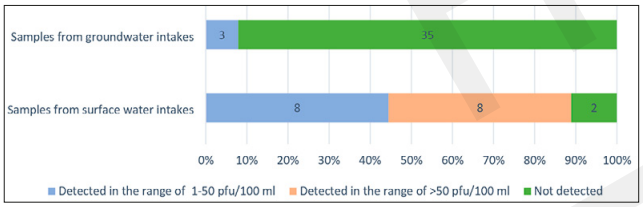

Figure 2. Occurrence of somatic coliphages in water samples depending on types of intakes

DISCUSSION

Most data concerning the phage occurrence in aquatic environments concern somatic coliphages primarily because they can be detected using simple, inexpensive and fast techniques [1]. Somatic coliphages can be present in large quantities in every aquatic environment exposed to faecal contamination of human or animal origin [12].

The results of tests for somatic coliphages in groundwater are often given as the percentage of intakes where somatic coliphages were detected in a specific sample volume [12]. In the presented tests, somatic coliphages were detected at 2 out of 7 groundwater intakes and in 3 out of 38 (8%) raw water samples collected at these intakes. In none of the samples did the somatic coliphage count exceed 50 pfu/100 ml in any of the samples which would warrant an intervention. Similar results, confirming the occasional detection of somatic coliphages and their low count in groundwater samples were obtained, for instance, in Canada [13]. The Canadian research suggests that somatic coliphages were present in 8.7% of

the samples collected from groundwater intakes [10, 13]. Based on these results, the authors found, among others, that somatic coliphages and F-specific RNA bacteriophages were not good indicators of the presence or absence of pathogenic viruses [14]. Tests of water samples from intakes, including household wells and rural and urban water mains, conducted in Spain showed that indicator bacteria were detected more often than bacteriophages in rural intakes and water mains. Bacteriophages, in turn, were detected more often than indicator bacteria in urban mains. In these tests, somatic coliphages were detected in 53.6% of samples collected from groundwater intakes, but it should be noted that they were shallow intakes, located in urbanised areas [15].

Unlike groundwater, surface waters are directly exposed to various types of contamination, including anthropogenic contamination, which may find its way into the water together with domestic sewage, run-off from farmland and atmospheric precipitation [16]. In the case of these waters, somatic coliphages were detected at all tested intakes and in most raw water samples (16/18–89%). Additionally, the confirmed presence of *E. coli* and intestinal enterococci suggested the presence of faecal contamination. Among the tested raw water samples, in 8/18 (44%) of cases, the determined somatic coliphage count exceeded 50 pfu/100 ml, which, as per the indications of the directive, should result in further tests after treatment to confirm the effectiveness of the treatment processes. Also, the conducted tests of treated water samples (acc. to Directive 2020/2184) did not show the presence of somatic coliphages, which confirms the effectiveness of the applied barriers. Also, tests conducted in The Netherlands by Lodder et al. showed that somatic coliphages were detected in all raw water samples collected at 10 surface intakes [17]. The determined somatic coliphage count ranged from 1.1–1.1 × 10^5 pfu/litre. The results of these tests also confirmed the presence of pathogenic viruses, such as rotaviruses (48 %), enteroviruses and reoviruses (80%), among others, in the water samples [17]. Lodder et al. found in their paper that the obtained results did not confirm the role of coliphages as indicators of the quality of water from the intakes, but they could be useful in determining the effectiveness of the treatment process [17]. French tests showed the presence of somatic coliphages at concentrations of 4×10^2 to 1.6×10^5 pfu/litre in surface water samples [18], and Czech studies, in turn, showed that somatic coliphages were present in the analysed water samples at concentrations of 0–25 pfu/ml [19].

The data concerning the occurrence and count of somatic coliphages, both in groundwater and surface water, suggests that their quantities are fairly small, and they are difficult to compare due to, in particular, the properties and technical limitations of the available testing methods (including the differences in the tested sample volumes), as well as the method used to present the results [20].

At the same time, it should be noted that somatic coliphages, as an indirect indicator of faecal contamination, should also not be regarded as an unambiguous indicator of the presence of pathogenic viruses in the water because there is no conclusive evidence of this, while the results of research in some cases are contradictory [1].

Tests of somatic coliphages are intended to determine the effectiveness of treatment processes against pathogenic viruses present in the water. According to the requirements of the new directive, the presence of somatic coliphages in raw water at concentrations > 50 pfu in 100 ml should make it necessary to conduct analyses after steps of the treatment train in order to determine log removal by the barriers in place, and to assess whether the risk of a breakthrough of pathogenic viruses is sufficiently under control [5]. It should be noted that neither the WHO nor other organisations in charge of water safety have defined the maximum parameter for viruses in drinking water, including drinking water [2, 21, 22]. The American organisation EPA, in guidelines 'Surface Water Treatment Rule (SWTR)' issued in 1989 for the filtration and disinfection of surface water, instead of defining the acceptable count for viruses, specified the rate of their removal and/or inactivation as 99.99% (4 log) in the disinfection process [23]. The Canadian guidelines of 2019, 'Guidelines for Canadian Drinking Water Quality' also define the required level of removal and/or inactivation of viruses by treatment barriers as at least 4 log reduction, also indicating that, depending on the quality of water in the intake, this level may be higher [24]. The latest recommendations and guidelines for requirements for water treatment, however, suggest a risk-based approach. The guidelines of the WHO indicate that the requirements for the efficiency of water treatment processes will differ depending on many factors [2].

CONCLUSIONS

The assessment of faecal contamination is an important tool in ensuring the safety of drinking water. The review of available knowledge and data in the context of the requirements included in the currently applicable regulations, indicates certain limitations of the microbiological indicators currently used for routine quality control of drinking water. Due to these imperfections, other parameters that could be used as models or indicators, such as somatic coliphages, became a subject of research because they could be used to monitor the effectiveness of the removal of smaller and more resistant pathogens (including viruses) from the water.

The presented results of the research confirm that somatic coliphages can fill the gap in the monitoring of the removal of pathogenic viruses from drinking water by the treatment barriers. However, it should be noted that this is an area that requires further improvement because data from European countries concerning the occurrence and concentration of somatic coliphages in raw water, are incomplete, primarily because of obsolete testing methods or the absence of such tests [20].

The results of the tests performed confirmed that the microflora of groundwater intakes is fairly limited and appears at low concentrations. Both the analyses concerning sanitary indicators and somatic coliphages showed that water from deep aquifers is protected against microbiological contamination, including faecal contamination. For surface water intakes – as well as for the identification of potentially dangerous incidents in groundwater intakes – it may be necessary to introduce the determination of somatic coliphages. The introduction of this operational parameter should improve the safety of drinking water regarding the appropriate microbiological quality.

In connection with actions intended to improve the health safety of water and implement the requirements of the new directive, somatic coliphages should be incorporated as a new indicator in the programme of operational monitoring

of raw water where the risk assessment supports this. The monitoring of somatic coliphages should be conducted by the water suppliers, based on WHO guidelines and the Water Safety Plan Manual [2, 5, 25].

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